GENE THERAPY IN THE TREATMENT OF LEUKEMIA-A REVIEW

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Abstract

Gene therapy can be broadly defined as the transfer of genetic material to treat a disease or at the very least to enhance a patient's clinical condition. Leukemia is a group of malignant disorders, affecting the blood and blood-forming tissues of the bone marrow lymph system and spleen. Although leukemia typically starts in white blood cells, other blood cell types can also develop leukemia. This review was designed to focus on gene therapy in patients suffering from leukemia. GLOBOCAN, a global observatory for cancer trends, reported 474,519 cases worldwide. The mortality rate is roughly 3.2 percent, with the age-standardized rates being around 11 per 100000. Around the world, around 2600 clinical trials involving gene therapy have already been completed. Information on gene therapy clinical activities from trial databases, government sources, and public sources, is combined in our database to provide a worldwide perspective. This review presents our analysis of clinical trials that, to the best of our knowledge, have been performed worldwide. We now have entries for 2597 trials that were conducted across 38 nations as of the November 2017 update. Gene delivery via retroviral vectors or oligonucleotides continues to be the key challenge in gene therapy. It concluded that among all the strategies in gene therapy of leukemia, molecular approaches were found much more significant. Further research suggested the development of some more genetic codon-targeted implementation to achieve more specific cancerous cell destruction. Gene transfer is also utilized to change the way tumors behave, make them more immunogenic, and give healthy hematopoietic stem cells medication resistance. As this field of science continues to progress, gene therapy will undoubtedly have a significant impact on the management of pediatric leukemia in the future.

Keywords: Gene therapy, leukemia, antisense retroviral vectors, molecular approaches, pediatric leukemia, review

INTRODUCTION

Despite substantial improvements in pediatric leukemia treatment over the past 30 years, a sizable patient group continues to be resistant to chemotherapy and bone marrow transplant treatments. Although leukemia typically starts in white blood cells, other blood cell types can also develop leukemia [1]. There is also concern that the cost of treatment for some individuals leads to unacceptable toxicity, such as cardiac and pulmonary problems, and an increased risk for infection and secondary malignancies emergence [2]. These factors make it important to investigate more therapeutic modalities with the intention of increasing overall increasing the survival rate of pediatric leukemias while lowering treatment-related mortality. Leukemia comes in a variety of forms, which are categorized mainly by how quickly it grows and how slowly it grows, as well as whether it originates in lymphoid or myeloid cells [7]. Leukemia types vary in their prognoses and available therapies.

Myeloid leukemia

The two most frequent subtypes are acute lymphoblastic leukemia and chronic lymphocytic leukemia, which affect the lymphoid chain, as well as acute myeloid leukemia and chronic myeloid leukemia, which impact the myeloid chain [10]. NK cell-related leukemias, mature B-cell and T-cell leukemia, and other less common kinds of leukemia can originate from mature WBC cells. Global cancer trends observatory GLOBOCAN recorded 474,519 cases globally [3]. The age-standardized death rate is approximately 11 per 100000, with a mortality rate of about 3.2 percent [6, 11].

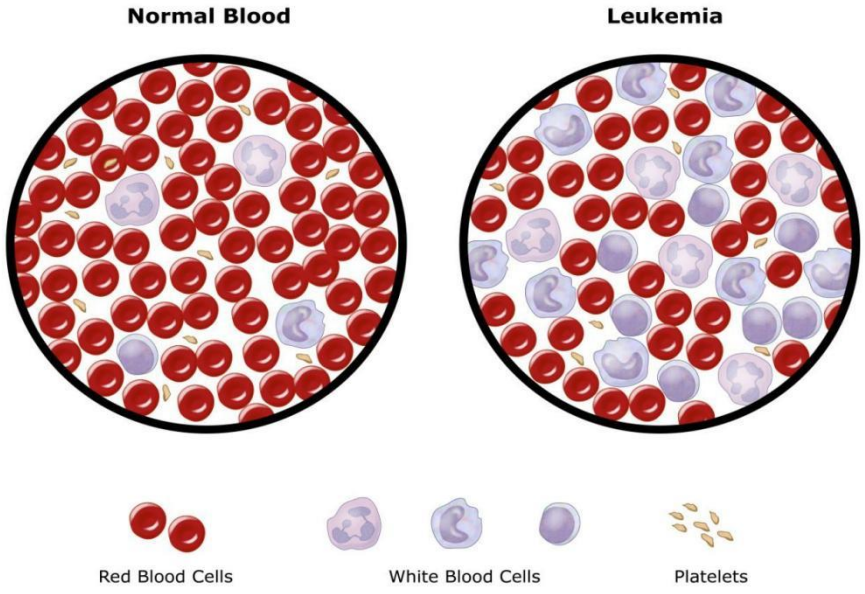

Fig 1. Depiction of normal blood and leukemia

Types of leukemia-

1. Acute lymphoblastic leukemia refers to B and T cell blastic transformation patients. It is the most common leukemia in pediatrics, with up to 80% of cases occurring in

children and just 20% of cases occurring in adults. The majority of young people's treatment is based on pediatric regimens, which have greater survival rates.

2. Acute myelogenous leukemia is the most prevalent adult acute leukemia, which contains myeloid blasts in excess of 20%. The prognosis can vary based on the molecular subtypes because it is the most aggressive malignancy [14].
3. Chronic lymphocytic leukemia refers to the expansion of clonal lymphoid cells. Those between the ages of 60 and 70 are most commonly affected.
4. Chronic myelogenous leukemia (ML) occurs from Philadelphia chromosome, a dysregulated tyrosine kinase on chromosome 22 [22]. It results from the reciprocal translocation and fusion of BCR on chromosome 22 and ABL1 on chromosome 9, which causes ML. In turn, this results in a monoclonal population of granulocytes that are defective, primarily neutrophils, basophils, and eosinophils [21, 26].

Numerous genetic and environmental factors have been seen in the development of leukemia as below -

- Exposure to ionizing radiation increases the risk of developing several distinct leukemia subtypes [4, 5].
- Exposure to benzene raises the risk of adult-onset leukemia, especially AML [8, 9].
- Prior chemotherapy exposure raises the likelihood of developing acute leukaemia, especially when using alkylating medications and topoisomerase inhibitors.
- The probability of eventually having another subtype of the disease increases if you have a history of any hematologic cancer.
- Viruses including the human T-cell leukaemia virus and the Epstein-Barr virus are linked to ALL subtypes.

Given the lack of knowledge regarding the disease's actual causes, it would be of great scientific interest to examine the part that various work-related risk factors play in the development of leukaemia. This analysis would also provide light on the disease's etiology [15].

RISK FACTORS FOR LEUKEMIA

Nowadays, it is common to find environmental radiation and solvent exposure to be risk factors for leukemia. However, AML can arise from a previously identified hematopoietic malignancy or as a consequence of DNA-damaging therapy for a previous malignant disorder [12]. 12% of adult AML patients had previously experienced myelodysplasia, and 8% had a therapy-related illness, 6% of them experienced myeloproliferative neoplasms in the past [16]. Therapy-related illness is mostly caused by such alkylating agents and chemotherapy radiation and topoisomerase II inhibitors are used for the treatment of lymphoma and are thus more common in older people [17].

SYMPTOMS OF LEUKEMIA

Symptoms depend, in part, on the type of leukemia. For instance, if you have a chronic form of leukemia, you may not have noticeable symptoms in the early stages.

Common signs and symptoms of leukemia include;

- Fatigue, tiring easily.
- Fever or night sweats
- Frequent infections
- Shortness of breath
- Pale skin
- Unexplained weight loss
- Bone pain or tenderness
- Pain or full feeling under your ribs on the left side
- Swollen lymph nodes in your neck, underarm, groin or stomach, an enlarged spleen or liver
- Bruising and bleeding easily, including nosebleeds, bleeding gums, a rash that looks like tiny red spots in the skin

DIAGNOSIS AND TESTS

1. Complete blood count: This blood test lets your healthcare provider know if you have abnormal levels of red blood cells, white blood cells, and platelets. If you have leukemia you will likely have higher than-normal counts of white blood cells
2. Blood cell examination: Your healthcare provider may take additional blood samples to check for markers that indicate the presence of leukemia cells.
3. Imaging and other tests: Your doctor may order a chest X-ray, CT scan, or MRI scan if symptoms indicate leukemia has affected your bones, organs, or tissue
4. Lumbar puncture: Your healthcare provider may test a sample of spinal fluid to see if leukemia has spread to the spinal fluid surrounding your brain and spinal cord.

GENE THERAPY STRATEGIES

Numerous alternative gene therapy approaches are being investigated in the search for novel leukemia treatment options. The usage of ribozymes, antisense oligonucleotides, and retroviral vectors are a few examples of methods used to attack cancer's molecular root causes. Another possibility, genetic labeling, is the exchange of cytokine genes and human leukocyte antigens (HLAs) to initiate defense against tumor cells. Through the use of several techniques, the biology of disease relapse after autologous bone marrow transplant has become better understood. After an allogeneic bone marrow transplant, the herpes simplex thymidine kinase

gene and other suicide genes have been used to lower graft-versus-host disease. There have been several modifications made to overcome gene delivery, despite it being a substantial barrier to gene therapy. This article will provide an overview of these gene therapy techniques designed to improve survival [19].

BENEFITS OF GENE THERAPY:

- Experimental gene therapy can attack cancer immediately and then evolve inside the body to keep the disease at bay.
- Gene therapies are used to prevent, treat, or cure certain inherited disorders such as cystic fibrosis, alpha-1 antitrypsin deficiency, hemophilia, beta thalassemia, etc.
- Gene therapy can potentially cure someone of a disease, especially in instances where no other medications have worked.
- Use genes to protect healthy cells from the side effects of therapy, allowing higher doses of chemotherapy.
- Gene therapy has better safety with tolerable adverse effects than chemotherapy for the treatment of cancer [30].

Molecular approaches

Congenital chromosomal abnormalities, disorders of DNA repair enzymes, poisons, viruses, and ionizing radiation have all been associated with leukemia; however, for the vast majority of people, the exact origins of their leukemia are still unclear [4, 5]. However, we continue to gain knowledge regarding the genetic and molecular characteristics of a patient's leukemia. Cancer cells exhibit a wide range of genetic aberrations, including point mutations, deletions, substitutions, and gene amplifications. Leukemias have received a lot of interest due to their frequent association with chromosomal abnormalities such as translocations and inversions. These chromosomal rearrangements typically lead to oncogene activation by one of two mechanisms.

In the t (8;22) translocation, which is common in Burkitt's lymphoma and the L3 subtype of acute lymphoid leukemia, the immunoglobulin gene enhancer and the c-Myc (cellular Myelocytomatosis oncogene) proto-oncogene are shifted near to one another (ALL). The c-Myc gene (cellular Myelocytomatosis oncogene), which is ordinarily healthy, is thus regulated improperly [25]. The t (9;22) chromosomal translocation in chronic myelogenous leukemia (CML) brings together the breakpoint cluster region (BCR) and all genes, generating a novel fusion gene.

Oligonucleotide strategy

Antisense therapy uses short, sequence-specific DNA molecules to modify the expression of genes [33]. The most straightforward scenario is when generated antisense oligonucleotides Watson-Crick base pair with a corresponding target RNA to create a duplex that either stops the production of ribosomal proteins or is degraded by ribonuclease H (RNase H). Targeting transcription factors with duplex DNA decoys is a second oligonucleotide method (Fig. la). By

attaching to certain DNA regions, proteins known as transcription factors serve as the regulating proteins in the production of genes [37].

The development of oncoproteins may potentially be prevented by diverting transcription factors that support abnormal gene expression. In a third technique, complementary oligonucleotides that bind with double-stranded DNA are combined to form a DNA triplex that prevents RNA transcription (Fig. lb). Theoretically, this approach makes sense because the majority of leukemia cells only contain one oncogenic DNA sequence per cell, as opposed to many copies of the matching mRNA transcripts.

Antisense nucleotide treatment

One way to stop the growth of leukemic cells is to use antisense oligonucleotides to inhibit the production of oncogenes. The effects of oligonucleotides that target BCR-ABL (breakpoint cluster region-Abelson) include inducing apoptosis during in vitro culture, increasing PCR-all-expressing cells' susceptibility to the cytotoxic drug, and specifically inhibiting leukemic cell proliferation.

Antisense phosphorothioates increase the survival of mice treated with human CML cell lines. You can also utilize antisense oligonucleotides to c-myc (cellular Myelocytomatosis oncogene), with varying degrees of diminished specificity, to stop CML cells from proliferating and forming colonies [36]. In IL-6 cells, conjugating c-myb (cellular myeloblastosis) oligonucleotides to transfenin-polylysine allowed the oligonucleotide to be internalized through receptor-mediated endocytosis, decrease proliferation, and reduce cell survival [27, 38].

Recent studies have shown that sense BCR-ABL (breakpoint cluster region-Abelson) oligonucleotides nonspecifically inhibit CML cell lines [21, 23], but the precise mechanism by which antisense oligonucleotides function is yet unknown [32, 28]. On the basis of pre-clinical research, clinical trials for oligonucleotide therapy for leukemia have already started. Using autologous CML marrow, antisense c-myc oligonucleotides for CML are being incubated in-vivo. In order to allow patients to get bone marrow transplants enriched for normal progenitors without CML progenitors, the aim of the oligonucleotides is to selectively cause apoptosis in CML progenitors. Sadly, despite receiving antisense-p53 phosphorothioates without any apparent negative effects, patients with acute leukemia did not see any therapeutic benefit [29].

It shows the following merits & demerits -

- Oligonucleotides are intriguing because of their potential for topical or intravenous delivery and putative sequence selectivity. They are also not susceptible to the replication-competent virus that is a common component of viral-based gene therapy systems.
- Since oligonucleotides do not permanently modify the genome, they do not carry the risk of germline modifications found in other in vivo gene therapy procedures.

- The technical challenge has been to develop oligonucleotides with a disease-specific function that do not degrade soon after injection, successfully reach the target cell, and remain stable inside the cell.
- By altering the backbone structure, it is possible to produce oligonucleotides that are resistant to serum and intracellular nucleases.
- The conventional phosphodiester backbone is most frequently replaced by a phosphorothioate or methyl phosphonate backbone. Unfortunately, these changes may decrease the affinity for RNA and have unintended negative effects due to interactions with cellular protein [31].
- The cellular absorption of oligonucleotides is enhanced without lysosomal disruption. Clinical trials for CML and melanoma are presently using liposomes to convey plasmid DNA because they can increase oligonucleotide stability and absorption.

Ribozymes

Site-specific binding of ribozymes to their target RNA molecules causes them to cleave the target RNA, separate, and then be free to cleave other target RNAs. This "revolutionary" discovery of a nonprotein molecule with a catalytic activity that functions as an enzyme will have a significant impact on gene therapy. In order to eliminate the leukemia-specific fusion product, ribozymes combined targeted antisense and enzymatic RNA cleavage.

The hammerhead ribozyme appears to be the most well-suited for gene therapy because it has a catalytic core surrounded by three helices, two of which create complementary flanking arms that connect to each side of the target region. The most efficient ribozymes require an X site in the target RNA (where X is adenosine, cytidine, or uridine). Specificity is obtained by surrounding the catalytic hammerhead region with sequences that are complementary to the target RNA. The most common length for flanking sequences is 12–14 nucleotides. Specificity is compromised by shorter sequences, whereas dissociation and efficiency are significantly impacted by longer sequences. Targeting the fusion site could increase selectivity for the chimeric RNA and reduce binding and cleavage to normal BCR and ABL when translocations, like the BCR-ABL fusion gene, are implicated in the malignant process.

The therapeutic use of ribozymes is nevertheless limited by three important obstacles. Second, the serum's RNase and other components quickly annihilate bare RNA. Clinical use of ribozymes given by liposomes may not be possible due to the high expense of producing significant quantities of liposome+ ribozyme preparations [13]. Last but not least, when ribozymes are delivered through retroviral vectors, additional approaches will be needed to kill leukemic cells that are not transduced by the vector containing the ribozyme.

Other nucleotide strategies

Due to their ability to attach to transcription factors and alter their activity, double-stranded oligonucleotides serve as molecular spies. For instance, the t(17;19) translocation results in the transcription factor fusion protein E2A-HLF (immunoglobulin enhancer-binding factor/hepatic leukemia factor) being present in juvenile pro-B cell acute lymphoid leukemia [35]. It has been determined that E2A-HLF binds to the 10 base-pair (bp) consensus sequence 5'-

GTTACGTAAT-3' from nuclear extracts of leukemia cells with tyrosine hydroxylase (17;19). Additional studies demonstrate that the reporter gene's transcription can be activated when this consensus sequence regulates the fusion protein. The importance of this fusion product in leukemogenesis and its potential as a molecular decoy target for gene therapy require more investigation. It was demonstrated utilizing molecular decoys in a different model system that the transcription factor NF-kB (Nuclear factor kappa B) may control its functional activity by lowering NF-a-dependent activation of the interleukin 2 (IL-2) gene in the Jurkat T-cell line and expression of the HIV enhancer in a B-cell line. Different double-stranded oligonucleotide forms, such as the hairpin and dumbbell structures, are more stable in serum than single-stranded oligonucleotides, which is a benefit of this method. Another strategy for suppressing gene expression is to use oligonucleotides that bind genomic DNA to form a triplex that blocks gene activation.

Gene therapy and cancer immunity

One mechanism by which cancer cells appear to avoid immune surveillance is the down-regulation of Major Histocompatibility Complex (MHC) proteins, which are required for T-cell recognition. After exposing mice to the parental tumor cell line, studies have shown that interferon-y expressing vectors used in retrovirus-mediated gene transfer of cancer cells increase MHC Class I and/or MHC Class II expression, incite a cytotoxic T-cell response, inhibit tumor cell growth in vivo, and develop long-lasting immunity in mice [18].

Another method to boost the immune response is to implant vectors that express allogeneic human leukocyte antigens (HLAs). Using liposome-mediated plasmid delivery, the HLA-B7 antigen gene was introduced into melanoma cells from HLA-B7-negative patients [34]. The local and distant tumor regression in five patients was caused by CD8 T cells. Regression of the local tumor was seen in four cases.

It supports the hypothesis that tumor antigens can be recognized by the immune system. In this instance, the immune response triggered by HLA-B7 activated T cells, some of which could recognize tumor antigens present on all melanoma cells, including those without HLA-B7, while others could recognize HLA-B7 on transduced cells.

Thirdly, enhancing T-cell immunity is the goal of activating accessory cells. For instance, hemopoietic cytokines like granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-2 have been injected into cancer cells by retrovirus-mediated gene transfer. The cytokine-secreting cells were radioactively irradiated, transplanted into mice, and inhibited tumor development when the mice were challenged with the parental cell line. In another experiment, the T-cell-co-stimulating molecule CD80 was injected into mouse leukemia cells to aid in the formation of an anti-leukemic response in vivo.

Antisense retroviral vectors

Retrovirus-mediated gene transfer has also been used to introduce antisense vectors into leukemic cells. The vectors produce complementary RNA sequences that interact with the target RNA to produce double-stranded RNA, which is anticipated to be promptly degraded by

intracellular enzymes. It's important to note that antisense retroviral vectors have not been as successful at expressing sequences that are complementary to the BCR promoter region as those that are directed towards the BCR-ABL junction. A number of antisense retroviral vectors that take advantage of BCR promoter sequences specifically inhibit the growth and proliferation of BCR-ABL expressing cell lines, in contrast to BCR-ABL negative leukemic cell lines [24].

Gene transplantation in bone marrow transplantation- retrovirus-mediated

Despite radionucleotides' thorough examination as labels, they are in vivo long-term utility is constrained by radiation exposure or the loss, repurposing, or sequestration of radiolabel.

The following factors make retrovirus-mediated gene transfer an effective method for labeling autologous cells:

- Retroviral vectors stably insert into the genome of the target cell.
- The designated gene does not get reused or hidden; it stays with the cell for the remainder of its existence.
- If the cell divides, the marker is present in every offspring.
- The polymerase chain reaction, which can identify roughly 1 vector-containing cell, is an incredibly sensitive method of vector detection.
- Retrovirus-mediated gene transfer is a straightforward technical technique that doesn't subject the marked cells to radioactivity or harmful compounds, which could change how the marked cells function [41].

Peripheral blood can be used to expand antigen-specific cytotoxic T lymphocytes (CTLs) ex vivo. Epstein-Barr virus (EBV)-specific CTLs have been generated in this way to combat EBV in vivo [59]. When examining the long-term advantages of CTL treatment, retroviral vectors were utilized to identify the CTLs. When EBV-specific CTLs were transduced ex vivo using a retroviral vector expressing the neo gene, transduced cells with EBV-specific activity were discovered 18 months after infusion [60].

Suicide-gene vectors are also being researched as a GvHD (Graft-versus-host disease) therapy option in the context of Allogenic bone marrow transplant (BMT) [61]. In the majority of research done to date, the suicide vector was herpes simplex thymidine kinase (HSV-TK). However, after being exposed to antiviral drugs like ganciclovir or acyclovir, vector-expressing cells are destroyed since these drugs have been enzymatically changed into a dangerous form. HSV-TK expression doesn't seem to harm cells in any way [62].

Drug resistance genes in leukemia therapy

It is possible to raise the bone marrow progenitors' tolerance to chemotherapy using retrovirus-mediated gene transfer of drug-resistance genes, allowing for dose escalation of the chemotherapeutic agents and the possibility of a greater favorable outcome. It is being investigated whether to transmit the multidrug resistance mutation 1 (MDRI), mutant dihydro-folate reductase genes, and methyl-transferase genes [40]. This method may not be appropriate for leukemias or other cancers of the bone marrow because they run the risk of accidentally

transferring the drug-resistance gene to cancerous cells. Due to the chimeric balance between healthy and leukemic stem cells, which may be altered, at least temporarily, by high-dose chemotherapy or interferon, CML may be an exception. When the normal progenitor cells are recovered from the autologous graft, they can be tested as potential targets for drug resistance genes [39]. Cell sorting of the autologous graft enables the enrichment of normal primitive progenitors from CML [42]. In this scenario, drug-resistant stem cells would be employed for autologous transplantation, and the administration of post-transplantation chemotherapy would facilitate their favored repopulation [43, 44].

TREATMENTS

CAR-T Therapy:

First gene therapy approved to treat leukemia. CAR-T cell treatment for leukemia has achieved outstanding success that should inspire additional attention and work to attain better clinical results using a wide variety of cancer types that have safer profiles. Indeed, there were lessons and guiding ideas. CAR-T research on anti-leukemia has successfully informed and directed therapeutic efforts in CAR-T therapy for cancers other than leukemia [55]. For instance, anti-patients with diffuse large B-cell lymphoma can inhibit tumor development with CD19 CAR-T cells. CAR-T cells designed to identify abnormally glycosylated cancer-associated antigen mucin 1 (MUC1) have demonstrated extraordinary effectiveness in inducing tumor regression in preclinical models of pancreatic cancer [57]. All of the success in these non-leukemia malignancies, especially in solid tumors and lymphoma, will provide priceless scientific and practical knowledge, which will enable the advancement of CAR-T treatment for leukemia. Future research should concentrate on locating relevant antigens that CAR-T cells can target therapeutically while protecting healthy tissues to preserve steady-state functioning [58]. Additionally crucial are standardization and optimization of the procedure to produce clinically relevant amounts in enough. Another top aim in this is expanding access to therapy for more patients. It is aided by the quickly evolving technologies and global partnerships, is our hope that the CAR-T therapy's full potential can be achieved to give the most effective benefits for both leukemia and those with other cancers [56].

TARGETED Therapy

With the use of more accurate sequencing tools, the molecular landscape of AML is rapidly evolving in the identification of germline predisposition syndromes, enhanced disease monitoring methods, innovative molecularly focussed therapies, and prognostic mutations [52]. Despite these developments have enhanced disease surveillance and risk categorization through measurable residual disease (MRD), there are still numerous difficulties, such as the best way to incorporate the prognostic algorithms to assess age-related clonal hematopoiesis (ARCH) mutation and nuanced improved knowledge of the clinical importance of mutations depends on the particular mutational variation, interaction and that have other co-occurring mutations [53]. Despite these obstacles, these developments have improved our understanding of leukemogenesis and helped us to identify its molecular substrates appropriate for monitoring diseases and delivering targeted treatments-enhancing effectiveness while reducing toxicity in

a negative way. It has spent the last three decades relying on cytotoxic regimens. These developments will only help with survival and patients with AML's quality of life [54].

CD40-ligand (CD154) gene therapy:

Recombinant CD40-ligand (CD154) expression in chronic lymphocytic leukemia cells can be achieved by transduction with an adenovirus vector with a fault in replication (Ad-CD154). Ad-CD154 transduced as well as cells of bystander leukemia grow rapidly antigen-presenting cells that are efficient and induce autologous CLL-specific cytotoxic in vitro hazardous T lymphocytes [47]. This research explored the clinical and immunological reactions to autologous stem cells Ad-CD154 cells seen in CLL patients [46]. There was an increase in de novo leukemia after a single bolus infusion of autologous Ad-CD154 transduced leukemia cells. Affected patients also developed interleukin-12 plasma levels were elevated and interferon-g, whose concentration matched the absolute blood $CD4^+$, T-cell counts, before therapy [45]. In general, patients reported more than a 24% increase in absolute blood T-cell counts rising one to four weeks after treatment. Patients suffering from this strategy's unique and efficient gene therapy.

CURRENT TREATMENT

The primary method of treating acute lymphoblastic leukemia typically contains four stages over two to three years: induction, consolidation, intensification, and long-term maintenance. Additionally targeted treatment is provided to avoid relapsing CNS. A more thorough therapeutic approach has resulted in an estimated 5-year overall survival of 90% in childhood acute lymphoblastic leukemia [48]. In adults, the outcome is more disappointing than the results seen in children, with 5-year overall survival at less than 45% [49]. The development of pediatrics-inspired regimens in older patients [50], and later in patients up to 50-60 years of age, has increased 5-year overall survival to 50% or more and up to 70-80% in disease subsets that are associated with a favorable prognosis. In older patients, however, the results remain poor, with 5 years of overall survival at less than 20% [51].

SIDE EFFECTS

- Hair loss: Blood cancer treatment often causes hair loss as a side effect.
- Nerve damage: Peripheral neuropathy is a condition of the nerves. It can be painful, but there are lots of things that can help.
- Brain fog: Lots of people with blood cancer get brain fog as a side effect of treatment.

CONCLUSION

Gene therapy techniques have decreased tumorigenesis in animal models and leukemia cell growth in pre-clinical investigations; a few of these techniques have also already entered clinical trials. Gene delivery via retroviral vectors or oligonucleotides continues to be the key challenge in gene therapy. It concluded that among all the strategies in gene therapy of leukemia, molecular approaches were found much more significant.

Further research suggested the development of some more genetic codon-targeted implementation to achieve more specific cancerous cell destruction.

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A SYSTEMATIC REVIEW ON HAIR TRANSPLANTATION

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ABSTRACT

When a patient has male- or female-pattern hair loss, hair transplantation is a safe and reliable therapy option. Alopecia is one of the most commonly presented problems in dermatology. A condition was loss of hair fall which leads to a lack of self-confidence and social withdrawal in young patients. When compared to their peers with good hair density. Patient selection, counselling, and planning the procedure have equal importance as that of the hair transplantation surgery itself. This activity reviews the techniques like Follicular Unit Transplantation (FUT) it is a commonly used technique for hair restoration. In FUT, a strip of tissue is excised from the occipital donor area resulting in a linear scar. Whereas in FUE, the scarring can be avoided as small individual follicular units are harvested and transplanted in the recipient area. Follicular Unit Extraction (FUE), is another technique for the hair restoration for the patient with patterned hair loss. which can elaborate by step by step by procedure Graft insertion and also Drugs used like Minoxidil, Finasteride used and other therapies like Biotin and Low-Level Laser Therapy (LLLT) can be explained in detailed.

KEYWORDS: Hair Transplantation, hair restoration surgery, follicular unit transplantation, follicular unit extraction.

INTRODUCTION:

Hair loss represents a distressing issue affecting a large portion of the population, including up to 85% of males & 40 % females, and its incidence increases with age for both sexes. Though many causes of hair loss exist, by far the most common etiology is androgenic alopecia (AGA), an androgen sensitive pattern of hair loss that affects both men and women. [1] (Indeed, non-surgical modalities exist which may halt the progression of androgenic alopecia and even help grow new hairs (i.e., minoxidil, finasteride, low level laser light therapy, platelet-rich plasma). [2]

Nevertheless, recent advancements in surgical hair restoration have made hair transplantation an increasingly effective, safe, and reliable way for patients distressed by androgenic alopecia to regain a more youthful and natural appearance of their hair. [3] The field of hair transplant surgery is undergoing prominent advancement with the goal of optimizing the results and ensuring better graft survival [4].

OBJECTIVES:

- Identify the anatomical structures, reasons for doing a hair transplant, and things that shouldn't be done.
- Describe the instruments, personnel, preparation, and method used in hair transplantation.
- Review the appropriate assessment of the clinical relevance and probable side effects of hair transplantation.

- Pre and after care measures for surgeries involving hair transplants and patient care
- Give an overview of the interprofessional team's initiatives for enhancing communication and care coordination to accelerate hair transplantation and enhance results.

ESSENTIAL NEEDS FOR HAIR REGROWTH:

- Regulating dihydrotestosterone levels in the blood
- By ensuring normal supply of balanced nutrients to the hair roots.
- Proper blood circulation to carry the nutrients to the roots in the scalp.
- Maintaining good health through general wellbeing of the individual.

The state of reduced anagen, or the growth phase, and prolonged telogen, or the fall phase, is what causes hair loss rather than an illness. In alopecia, this is what causes hair loss. Both internal and external causes may contribute to hair loss.

EXTERNAL FACTORS FOR HAIR LOSS	INTERNAL FACTORS FOR HAIR LOSS
The lifestyle and environmental factors do influence the hair health and growth cycle. Common causes like: 1. Smoking 2. poor scalp hygiene 3. alcohol intake 4. lack of sleep, stress. 5.pollutions, fumes, exposure to endocrine disrupting chemicals 6. crash dieting, fat diets, high- protein diet, less intake of fluids. 7. physical factors	Numerous elements that are internal to the body might cause deficient conditions that inhibit hair growth. Common causes like: 1. Iron deficiency, vitamin deficiency, calcium deficiency, zinc deficiency 2. Thyroid issues 3. insulin resistance 4. metabolic disorders like: gout, PCOD, chronic illness, mental stress, 5. Following surgery and individuals with seborrheic scalp

CLASSIFICATION OF HAIR LOSS:

They are mainly two types hair loss patients

1. Male pattern alopecia (By Norwood classification)
2. Female pattern alopecia (By Ludwig classification)

CLASSIFICATION OF MALE PATTERN ALOPECIA:

The Norwood classification system (1975), which is a system for identifying male pattern hair loss, is the most widely used classification. The Hamilton system from 1941 has been refuted.[5]

NORWOOD CLASSIFICATION TYPES OF HAIR LOSS:

Type 1: Present the normal hairline with minimal recession of the frontotemporal area.

Type 2: Patients present a symmetrical and mild recession of the frontotemporal area along with thinning of the hair.

Type 3: Patients define balding with minimal or no in the frontal areas and a deep recession.

Type 4: Patients present with significant recession and loss of both frontal and vertex hair with a bridge of her between the two still retained.

Type 5: Category patients present a progression of the type four category with only thin narrow bridge of hair between the frontotemporal and the vertex areas.

Type 6: Represent the loss of hair that separates the frontal and the posterior vertex areas with further progression in lateral and posterior zones.

Type 7: Represents the most severe from of band of hair remaining in the low occipital and temporal areas with extensive miniaturisation of the hair.

Basically, in the above classification, the two areas of hair loss -a bitemporal recession and thinning crown gradually enlarge and coalesce until the entire front, top and crown of the scalp are bald.

To regain the crowning glory, medical treatment gives good results up to 3 type patients while hair transplantation is the only choice for patients between types 4 and 6.

Routinely clinicians come cross variations of the classification which fall outside the Norwood classification, they are as follows: differential use pattern alopecia, diffuse and patent alopecia.

2.Classification of female pattern alopecia:

The Ludwig classification (1977) uses three stages to describe female pattern genetic hair loss

Association of oral and maxillofacial surgeons of India.

Ludwig scale classification types:

Type 1: Mild

Type 2: Moderate

Type 3: Extensive

In all three Ludwig stages, there is hair loss on the frontal hairline. The back and sides may or may not be involved.

In Ludwig type 1: Early thinning happens, but it can be easily hidden by regular check-ups. Patients with type 1 have insufficient hair loss to support surgical hair restoration.

The midline area of women who have type 2 hair loss has significantly widened, and the donor area on the sides and back of the scalp is stable, hair transplantation can be necessary

In Ludwig type 3: The base of the scalp seems thin and transparent. This is frequently accompanied by generalised scalp thinning. Patients who have reached this stage frequently have insufficient donor hair to warrant surgical hair restoration.

Every woman suffering with hair loss needs to have a precise produced, ideally by a dermatologist with experience. This is especially crucial given the scattered hair loss that typically, women develop, but occasionally, a number of medical disorders that are curable, no matter what only women with stable hair on top can determine the level of hair loss hair transplant candidates for the scalp's side and back transplantation.

Other infrequent alopecia's observed among the patients visiting hair clinics are as follows:

Alopecia areta: It's an autoimmune disorder which starts with small round and punched out areas with no hair. Lesion has very smooth skin and not even a single hair is present in the lesion.

Alopecia totalis: There is total loss of scalp hair.

Alopecia universalis: There is total hair loss in the body including the eyebrows.

Turban alopecia: It caused due to tight turban usage. It affects frontal and parietal areas mainly and is seen among Sikhs.

Trichotillomania: A psychiatric disorder where the individuals deliberately pull their hair every day leading to hair loss in those areas.

Medications for Hair loss:

Only 2 drugs have been approved by FDA and have proven their efficacy in the management of hair loss with good success.

Minoxidil:

This is the only over-the-counter medication for hair loss approved by the FDA for use by both men and women. It won't rescue a receding hairline. It does stimulate hair growth, although scientists aren't quite sure how it works.[6][7]

Minoxidil is available as Rogaine or Theroxidil, or in generic form. It's sold as a liquid or foam and in two strengths: 2% and 5%.

- **Effectiveness:** Minoxidil works for about 2 out of 3 men. It's most effective if you're under age 40 and have only recently started to lose your hair.
- **How to use it:** Twice a day, when your hair is dry, apply minoxidil on your scalp where the hair has started to thin. Then be patient. You may not notice changes for 4 months or more.
- **What it doesn't do:** Minoxidil does not cure baldness. If you stop using it, you will start losing hair again. Your hair may fall out faster than before.
- **Side effects:** You may have redness, itching, dryness, flaking, or other scalp irritation, though this is uncommon. It's more likely if you use the stronger 5% solution.

Finasteride:

This medication stops your body from making the hormone at the root of male pattern blandness DHT (dihydrotestosterone). It is available under the brand name Propecia. Learn more about spotting the early signs of having high DHT.[8][9]

- **Effectiveness:** Finasteride is very effective. It slows or stops hair loss in nearly 90% of men. About two-thirds of these men also regrow some hair.
- **How to use it:** Finasteride is a pill. Usually, you take it once a day. Your dermatologist may recommend using it in combination with minoxidil.
- **What it doesn't do:** Like minoxidil, it doesn't cure hair loss. If you stop taking it, you will lose hair again.
- **Side effects:** Finasteride can cause erectile dysfunction and other sexual side effects, though this is unusual. If it happens to you, it will likely clear up once you stop taking finasteride. But for some men, that can take 3 months or more.

Biotin and Low-Level Laser Therapy (LLLT):

Biotin is a B vitamin that is essential for your health. You most likely get plenty of it in your diet in egg yolks, yeast, liver, and other foods. That's good news because too little biotin can cause hair loss. Does that mean that taking mega-doses of it will give you more hair? Probably not. No scientific studies have shown biotin to prevent or treat hair loss. Learn more about biotin and hair loss. [19]

You may have heard that laser combs, brushes, hoods, and caps can help halt hair loss. The theory is that when hair follicles absorb laser light at a certain level, it stimulates hair to grow. But there's not enough evidence that any of these devices restore hair or prevent balding.

Platelet - rich plasma (PRP):

PRP (platelet – rich plasma) therapy for hair loss is a three- step medical treatment in which a person's blood is drawn, processed, and then injected into the scalp.

Some in the medical community think that PRP injections trigger natural hair growth and maintain it by increasing blood supply to the hair follicle and increasing the thickness of the hair shaft. Sometimes this approach is combined with others hair loss procedures or medications.

PRP Therapy Process:

PRP therapy is a three - step process. Most PRP therapy requires three treatments 4-6 weeks apart. Maintenance treatments are required every 4-6 months.

Step 1:

Your blood is drawn – typically from your arm- and put into a centrifuge (a machine that spins rapidly to separate fluids of different densities).

Step -2:

After about 10 minutes in the centrifuge, your blood will have separated into in three layers:

- Platelet poor plasma
- Platelet rich plasma
- Red blood cells

Step -3:

The platelet rich plasma is drawn up into a syringe and then injected into areas of the scalp that need increased hair growth. There hasn't been enough research to proven whether PRP is effective. It's also unclear for whom and under what circumstances its most effective.

According to a recent study. Although PRP has sufficient theoretical scientific basis to support its use in hair restoration, hair restoration using PRP is still as its infancy. clinical evidence is still weak. [20].

Postoperative Minoxidil, Platelet-Rich plasma, Finasteride, Low-Level Therapy:

It has been proposed that the vasodilatory effects of minoxidil enhance postoperative wound healing. Minoxidil 5% must be administered twice daily in the recipient and/or donor areas starting 5-7 days after surgery. The danger of postoperative haemorrhage and scalp itchiness has increased, nevertheless. A lower dose or a reduction in frequency may be helpful if irritation occurs. The use of minoxidil should be stopped and a topical corticosteroid provided if it results in irritating folliculitis.[10]. Studies have looked into the effectiveness of using plasma rich in growth factors (PRGF) technology as an adjuvant therapy for FUE surgery. Proliferation and migration of follicular cells were induced by autologous growth factors. PRGF decreased postoperative crusting, the time it took for hair to grow back, inflammation-related pain, and itching while also enhancing the integrity of perifollicular structures and extracellular matrix proteins [11]. compared PRP in the root of the grafts in two sites of hair transplantation in a small cohort of patients. On the scalp, two 2.5 cm2 regions were grafted with about 20 grafts per cm2. The area where PRP-enriched grafts were placed showed a greater follicular unit survival rate and density during a 12-month follow-up. [12]. There was no discernible difference between the groups in terms of hair growth in a trial that evaluated the outcomes of low-level light therapy irradiation with placebo in hair transplantation where the hairs were irradiated prior to transplantation ($P > 0.8$). [13]

Planning of hair transplant surgery:

Since a hair transplant is a surgical treatment, the surgeon typically has plenty of time to plan it out. Planning begins even before the first patient consultation. The doctor must determine the necessary number of grafts during the initial consultation by evaluating the patient's preferences, the quality of their hair, and the severity of their alopecia. The result may vary depending on factors such as age, medical history, alopecia in the family, facial features, and hair qualities (calibre, texture, and colour). From the surgeon's point of view, this is very important because the quantity of grafts used heavily influences the transplant result. Additionally, as the cost of the transplant is also based on the quantity of grafts used, it affects the patient as well. Additionally, the initial interview assists in identifying the patients with and the contraindications.

Morden techniques of hair transplantation:

Two types of hair transplantation techniques like

1. Follicular Unit Transplantation (FUT)
2. Follicular Unit Extraction (FUE)
3. Graft Insertion

Follicular Unit Transplantation Technique:

The donor area of the scalp's occipital region is used to harvest a strip of graft that is approximately 10-20 cm long after that, each FU is separated off the strip. With enlargement. These are subsequently inserted into the slits already made in the frontoparietal region of the scalp, '' receiving area''.

Figure 1,2: Mechanism of Follicular Unit Transplantation. Donor area suture site soon after the strip harvesting in Follicular unit transplantation (FUT).

The advantages of the FUT technique over the conventional methods are:

1. Minimal bleeding from the donor area.
2. The single strip provides 1000-1500 grafts
3. Placement of individual FU in each slit gives a denser packing and cosmetically better results.

The various disadvantages of this method include:

1. Skill based method.
2. Require careful handling of delicate hair follicles.
3. Needs microscopic dissection of hair units, hence time consuming and laborious.
4. More expensive.
5. The donor area linear scar may widen and may lead to poor cosmesis.
6. Postoperative pain is more.

Follicular Unit Extraction Technique (FUE):

Individual FU are extracted from the donor area and applied to the recipient's frontoparietal scalp via the FUE procedure. Originally, known as the "FOX procedure," FUE, FUSE (Follicular Unit Separation Extraction) method, Wood's technique, or FU Isolation method is rapidly becoming a substitute to the strip technique. Bernstein and Rassman started developing the FOX procedure, proclaiming it as a new surgical method for hair restoration without strip harvesting.

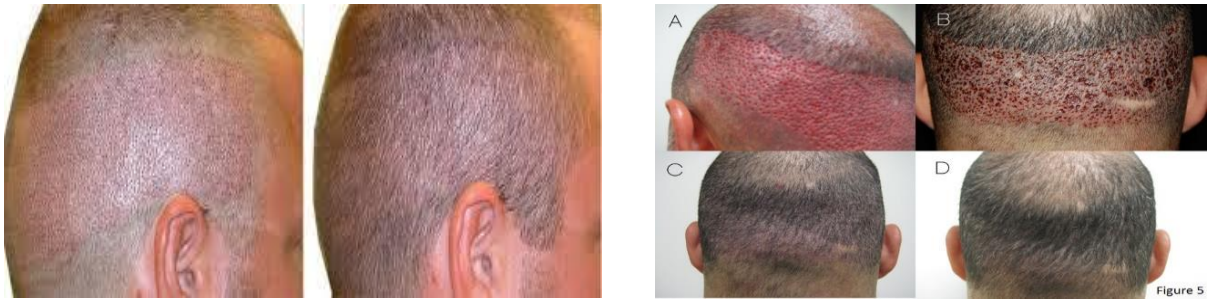

Figure 3,4: Follicular unit extraction. Donor area soon after the individual follicular unit's grafts harvesting in follicular unit extraction.

TABLE 2: DIFFERENCES BETWEEN FUT& FUE: [14-17]

	FOLLICULAR UNIT TRANSPLANTATION(FUT)	FOLLICULAR UNIT EXTRACTION (FUE)
Hairstyle in donor area	Short hair not possible	Short hair possible
Harvesting technique	Single strip	Individual follicular units
Stitches	Required	Not required
Microscopic dissection	Required	Not required
Postoperative bleeding/pain	May occur	Uncommon
Never damage	Possible	Uncommon
Reaction to suture material	May occur	Uncommon
Shaving of head prior to surgery	Not required	Required
Fatigue	Not tiring due to shorter procedure	Tiring due to longer procedure
Healing time		
Donor area	2-3 weeks	1 week
Recipient area	10-14 days	10-14 days
Recovery time	2-3 weeks	1 week
Patient is fit to return to work	5-7 days	The day after
Visible scarring	Present	Absent
Large areas	Possible faster	Tedious
Staff requirements	More	Less
cost	Cheaper	Expensive
Natural result in recipient area	Yes	Yes
Pain	Yes	Yes

RECIPIENT INFLUENCE VS. DONOR DOMINANCE:

The tendency of the transplanted hair grafts to retain the characteristics of the donor location following transplantation to the recipient site is known as donor dominance, according to Orentreich. This aids in hair transplantation because balding spots in the non-permanent parts of the scalp are covered with hair from the permanent donor zone of the scalp. As we all know, body hair follicles have a brief anagen phase, so when transplanted, they don't match the features of scalp hair, such as length, thickness, and curls. It was discovered that the recipient area affects the donor hair's growth cycle and rate in order to imitate recipient hair features. [18,19,20]

FUE technique with non-scalp hair as donor:

The main benefit of FUE is that it allows for the use of body and facial hair, which is necessary in severe cases of baldness with little scalp tissue available for donor. [22] Even patients who have already had surgery and have used up all of their donor tissue can still get good results. However, the thickness, length, and hair cycle of body and beard hair are very different from those of scalp hair.

Eyelash, brow, and beard transplant:

Patients who want denser beard, eyebrow, or eyelashes may also benefit cosmetically from hair transplantation. Additionally, it can be used to restore eyelashes, eyebrows, and beards in people with alopecia areata (stable), traumatic scars, trichotillomania, acid burns, etc.

Graft Insertion:

Extracted grafts Using small-angled forceps, the grafts are placed into the recipient incisions. The grafts must be implanted using a non-traumatic method. To insert the FUs into the recipient slits during FUT, subcutaneous tissue beneath the hair bulbs grasps them. Following a "No root touch" strategy in FUE, where the FUs are held by the upper 1/3rd region without harming the bulb, is advocated. To ensure that the grafts are at the same level as the surrounding skin, consistent pressure is used [Figure 10]. Avoid burying the grafts below the level of the skin if possible because doing so might cause a pitted appearance and epidermal cysts. If the grafts are too far above the surface, the look will resemble cobblestones.

The techniques of graft insertion are as follows:

1. During the "stick and put technique," a recipient is slit, and a helper immediately inserts hairs into the region.
2. establishing every recipient site at once, followed by individual graft placement.
3. utilising implanters, a technique involving direct scalp implantation of hollow-end pen-shaped instruments containing an extracted graft.
4. Making all of the recipient sites and using an implanter to place all of the grafts at once.

Figure 5: Implantation of follicular units using fine angled forceps.

[23] developed an alternative method for expediting the transplantation process. The follicular units must be carefully prepared before being implanted with an implantation device. The "Choi Implanter" is a useful device into which follicular units are loaded. To implant the graft, the needle is inserted into the scalp and the plunger is pressed. A three-person team of two loaders and one planter is typically required to implant 12 grafts per minute, or approximately

700 grafts per hour. Alternatively, the slits can be pre-made by the surgeon and "filled" with the implanter by the assistants. This ensures proper graft density, and the surgeon retains complete control over the angle, density, and direction of the grafts. [24]

Female Hair Transplant:

A female hair transplant is not the same as a male hair transplant. Hairs are not trimmed in this case, the hairline has more rounded temporal peaks, and the transplant is planned with a wide base at the hairline and a tapering design posteriorly, corresponding to the FPHL's 'Christmas tree' pattern alopecia. [25]

Megasession:

FUE megasession is defined as transplanting more than 3000 grafts in a single surgery. A team of 1-2 surgeons and 4-5 assistants will perform the transplant. Surgeons will be responsible for graft harvesting and slit creation, while three assistants will be responsible for graft dissection and two assistants will be responsible for graft implantation. Most hair restoration surgeons recommend harvesting only 50% of the donor density because more than that may result in a visible decrease in donor hair density. FUE megasession has the advantage of reducing surgery frequency, and patients with severe AGA are encouraged to seek hair restoration treatment.[26]

Summary:

Hair transplantation is considered to be an instant long-lasting remedy for patterned hair loss. Patient selection, counselling, and planning the procedure have equal importance as that of the hair transplantation surgery itself. FUT and FUE are the two commonly used techniques of hair restoration. In FUT, a strip of tissue is excised from the occipital donor area resulting in a linear scar. Whereas in FUE, the scarring can be avoided as small individual FU are harvested and transplanted in the recipient area. FUE is emerging as a most opted hair restoration procedure in patients with patterned hair loss.

Declaration of patient consent:

The authors attest that they obtained all necessary patient consent forms. The patient(s) has/have given his/her/their consent in the form for his/her/their images and other clinical information to be published in the journal. The patients understand that their names and initials will not be published, and that while every effort will be made to conceal their identities, anonymity cannot be guaranteed.

Conclusion:

Surgeons who perform aesthetic surgeries on the face must have a diverse set of skills in order to address all aspects of beauty that are defined by the individual elements. The most important of these is the framing of the face like a photo frame with hair transplantation, which allows us to focus our vision on the finer elements of facial beauty, such as the nose, lips, eyes, teeth, jawline, or a good smile. If surgeons adhere to the principles of hair transplantation surgery, they will produce patients who are highly satisfied with the FUE or FUT procedure's micrograft’s. By embracing this sophisticated form of art as a tool.

Thus, understanding the above logical mathematical principles with regard to hair loss enables the surgeon to plan and economize donor graft supply to achieve a balanced, aesthetically pleasing appearance in the long run and to avoid mistakes and short-sightedness.

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Review Article

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Review on Chromatographic Fingerprint Analysis of Herbal Medicines

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Abstract

The concept of biological chromatographic fingerprinting for quality control of herbal samples is still relatively new. It was initially developed using HPLC, and more recently, thin-layer chromatography was used to extract the botanical profiles of herbal samples (TLC). This study provides an overview of the use of liquid chromatographic methods for the botanical fingerprint analysis (BFA) of sophisticated herbal specimens. The prospects for biological TLC fingerprint development are discussed in more detail since it is a relatively novel option. Along with previous research, some novel findings are presented and recognized. The objective of the paper is to awaken scientists to the peculiar solutions provided by biological fingerprint construction.

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INTRODUCTION

Overview of Chromatography

Greek words chroma, which signifies "colour," and graphene, which signifies "to write," are the origin of the word "chromatography." Chromatography is a technique for both qualitative and quantitative studies that allow for the separation, purification, and identification of various components from a mixture. Chromatography is used to separate polarity, enzymes, and net charges based on interactions between hydrophobic molecules. Chromatography is a physical technique for separating substances. TSWET, known as the "father of chromatography," developed the method to separate various plant pigments, such as chlorophylls and xanthophylls, bypassing these pigments down a glass column filled with finely split calcium carbonate [1].

Chromatography consists of two phases, which include:

1. Stationary phase
2. Mobile phase

Stationary Phase

In chromatography the unmoving phase is known as the stationary phase with the sample. In high-performance liquid chromatography, "stationary phase" is composed of silica gel, alumina, plus glass. Glass plate coated with silica gel serves as the "unmoving phase" in TLC. In paper chromatography, a paper strip is alluded to as a "stationary phase."

Mobile Phase

In chromatography, the term describes the phase that moves with the sample. A "Mobile phase" is used in high-performance liquid chromatography, for occurrence. In TLC, the "Mobile phase" is a combination of solvents. A "mobile phase" is a solvent in paper chromatography.

This method involves applying the mixture to a stationary phase (solid or liquid), allowing a pure solvent—such as water or any gas—to move slowly over the stationary phase, and allowing the components to be transported individually in accordance with their solubility in the pure solvent (Figure 1).

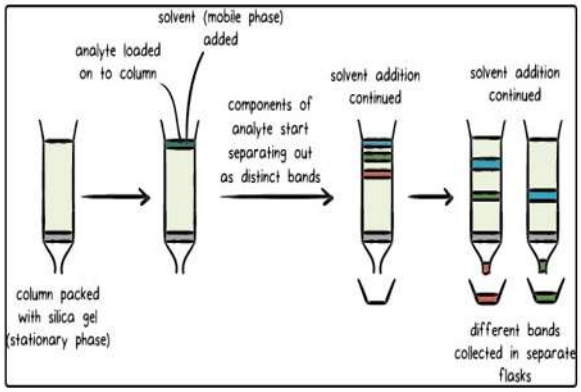

Figure 1: Principle of Chromatography

Fingerprinting

A pattern or an impression that is extremely exact enough to serve as a distinguishing feature for that specific entity.

Chromatographic Fingerprinting

Chromatographic fingerprinting is an effective, extremely accurate way to check the purity of herbal remedies.

It is used to determine the individual herbs that are added to medicinal herbs in addition to determining their identity and quality.

Several Key Chromatographic Fingerprinting Definitions

1. A chromatographic fingerprint is a pattern of chemically distinctive elements found in the specific extract, according to the state drug administration of China (2000).

2. According to Reich E & Schibli A, (2007): An enhanced fingerprint of a plant species serves as the "chemical signature" of a specific herbal entity and allows for the acquisition of a wide range of data about the sample. It also helps to understand the "Total Ingredient Patterns" of that plant species. The fingerprint of a certain plant species is established and serves as a restriction.

3. Herbal sample chromatographic fingerprints or spectroscopic signals, whose comparison enables unmistakable sample identification, according to Lukasz Liesla (2012).

Major Types of Chromatographic Fingerprinting

High Performance Liquid Chromatography (HPLC)

HPLC, is an analytical technique for segregating, characterizing, or quantification each constituent in a mixture. The mixture is separated by using techniques of column chromatography, and it is recognized and analyzed employing spectroscopy. HPLC is basically a dramatically improved version of column liquid chromatography. By instead allowing a solvent to run through a column under gravity, high voltages of up to 400 atmospheres are utilized to drive the solvent through (Figure 2).

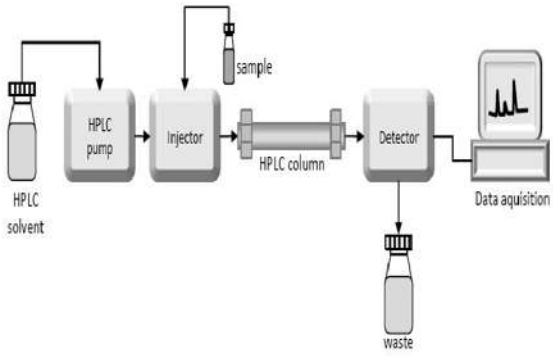

Figure 2: Block Diagram of High-Performance Liquid Chromatography (HPLC)

Thin Layer Chromatography (TLC)

The ingredients in a mixture were segregated utilizing thin layer chromatography (TLC), an affinity-based approach. TLC is a separation method involving diverse purposes, including the assessment of the both qualitative and statistical specimens.

Example: Pesticides are one of the many substance classes that TLC can be used to analyze. Steroids Alkaloids Lipids Nucleotides Glycosides Carbohydrates Oleic acid (etc...,).

A glassy, plastics, or aluminum plate surface that has been sparsely covered with an adsorbent material, usually silica gel or aluminum oxide, exists to serve as the stationary phase in TLC. After the sample was therefore spotted onto one end of the TLC plate, it is mounted directly into a closed chamber comprising an organic solvent (mobile phase). Sample components transfer over a variation of distances when the mobile phase is driven up the plate by capillary forces owing their special properties for the stationary and mobile phases. Once the solvent has reached the top of the plate, it is extracted from either the developing chamber and left to dry. Upon these plates, the segregated constituents display as marks, and the retention factor (Rf) across each constituent

is assessed (Figure 3).

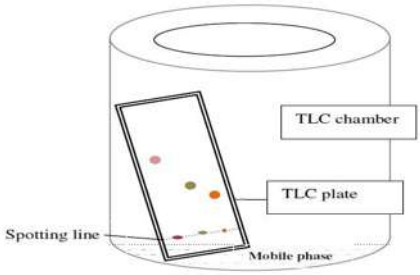

Figure 3: Block Diagram of Thin Layer Chromatography (TLC)

Retention Factor (Rf)

The behavior of a molecule is often explained using the improved specific, or Absorption spectrum, of that substance on a TLC. Every chemical, under the same conditions, has a numerical value for the binding constant, or Rf. The Rf for a certain molecule will only be incessant from one session to the next if the chromatographic parameters listed below are aligned: Temperature, the solvent system, the adsorbent, the thickness of the adsorbent, the quantity of material spotted; Since it can be difficult to keep these variables consistent from experiment to endeavor, comparative Rf values are frequently taken into consideration. The techniques are presented as perhaps "Relative Rf" and are supplied in relation to a benchmark.

Botanical Fingerprinting of Herbal Medicines based on Liquid Chromatography

The quality control of herbal samples uses a relatively recent concept called biological chromatographic fingerprinting. It was initially created using HPLC, and more recently, thin-layer chromatography has been used to extract the biological profiles of herbal samples (TLC). This study provides an overview of chromatography by using liquid methods for the analysis of biological fingerprint for sophisticated botanical extracts. The prospects for biotic thin layer chromatography fingerprint development are discussed in more detail since it is a relatively novel option. Along with previously cited data, some new speculations are presented and addressed. The goal of the thesis is to raise awareness among scientists of the distinctive benefits provided by the creation of biological fingerprints [2].

The creation of fingerprints has grown in importance as a quality control technique for herbal samples due to the steadily increasing demand for medications from natural sources. The WHO has approved fingerprint analysis of a tool to ensure the purity of herbal sample. It was used for recognize firmly similar botanicals, spot forgeries, manage the eradication process, conversely assessment of caliber for a finished product. Botanical fingerprints are a collection of distinctive chromatographic that, when compared, provide clear unmistakable sample determination [3].

Numerous chromatographic techniques, including thin-layer chromatography, high-performance liquid chromatography, and greater speed current counter chromatography, have been used to create fingerprints. However, pinpointing with absolute confidence of gesture signals like peaks, bands, etc. to verify the identification of the sample, must appear in the generated fingerprint, is difficult. Extrapolation of the sample perhaps comparable for predetermined established Botanical Reference Sources was a group to benchmark cannabinoids for that purpose. New approaches to fingerprint matching are needed because it is challenging to define and get BRM for every plant species. As more chromatographic and/or spectroscopic signals are now available to enable more thorough data analysis, the concepts of multiple fingerprint synthesis and multidimensional fingerprinting have recently attracted a lot of attention. While multidimensional fingerprinting uses hyphenated detectors that record hydrolyzing compounds, such as DAD and MS, multiple chromatographic fingerprints are made up of multiple chromatographic profiles [4, 5].

Obeying amalgamation, the data set is often scrutinized using a similarity measure or sophisticated analytical method such as nonparametric calibration, clustering algorithms, combined linear discriminant analysis. It is suggested to use a data fusion-based technique when there are several fingerprints. Building customary fingerprints, albeit one that are then used to generate data. The correlation coefficient measure is typically used to determine how similar the fingerprint of tested material and a reference chromatographic profile are.When it comes to herbal remedies, biological activity is a crucial factor. Mainstream fingerprint detection employing chromatography, nevertheless only provides research with numerical data. The fact that substances may exert stronger biological action when present in low concentrations as opposed to being present in higher concentrations is a crucial issue. Therefore, it is crucial to incorporate biological activity screening into the study of chromatographic fingerprints. Biochemical fingerprinting analysis has been introduced to check the presence of the most active chemicals in natural samples. It was initially created using high-performance liquid chromatography, and it not only offers appropriate information, but also the chance to discern

the additives from the myriad other bioactive constituents in natural specimens. researchers provides thorough reviews of HPLC biological fingerprinting techniques [6, 7].

HPLC-Based Botanical Fingerprinting

An idea of a botanical fingerprint was initially created to ensure the lethality of sophisticated orthodox Chinese therapies. A group of experimental and computational signals one which allow the cannabinoids in a daunting herbal sample to be ascertained that can be referred to as a biological fingerprint, which is analogous to the concept of a typical chromatographic fingerprint. Additionally, botanical impressions were incorporated with conventional computational patterns commonly used in order to get more detailed data on the complicated sample. However, the fundamental objective of creating a bio fingerprint is to identify specific active substances that are a facet of an intricate array are qualified to get potential treatments. Additionally, bio fingerprint analysis can be utilized to model and assess the in vivo effects of active substances [8].

Examples include: Contact with cell membranes. Serum proteins, enzymes, and receptors (etc.,).

Therefore, bio fingerprints combine the qualitative and quantitative data from classical chromatographic fingerprints with biological activity. The bulk of bio fingerprints have been created by interactions between tiny molecules and biomacromolecules. The potential interactions between the substances in herbal formulations and Investigations were done on Genetics, biological fluids, hepatic purée, and oligos. ubiquitously an editorial with immobilized proteins or other macromolecules is linked with an RP-HPLC column. Given that many drugs target Genome on a genetic level, including anticancer, antiviral, and antibacterial ones, the most widely used approaches have been those utilizing DNA [9].

Two methods have been used to create biological profiles: affinity chromatography with DNA immobilized on silica gel and microdialysis followingits linkage with Genes. First instance of the researchers combined a silica monolithic ODS column with an immobilized DNA column. Under these chromatographic circumstances, the researchers were able to generate discrete biometrics for Druze (See Figure 4). The main disadvantage of such a solution is the DNA degradation that results in a drop in column efficiency; as a result, the stream needs to be kept at 4 degrees Celsius. The authors examined the In between chromatographic traces of the contact with DNA the case of the approach with the microdialysis step. In addition to data multiplication, which makes comparative research easier, DNA-binding fingerprints offer the chance to identify substances that may be employed as DNA-target medications. In addition, bio-fingerprinting chromatogram analysis offers an alternative to the standard method for identifying bioactive substances in complicated samples [10].

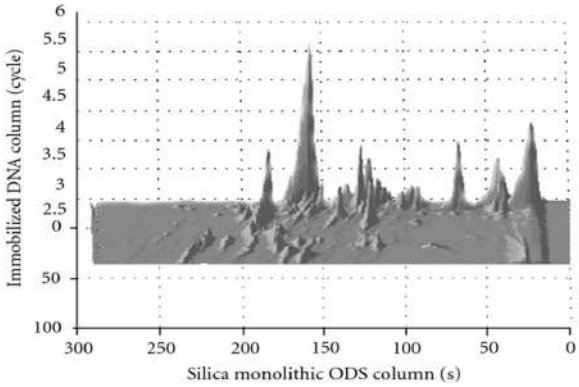

Figure 4: Rheum Palmate L.'s Binary Chromatographic Fingerprint was Acquired

Lactoferrin in supernatants, which graffiti on a column packing's external, has been used to create biological fingerprints in addition to DNA-interaction profiles. One of the most significant drug-binding macromolecules in human plasma is this protein. To screen a traditional Chinese medicine prescription made up of ten medicinal components, Wang et al. used monolithic ODS columns in the second direction and silica-bonded human serum albumin columns in the first. The authors stress the significance of using an HSA-immobilized column in the first dimension since ODS, a second-dimensional column, is better suited for MS detection and is characterized by a higher peak capacity. This multidimensional liquid chromatography technique was used to separate 100 molecules that interact with HSA, and 19 of those compounds were identified (see Figure 5).

Fast separation was made possible by the use of monolithic columns, which exhibit good mass-transfer qualities and high permeability. The authors stress the necessity of combining biochromatography fingerprints with conventional chromatographic patterns (2D system), as the latter's applicability as a single fingerprint approach is constrained by low column efficiency and peak capacity. It has been discovered that 2D bio chromatography is useful for analyzing biological fingerprints of complicated substances, such as traditional Chinese medicines. The HPLC and microdialysis steps have also been used to examine how components of herbal samples interact with

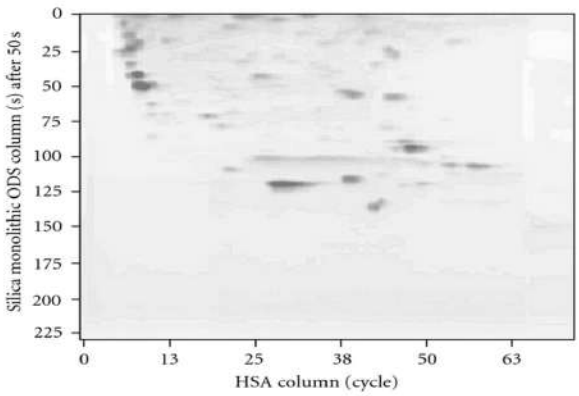

Figure 5: Xingang Decoction's Binary Chromatographic Fingerprint was Obtained

human plasma proteins and cells. One benefit of the suggested solutions is the ability to directly inject samples into the HPLC system during microdialysis. The degree of binding of Rhizome Chuanxiong components to human serum albumin (HSA) and other human blood serum proteins was examined by the authors in the situation of analyte-protein interactions. It was demonstrated that pH had an impact on the analytes' degree of binding, which was attributed to changes in the ionization levels of the active herbal elements and the conformation of the HSA binding site. It has also been investigated how elements of herbal samples interact with human plasma proteins and cells using the HPLC and microdialysis procedures [11].

The ability to directly inject samples into the HPLC system during microdialysis is one advantage of the offered solutions. In the context of analyte-protein interactions, the authors evaluated the degree of binding of Rhizome Chuanxiong components to human serum albumin (HSA) and other human blood serum proteins. It was shown that pH affected the degree of binding of the analytes, which was related to variations in the ionization levels of the active herbal components and the conformation of the HSA binding site. Additionally, it is stated that microdialysis, which is carried out before HPLC analysis, may be constrained by the low recoveries of particular chemicals. The development of biological fingerprints based on the interactions between analyte and cancer cells can be a useful approach for locating prospective anticancer medicines in challenging natural samples.

Immobilized liposome chromatography (ILC), first described by Mao et al., can also be used to determine the biological profile of a herbal sample. ILC can be a useful tool for researching how drugs interact with membranes. Since immobilized liposomes resemble the bilayer structure of phospholipid cellular membrane, this approach can be used to identify substances that can pass through biological membranes.

The authors tested an Angelica sinensis sample using this method to gauge the permeability of its constituent parts. When compared to models based on interactions with ODS column surface, it is believed that ILC is a better model for drugs' permeability testing because of the intricacy of drug-biological membrane interactions in it (combination of hydrophobic, ion pairing, and hydrogen bonding). The main downsides of ILC are restrictions on the use of various organic solvents and mobile phase additives, which may result in liposome bilayer breakdown. The permeable substances were identified using a supplementary RP-HPLC approach because the ILC and MS could not be connected directly. It has also been claimed to screen a complex traditional Chinese medication called Longden Xingang Decoction by coupling an ILC column with an RP column (See Figure 6) [12].

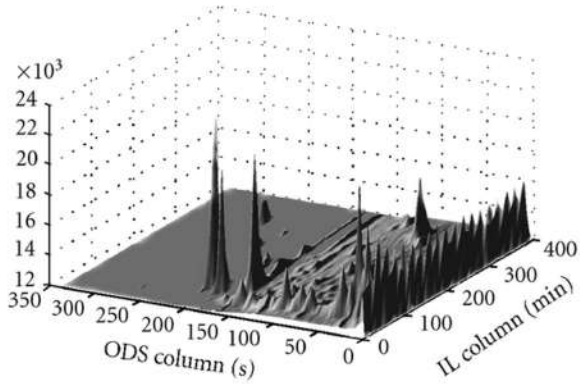

Figure 6: A 3D Chromatogram for Longden Xingang Decoction was Produced

Based on their interactions with the ILC column, the authors discovered eight flavonoids and two iridoids that might pass through biological membranes. According to the authors' analysis, this two-dimensional approach exhibits excellent adaptability for the analysis of complicated natural samples using biological fingerprinting. Studies on the utilization of cell membranes immobilized on the packing of chromatographic columns for biological fingerprinting have also been described. Zhang et al. reported on the coupling of chromatographic and metabolic fingerprint for the quality control of common traditional Chinese prescription, and they presented an intriguing method for biological fingerprint analysis. The use of HPLC-UV technology allowed for the acquisition of the chromatographic profile. After giving rats an intravenous admin-

istration of the tested formulation, the metabolic HPLC fingerprint was collected. Plasma samples were properly prepared before being analyzed using HPLC-UV and HPLC-MS. The combination of chemical and metabolic fingerprints is a good technique for quality control and exposing potential mechanisms of action of herbal samples, according to the scientists' findings. The notion that herbal active chemicals ought to be present in blood (but also in urine) after administration can serve as the foundation for the metabolic fingerprint theory. Another group of bio-fingerprinting methods focuses on fusing a sample's antioxidant profile with a typical chromatographic fingerprint. In this instance, the sample only requires one column for separation; nevertheless, the separated chemicals are derivatized after the column to examine any potential antioxidant activity [13].

There are three primary types of assays for measuring antioxidant activity:

1. Those that use actual ROS.
2. Those that employ a generally stable single oxidizing reagent.
3. Those that use electrochemical detection.

Netherlander et al. have studied the use of high-resolution screening techniques (HRS) in conjunction with biochemical detection. The interactions between substances and used derivatizing agents in antioxidant bio-fingerprinting are chemical rather than biological. Consequently, the question of whether these fingerprints should likewise be considered "biological" emerges. Because the antioxidant mechanism of naturally occurring chemicals in vivo resembles that seen in vitro. Consider the transfer of an electron or hydrogen to a radical. For the sake of this paper, these techniques have also been categorized as biological fingerprints.

Chang et al. described how to build an antioxidant activity fingerprint of Dashan injections using high-performance liquid chromatography and chemiluminescence detection (see Figure 7). Phenolic substances that can neutralize hydrogen peroxide exhibited negative peaks in the fingerprint of antioxidant activity.

Since this preparation's protective effect on reperfusion injuries has been associated with antioxidant capabilities, an antioxidant activity fingerprint has been proposed for It. To analyze the samples under investigation, a data fusion-based approach was used, which merged information contained in antioxidant and chemical signatures. Between chromatographic profiles and activity fingerprinting, a

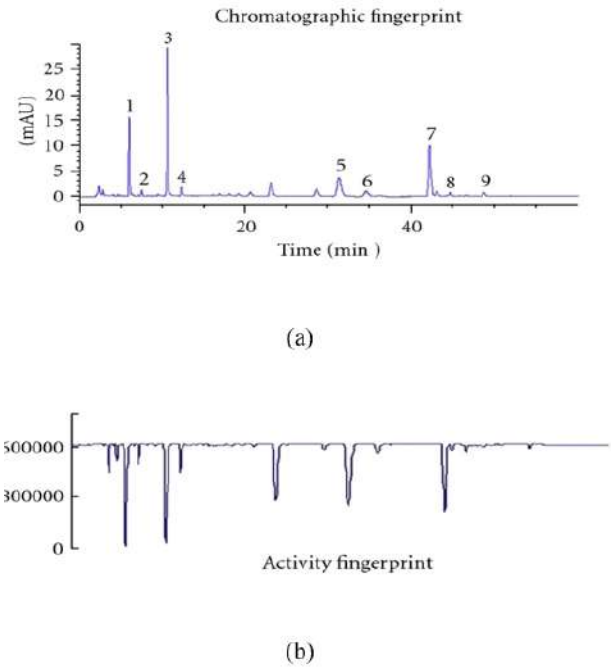

Figure 7: Antioxidant Activity Fingerprints

large discrepancy was seen. These findings call into question the conventional wisdom that samples with comparable chromatographic (chemical) profiles are likely to share similar characteristics. The authors concluded that a simultaneous generation of chemical and biological fingerprints is more thorough than a typical strategy for quality control of complicated herbal samples. For the purpose of quality control of Dashan samples, it has been demonstrated that antioxidant-activity-integrated fingerprints predominate over conventional chromatographic profiles. Using relatively stable free radicals like DPPH or ABTS, post-column derivatization has been used often in HPLC online tests for antioxidants. This method always yields two profiles: a chemical profile (also known as "normal") with positive peaks and an antioxidant profile with "negative" peaks. For the screening of intricate natural samples, various solutions have been put forth. Recently, Netherlander et al. and van Beek et al. reviewed them. Multivariate calibration approaches can also be used to predict the antioxidant activity of a sample from its chromatographic fingerprint, as demonstrated in a number of articles. The authors have demonstrated that adequate quality control of herbal samples may be achieved by combining the data from chromatographic fingerprints with those from spectrophotometric antioxidant assays. There have also been attempts to introduce techniques for identifying specific enzyme inhibitors using RP-HPLC; however, these techniques have a number of significant drawbacks, including the need for a sig-

nificant number of enzymes, the length of the reaction time [14, 15], and the general unsuitability of organic solvents as mobile phase components for studying analyte-enzyme interactions [16, 17].

TLC Based Botanical Fingerprinting

Thin-layer chromatography is said to be the best technique for creating fingerprints from herbal samples. The benefits of this method are widely acknowledged and have been outlined in a number of publications. Only a small number of research teams involved in phytochemical analysis are aware of its potential for biological detection, though. Casella et al. established the idea of biological fingerprint development in TLC by creating a so-called "binary chromatographic fingerprint" that combined chemical and biological detection techniques.

Vanillin reagent was used to spray the plates in the former scenario, while a methanolic solution of the stable free radical DPPH was used to apply biological fingerprints (see Figure 8).

Using the publicly accessible image processing tool ImageJ, genuine chromatograms were obtained in addition to video cameras that were documented for fingerprint comparison. The usage of densitometers is indeed a challenging task when dealing with findings that change over time, such as the one produced with DPPH as the derivatizing agent, but the application of this software makes it possible to analyze fingerprints that are documented in the form of video scans. Using this technique, four Salvia species—S. officinalis, S. triloba, S. Camarines, and S. Lavandula folia—were identified as a rich source of free radical scavengers that are active in vitro. S. triloba can be further examined as a potential substitute for the pharmacopeial S. officinalis as a result of the comparison of both chemical and free radical scavenging fingerprints. Additionally, the scientists stress that combining chemical and biological fingerprints provides a more thorough examination of the analyzed samples because some characteristics that are hardly visible in a chemical profile may be more noticeable in a biological one. For the quality control of medicinal products incorporating Salvia officinalis extract, a similar approach has been used. We compared the chromatographic profiles obtained for botanical reference material with the chemical and biological fingerprints of correctly processed chromatographic formulations (BRM) [18, 19].

It was determined that the suggested method can be used to successfully conduct an extensive quality control on final goods containing sage extract. The concept of creating binary chromatographic fingerprints using thin-layer chromatography is not new; Chen et al. has previously discussed it. But in the aforementioned articles, the idea of binary chromatographic fingerprinting is considerably different. The word "binary" refers to fingerprints acquired separately for glycoside and aglycone fractions in the work by Chen et al., which are bordered by a peak that appears in both profiles. The goal of Ciela et alpaper.'s is to combine the biological and chemical chromatographic fingerprints to gather more data for species separation and bioactive molecule identification [20].

The main application for biological detection in TLC has been affect guided analysis, which aims to isolate molecules with desired activity. But as has already been demonstrated, it may also be used to check the quality of various herbal samples. The potential of thin-layer chromatography for carrying out straightforward benchtop bioassays has recently been examined, and various writers have provided ideas for its continued development. In addition to the use of TLC for determining free radical activity described above, it can also be used to check natural samples for the presence of certain enzyme inhibitors or to find substances with antiviral characteristics. There is an increasing demand for discovering new medications due to the large number of people suffering from neurodegenerative illnesses such as Alzheimer's disease and the small number of licensed medications. Scientists whose research is focused on the development of novel possible medications to treat Alzheimer's disease have recently become increasingly interested in screening natural samples for the presence of acetylcholinesterase inhibitors, using straightforward TLC benchtop bioassays. The search for new solutions to improve the performance of TLC tests is still ongoing. The Department of Inorganic Chemistry's most recent findings have demonstrated that low-temperature TLC can be used to test volatile samples for the presence of AChE inhibitors. (Figure 9) provides an illustration of the application of the TLC inhibitory test for particular volatile samples. Low-temperature TLC bioassays may be the preferred technique for biological fingerprint development and effect-directed analysis of volatiles [21].

The preferred analytical approach for essential oils is typically GC-MS, but using this method precludes the use of effect-directed analyses. As a result, it can be said that TLC biological fingerprinting can be a useful tool when dealing with volatile samples whose composition is fluctuating, which could affect how effective it is when used for medical purposes. The prudent application of thin-layer chromatographic fingerprint analysis should be taken into consideration. Only when the analyzed extract

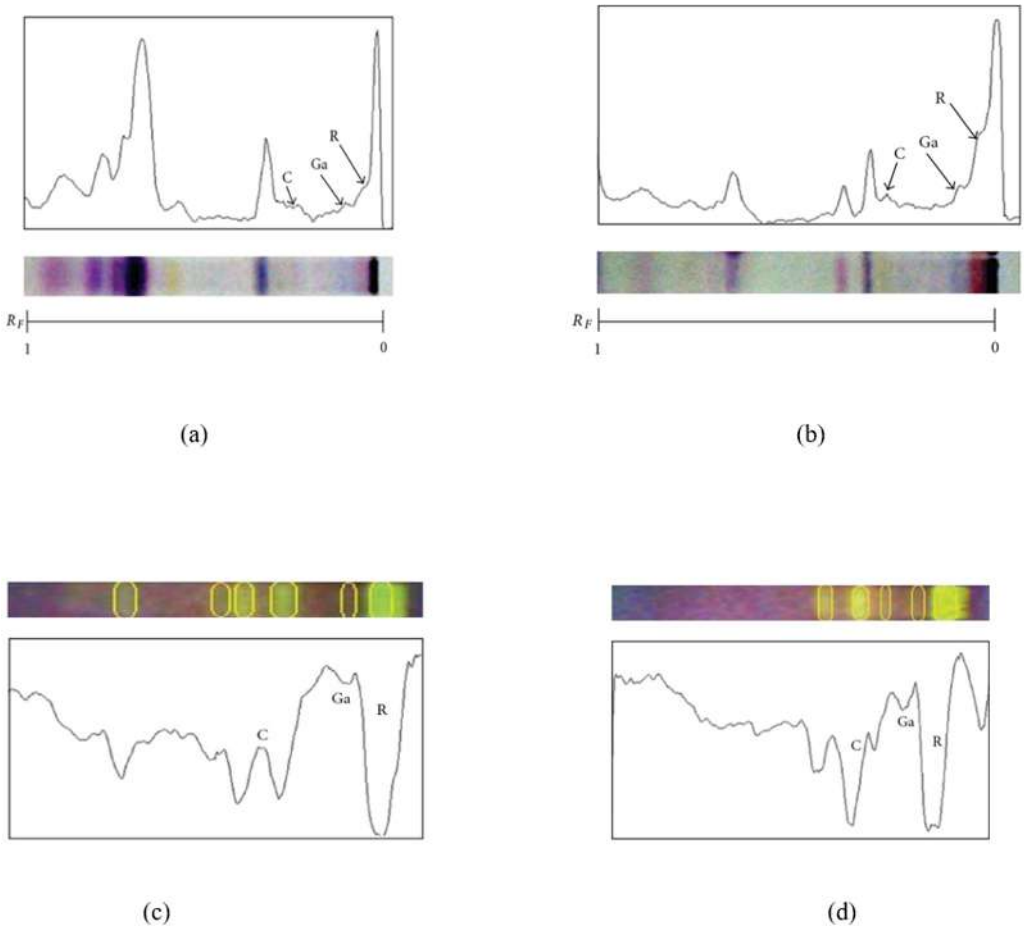

Figure 8: Analysis of the TLC Fingerprints for the Extracts Extracted from the Two Salvia Species

or formulation is intended to be used due to its properties evaluated in the study should TLC bio fingerprints be produced. For instance, there is no need to run AChE inhibitory tests on materials that won't ever be utilized to treat dementia of Alzheimer's type. Recently, it has been apparent that many DPPH screening procedures, including TLC-DPPH tests, are being abused. Numerous papers assert a direct relationship between the findings of DPPH studies and pharmaceutical action, however there is no evidence for this assertion. One of the factors contributing to the success of the TLC-DPPH test could be its simplicity. Houghton et al. have identified the most typical misuse of straightforward in vitro testing. One of the factors contributing to the success of the TLC-DPPH test could be its simplicity. Houghton et al. have outlined the most typical misuse of straightforward in vitro testing.They might manifest, for instance, as a result of interactions between the examined chemicals and the adsorbents brisk areas after being used [22, 23].

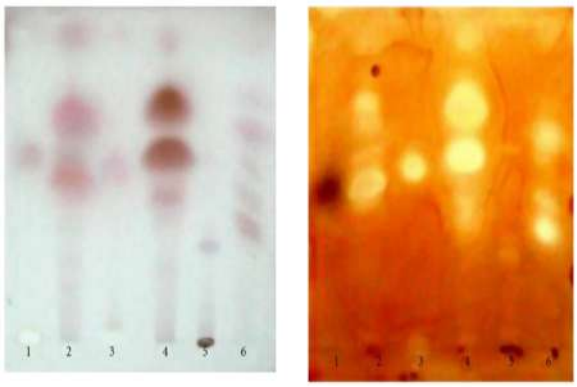

Figure 9: Comparison of the Botanical Specimens that Shows Pain Reducing Activity

Insights on the Development of Biological Fingerprinting Analysis

Since the concept of a bio fingerprint is novel in the analysis of multicomponent herbal samples, as was already said, there is a lot of room for future improvement. In the future, herbal samples can be screened regarding the potential for activated substances it may converse to certain sensors using HPLC-based bio fingerprint analysis. The frequent issues encountered when researching analyte-enzyme interactions may potentially receive new approaches [24].

To find the bioactive chemicals in plant extracts, new detection methods and hyphenations may also be suggested. According to Yu et al., BFA and omics technologies have the potential to be combined to find bioactive components when using conventional Chinese remedies. BFAS may see therefore potent while evolving technique for medicinal investigation as a result. Thus, goals investigation focused on effects and a fingerprint's pattern in the context of planar chromatography, specialized chromatogram development modes (such like multifunctional breakups, 2D TLC, or even multichannel partitioning) might become paired utilizing both environmental as well as physical monitoring. This strategy may be advantageous, particularly when dealing with extremely complicated samples, such polyherbal mixtures. In the literature, special chromatogram generation modes have been well described along with examples of how they might be used to create fingerprints. Recent descriptions of chemometrics investigation, both guided but also unguided, involving 2D pictures have else proven useful for distinguishing between closely related plant species [25].

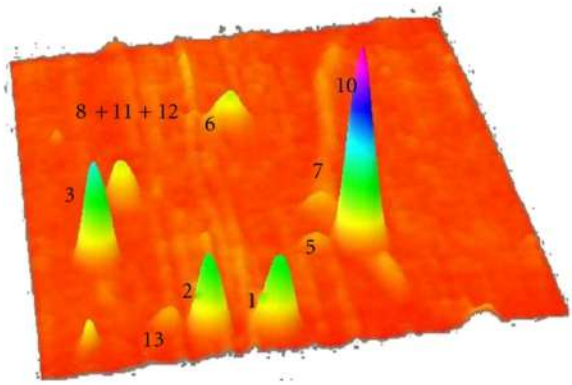

Figure 10: Plot in Three Dimensions for a Collection of Standard Substances that were Separated Using Two-Dimensional Thin-Layer Chromatography

In addition to chemometric pre-treatment, Wayne Rasband of the National Institutes of Health in the United States has suggested creating an average fingerprint utilizing with unlimited programme view. This solution has been proposed to address the TLC issue of RF values shifting. The ImageJ program's "Calculator" feature is used to create the average fingerprint. The procedure's comprehensive description is available elsewhere. For various hyphenations, including super hyphenations, planar chromatography is the best option (hyper nations).

It has recently been demonstrated that combining minimal condition TLC about MS monitoring or even vapor phase chromatogram is effective for creating fingerprints such as aromatherapy originating several sorts of salvia. The examination of biological fingerprints can also use all the aforementioned solutions in more detail. The first study describing the use pertaining to TLC in 2D in conjugation because of dipenylpicrylhydrazyl (DPPH) smears to examine previously, intermediate vegetative related compounds presently was thus released. All scholars demonstrate an intriguing method while presenting relevant information using this view application (Figure 10) [25–27].

CONCLUSION

Liquid chromatography with biological detection offers the chance to thoroughly investigate herbal sample. In addition to generating more data, bio fingerprints make it possible to check vegetation samples due to existence based on novel essential ingredients (effectually-focused evaluation). Spectrometric as well as chromatography data could be used to separate the bioactive substances. HPLC and TLC can be thought of as complementing procedures because while HPLC may make it easier to realize particular bio-fingerprint solutions, TLC may perform better in other circumstances.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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HORMONE REPLACEMENT THERAPY IN POSTMENOPAUSAL WOMEN-A REVIEW

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ABSTRACT:

Hormone replacement therapy [HRT] began out in the year of 1960s, with very high popularity in the 1990s.The first clinical trials on HRT and chronic postmenopausal conditions were began in the USA in the 1990s.After the declaration of the primary results of the Women's Health Initiative [WHI] in 2002, which confirmed that HRT had more adverse than useful effects, so HRT was dropped. The negative results of the study received extensive publicity, developing panic among a few users and new guidance for doctors on prescribing HRT. The clear message from the media was that HRT had greater risks than benefits for all women. In the following years, a reanalysis of the WHI trial was performed, and new studies confirmed that the usage of HRT in younger women or in early postmenopausal women had a useful effect on the cardiovascular system, decreasing coronary disease and mortality Rate. However, this the public opinion on HRT has not modified yet, leading to important negative outcomes for women's health and quality of life. Hormone replacement therapy prescribing practices have developed over the last few decades guided by the changing knowledge off the treatment's, risks and benefits. since the women's health initiative WHI trial results in 2002, consisting of post-intervention analysis and cumulative 18-year follow up, it has become clear that the risks of HRT are low for healthy women less than age 60 or within ten years from menopause. For those who are experiencing bothersome vasomotor symptoms, the advantages are likely to outweigh the risks in view of HRT's efficacy for symptom management. HRT also has a role in preventing osteoporosis in suitable applicants for treatment. A complete overview of the therapies, routes and formulations of currently available HRT's, as well as HRT's benefits and risks by results of interest are provided to facilitate clinical decision making.

***Key words**: Postmenopausal women, Hormone replacement therapy*

INTRODUCTION:

Menopausal syndrome has received more attention in the 20th century, although it took a while for scientists to understand the true effects of hormone depletion on women's health. The "Hormone deficiency syndrome", which encompassed late chronic disorders like osteoporosis, cardiovascular events, Alzheimer's disease, and vaginal atrophy in addition to hot flashes, was named as the group of conditions related with menopause [1]. Over the past few decades, prescribing procedures for hormone replacement therapy have changed in response to evolving knowledge of the treatment's dangers and advantages. Preamirin®, an estrogen medication, was the first to receive FDA approval for the treatment of hot flashes at the turn of the 20th century [2].Women's position and life expectancy were altered by the feminist movement in the 1960s, which promoted menopause treatment with the idea that women should remain "feminine forever," notably in European nations. Wilson's book, "Feminine Forever," which was released in 1966 and made the premise that "menopause is a hormone deficiency disorder, treatable and fully preventive, simply take estrogen medication"[3]. Hormone replacement therapy (HRT) was promoted as a treatment that may help women escape the perils of estrogen deficiency and maintain their femininity. The discovery that unopposed estrogen supplements were linked to a higher risk of endometrial cancer in the 1970s had a negative effect on HRT's reputation[4].Endometrial cancer may be associated with the treatment of conjugated estrogen alone, according to Ziel et al.[4]

But in the following years, scientists learned that lowering estrogen levels and mixing them with progesterone might lower the risk of endometrial cancer [5].Initially, the FDA only approved HRT for the treatment of hot flashes and not for the prevention of chronic illnesses, but in 1988, osteoporosis prevention was added to the list of FDA-approved uses. Additionally, during the same time period, a number of observational studies revealed that HRT had a number of advantages, including the ability to prevent chronic diseases in addition to treating menopausal symptoms [6,7,8,9]. The notion changed from "feminine forever" to "healthy forever" as a result. The American College of Physicians created the first guidelines for using HRT as a preventative therapy for postmenopausal women's chronic diseases, and this led to an increase in the use of HRT [9], progestin on the cardiovascular system and other benefits of estrogen [10].

The FDA mandated that this purported HRT-induced cardiovascular benefit be verified by randomized clinical trials because of the alleged beneficial effect of HRT on cardiovascular health. The Heart and Estrogen/Progestin Replacement Study (HERS), a study of secondary prevention of cardiovascular disease, was the first randomized clinical trial [11]. 2736 postmenopausal women with coronary heart disease were enrolled in this trial. The 0.625 mg of conjugated equine estrogens and 2.5 mg of medroxyprogesterone acetate given to enrolled women was randomly assigned to receive HRT or a placebo[12].

After four years of follow-up, there was no difference between the two groups, however after one year, there was a rise in coronary events (nonfatal myocardial infarction or coronary heart disease (CHD) deaths in the HRT group [11]. The following years saw a reduction in this generated growth. In (1998), The Women's Health Initiative (WHI), the biggest randomized trial to date that evaluated the impact of HRT on the most prevalent causes of mortality and disability in postmenopausal women, including cardiovascular disease, cancer, and osteoporosis [12]. Women without uteri (10,739 participants) received 0.625 mg of conjugated equine estrogen or a placebo, while women with uteri (16,608 participants) received 0.625 mg of conjugated equine estrogen plus 2.5 mg of medroxyprogesterone acetate. After an average

follow-up of 5.2 years, the WHI's initial findings were released in 2002[12]. Increased rates of breast cancer and coronary heart disease were seen in the group of women with intact uteri, along with a decrease in osteoporotic fractures and colon cancer [12].With these findings, it appeared that the risks outweighed the advantages, and the research was abruptly stopped. The statistics were widely reported in the media, which caused alarm among HRT users and forced medical professionals to change their recommendations for HRT prescriptions. The message was that HRT had more hazards than advantages and didn't specify the type or mode of administration. However, there was no distinction in users' ages. The trial with just estrogen (conducted on women who had undergone hysterectomies) went on, and the early findings were released in 2004 [13]. Additionally, this trial was terminated early after just 6.8 years of follow-up because there was evidence of a little increase in the risk of an ischemic stroke without any other appreciable cardiovascular benefits [13].

It has been considered that the varying ages of the included women may have caused to the conflicting results between the WHI and earlier observational studies. While women involved in the WHI trial were asymptomatic, older (average age 63.2), and typically more than 10 years after the onset of menopause, several observational studies had included symptomatic women who had begun HRT close to the onset of menopause. There may be a "window of opportunity," or time right before menopause, when the advantages of HRT outweigh the hazards [14].

Following a 13-year follow-up period, several findings from the WHI, including an age separation of the cardiovascular outcomes, were reported [15,16,17,18]. The use of HRT in younger women (50-59 years old) or in early postmenopausal women (within 10 years of menopausal onset) had a positive effect on the cardiovascular system, lowering coronary diseases and all-cause mortality, according to a reanalysis of the WHI. A significant controlled Danish study that was published in 2012 showed that healthy women who took combined HRT for 10 years after menopause had a lower chance of developing heart disease and dying from it [19].

Because of this, the history of HRT over time has revealed a trend with two utilization peaks: a first rise in the 1960s and a second, greater spike in the years 1999-2000, prior to the release of the WHI data. After these years, the use of HRT fell off dramatically in several nations [20]. For instance, the usage of HRT drastically decreased by 46% in the United States [21], and by 28% in Canada [22], and comparable results were seen in European nations like Germany [23,24] or the United Kingdom [25].

EFFECT OF HRT ON BREAST CANCER:

Data on the effect of HRT on breast safety and breast cancer mortality have generated debate throughout the years. The majority of observational studies and meta-analyses conducted in the 1990s revealed no increased risk of breast cancer associated with estrogen use [26]. However, administration of combined estrogen-progesterone therapy was associated with certain elevated hazards related to dose and duration of use [27].

After 5.6 years of treatment (about 6-7 years), the WHI revealed an elevated risk of breast cancer in women treated with a combination of conjugated estrogen and medroxyprogesterone acetate [12], a risk that was considerably greater than in placebo users.

Conversely, among women receiving just conjugated estrogen therapy, the incidence of breast cancer was markedly lower than in placebo users [28]. After the publication of the WHI trial, one significant but false assumption was made by the media: HRT causes breast cancer "de novo." The growth of an occult tumor that was existing prior to the start of medication and de novo tumor development were not differentiated in this study. The biology of occult cancers has been studied, and the current consensus about breast safety [29].

DRAWBACKS WITH HRT WITHDRAWAL:

Some people predicted a concurrent fall in the incidence of breast cancer after the publication of the WHI results and the corresponding decline in HRT use. However, the effects of stopping HRT varied significantly by country. The first data about the prevalence of breast cancer between 2001 and 2003 were published by Clarke and colleagues [30]. The use of HRT in northern California decreased by 68% after the WHI release, while at the same time, the incidence of breast cancer decreased by 10%. Similar theories on the relationship between declining HRT use and a decline in breast cancer incidence were put forth in a study conducted in California between 2001 and 2004 [30]. These findings were consistent with the Surveillance, Epidemiology and End Results (SEER) research, which revealed a 6.7% decline in the incidence of breast cancer in 2003 [21]. Following a decline in HRT use, breast cancer incidence was seen in other western nations [31].Between 2002 and 2005, the incidence of breast cancer decreased by 8.8% yearly in Germany, and the usage of HRT decreased by approximately 50% [32]. With a decline in HRT use from 32% to 11% between the years 2001 and 2007, the breast cancer rate in France decreased by 14.7% from 2003 to 2007 [33]. Breast cancer incidence in Australia decreased by 6.7% in 2003, while between 2001 and 2003, the usage of HRT decreased by 8% [34]. From 2002 to 2004, there was a 15% decline in the usage of HRT in Canada, which resulted in an annual decrease in breast cancer incidence of 8% [35]. The absolute fall in therapy after 2002 did not result in a decrease in the incidence of breast cancer in Italy, the Netherlands, or Spain [31]. Additionally, in the UK, the use of HRT peaked in 2000 at 25% and then started to decline. Despite this, the incidence of breast cancer decreased by 0.8% year from 1999 to 2006 [36]. It's interesting to note that, at least two years before the WHI publications, the incidence of estrogen receptor-positive breast cancer peaked in the USA in 1999 before beginning to decline in 2000 [31,37]. Additionally, after a few years, there was a fresh increase in the incidence of breast cancer (both ductal and lobular), and by 2012, the rates were similar to those in 2001. This could imply that variables other than HRT can account for the rise in breast cancer. It's likely that modifications to the country's breast cancer screening systems can account for some of these statistics. High rates of mammography, which have been made available to younger women, may have contributed to an increase in breast cancer incidence in the years immediately following the implementation of screening, which was then followed by a decline due to the early diagnoses made in the previous year’s [38,39].

EFFECT OF HRT ON ENDOMETRIAL CANCER:

Endometrial cancer risk has been linked to the use of estrogen that is not countered [4]. As a result, recommendations state that estrogen therapy in women with uteri should be

provided along with a progestin molecule. In the HERS trial, which revealed seven fewer endometrial cancer cases per 10,000 in women using HRT than in placebo users [40], there was strong evidence of a decreased risk of endometrial cancer associated with combined HRT. In a similar vein, the Million Women Study found that using HRT in combination decreased the incidence of endometrial cancer [41]. Sequential therapy is less safe for the endometrium than continuous combination therapy [42,43]. Sequential therapy caused an enhanced risk of endometrial cancer when used for longer than 10 years, according to a sizable Finnish case-control study [44]. The functions of various Progestogen varieties must still be date, the two most widely used progestin molecules, showed no differences in their ability to prevent endometrial cancer in two observational studies [41,44]. According to the literature, 200 mg/day of oral micronized progesterone given consecutively for 12–14 days each month is beneficial for protecting the endometrium for up to five years [45]. However, combination therapy with oral micronized progesterone or dydrogesterone was linked to a higher risk of endometrial cancer for treatments lasting more than five years in the E3N cohort research [46,47].

DRAWBACKS WITH HRT WITHDRAWAL:

Between 2001 and 2012, endometrial carcinoma rates rose as a result of the WHI publication and the decrease in HRT prescriptions [48]. Similar changes in endometrial cancer mortality were seen in England after 2002, where there were nine more endometrial cancer fatalities annually [39].

EFFECT OF HRT ON CARDIOVASCULAR SYSTEM:

Postmenopausal women are more likely to experience cardiovascular events, especially if they have severe vasomotor symptoms. Women with hot flashes had higher subclinical CVD, including greater aortic calcification, worse endothelial function, and higher intima media thickness, than women without menopausal symptoms, according to the Study of Women's Health Across the Nation (SWAN) [49,50,51]. HRT has a positive effect on the risk factors for CVD, according to clinical studies [52, 53,54,55,56, 57]. The clinical effectiveness might be connected to early therapy, but it might also be related to giving HRT to people who, because of their symptoms, have a higher cardiovascular risk. This was not the case in the WHI, where asymptomatic women were given HRT years after menopause. Therefore, it is not shocking that the WHI trial's findings did not support the idea that HRT protects against coronary heart disease, stroke, and venous thromboembolism [12]. However, a reanalysis of the data using age stratification revealed that the administration of HRT decreased the rate of coronary artery disease and all-cause mortality in women within 10 years of the onset of menopause [17]. Two subsequent meta-analyses [58;59], which combined data from 23 and 30 randomized clinical trials, respectively, found a reduction in CVD and all-cause mortality in HRT users under the age of 60 or in those whose menopause started within the previous 10 years. The Early versus Late Intervention with Estradiol (ELITE) study [60] and the Kronos Early Estrogen Prevention Study (KEEPS) [61],two more recent randomized trials that investigated the timing hypothesis, have revealed a protective effect of HRT in young women approaching menopause. The same

results were later reported by the Danish Osteoporosis Prevention Study (DOPS) after 10 years of HRT treatment versus a placebo [19].

DRAWBACKS WITH HRT WITHDRAWAL:

According to the aforementioned data, it is possible that the failure to treat postmenopausal women who are experiencing symptoms led to an increase in cardiovascular events after 2002. A Finnish study found that women who stop using hormone replacement therapy (HRT) are more likely to die from myocardial infarctions and strokes, especially if they are under 60 years old [62]. Unfavorable changes in myocardial infarctions and strokes were observed after the HRT fall following the WHI publication, according to an English ecological study [39].On the other hand, a US ecological study conducted after 2002 found a link between the decline in HRT use and a drop in the frequency of acute myocardial infarction [63].

EFFECT OF HRT ON FRACTURES:

Inflammation has a multifaceted role in the development of postmenopausal osteoporosis, which is a result of estrogen deprivation [64, 65]. Any type of HRT is capable of lowering bone turnover, lowering bone reabsorption, and raising bone mineral density, according to clinical studies. Inflammation has a multifaceted role in the development of postmenopausal osteoporosis, which is a result of estrogen deprivation [64,65]. Any type of HRT is capable of lowering bone turnover, lowering bone reabsorption, and raising bone mineral density, according to clinical studies. The WHI was the first randomized experiment to demonstrate the HRT's unmistakable efficacy in lowering vertebral and hip fractures by roughly 34% [12]. The DOPS study used the exact same data. The outcome was shown in a group of healthy women who were not specifically afflicted with osteoporosis, i.e., in a clinical setting where other antiosteoporotic treatments had failed to exhibit a preventative effect [66].

DRAWBACKS WITH HRT WITHDRAWAL:

Five years after stopping HRT, the WHI study's follow-up revealed no increase in fractures [67].Women who used HRT for more than five years revealed preserved positive effects on bone in another observational trial (after 15 years of follow-up) [68]. However, a sizable investigation by Islam and colleagues indicated that postmenopausal women's fracture risk rose in the three years after the WHI's release [69]. According to an Italian study, less HRT use was linked to 43,000 more bone fractures annually in the USA [70]. An even more recent long-term observational study found that women who stopped using HRT had a greater incidence of fractures than those who continued treatment, which has a significant impact on women's health [71].

TABLE1: RISK-BENEFIT PROFILE OF MENOPAUSAL HORMONE THERAPY;

SYMPTOM	FORMULATION	BENEFIT	RISK
Vasomotor symptoms	Estrogen +Progestogen	More	Less
osteoporosis	Estrogen +Progestogen	More	Less
Coronary Heart Disease	Estrogen Estrogen+ Progestogen	More May be	May be chance of risk in women many years past menopause
Stroke	Estrogen Estrogen+ Progestogen	Less Less	More More
Type 2 Diabetes	Estrogen Estrogen+ Progestogen	More More	Less Less
Venous Thromboembolism	Estrogen Estrogen+ Progestogen	Less Less	More More
All-Cause Mortality	Estrogen Estrogen+ Progestogen	May be May be	May be May be
Breast cancer	Estrogen Estrogen+ Progestogen	More More	Less Less
Endometrial cancer Colon cancer	Estrogen Estrogen+ Progestogen Estrogen	Less More May be	More Less Less

	Estrogen+ Progestogen	More	Less
Fractures	Estrogen	More	Less
	Estrogen+ Progestogen	More	Less

Table 1: [72].

CONCLUSION:

A potent pathogenetic therapy for all postmenopausal disorders has a contentious past that is related to the contentious history of HRT. Its effects on symptoms are and were evident right away, which initially led to quickly rising estrogen use. HRT use was restricted as a result of a lack of understanding regarding its adverse effects and difficulties, notably in the endometrium and the widespread use of HRT was made possible by the subsequent association with progestin, with positive effects on many aspects of women's health. Unfortunately, the publication of the WHI experiment, which was poorly planned, assessed, and reported, quickly put an end to the rise in HRT use and its consolidation. Even if the epidemiological statistics were not robust enough to clearly show a harm to women's health, the damage done was significant, essentially leaving many symptomatic women without an effective treatment. Further studies and analyses have solidified the idea that HRT is highly beneficial when administered to symptomatic women within 10 years of the onset of menopause or to symptomatic women under the age of 60, despite the fact that the majority of the evidence obtained was only with oral conjugated estrogen with or without medroxyprogesterone acetate. The harm is still there, though, and modest HRT use—which is unjustified—is nevertheless prevalent globally.

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TREATMENT APPROACHES FOR THE COVID 19

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ABSTRACT

The global pandemic of novel coronavirus disease [COVID-19] caused by severe acute respiratory syndrome coronavirus [SARS-COV-2]. Treatment approaches in the covid-19 changing swiftly, in the initial phase of covid-19 managed with repurposing antiviral drugs, these drugs have been attempted with overestimated efficacy owing to limited clinical evidence. Most of the early clinical trials have the defects of study design, small sample size, non-randomized design, or different timings of treatment initiation. Remdesivir can improve the patient recovery time, lopinavir and ritonavir does not show any effect in severe covid -19 patients, molnupiravir suggest to the non-severe covid -19 patients, Adjunctive immunomodulators [interleukin-6 inhibitor and jak pathway inhibitors] are beneficial for severe covid-19 patients with cytokine release syndrome, triple therapy of ribavirin, lopinavir and interferonβ-1b shown negative conversion in early stage but ribavirin is a teratogen. Convalescent plasma therapy can be potentially lifesaving in critical ill patients. Low molecular heparin should be initiated in all hospitalized covid-19 patients and dose based on the coagulation profile and risk of thromboembolism. Steroids must be used in low doses and for short period. Vaccination therapy is the specific therapy for covid -19, most of the people have read false information regarding vaccination but vaccines are safer and more effective with fewer side effects.

KEY WORDS:

Coronavirus disease-2019[COVID-19], severe acute respiratory syndrome [SARS-COV-2].

INTRODUCTION

Covid-19 is an infectious disease caused by the SARS-COV-2, Globally 630,832,131 people effected with covid-19, including 6,584,104 deaths are reported as per the world health guidelines. The SARS-COV-2 virus cause immune dysregulation, it leads to cytokine storm and finally cause organ damage in Patients.so far there is no effective therapy for covid-19, the proposed therapy based on SARS and middle east respiratory syndrome [MERS], in the initial phase of covid 19 can be managed with repurposing antiviral drugs, after that covid-19 treated with newer antiviral drugs, immunomodulators, and adjunctive treatments [10]. Convalescent plasma therapy can be potentially lifesaving in critical ill patients [55]. Low molecular heparin should be initiated in all hospitalized covid-19 patients and dose based on the coagulation profile and risk of thromboembolism [5]. Dexamethasone and other glucocorticoids are used in the treatment of covid-19 with low doses for short period [8]. In the year of 2021 vaccine therapy can be developed, covid-19 vaccination begun, most of the people have read false information regarding vaccination but vaccines are safe and more effective, Vaccines cause some side effects that is the body creating antibodies to fought for covid-19 and it indicates vaccine working in the body [5].

GENOME STRUCTURE AND PATHOPHYSIOLOGY:

The origin of the SARS-COV-2 genome has been linked to the SARS-COV-1 and MERS-COV viruses. SARS-COV-2 is form of beta corona virus family, single-stranded RNA, enveloped virus, it is present in 50-200 diameter.one different encoded structural protein is the spike glycoprotein. The spike glycoprotein consists of three S1-S2 heterodimers [5].

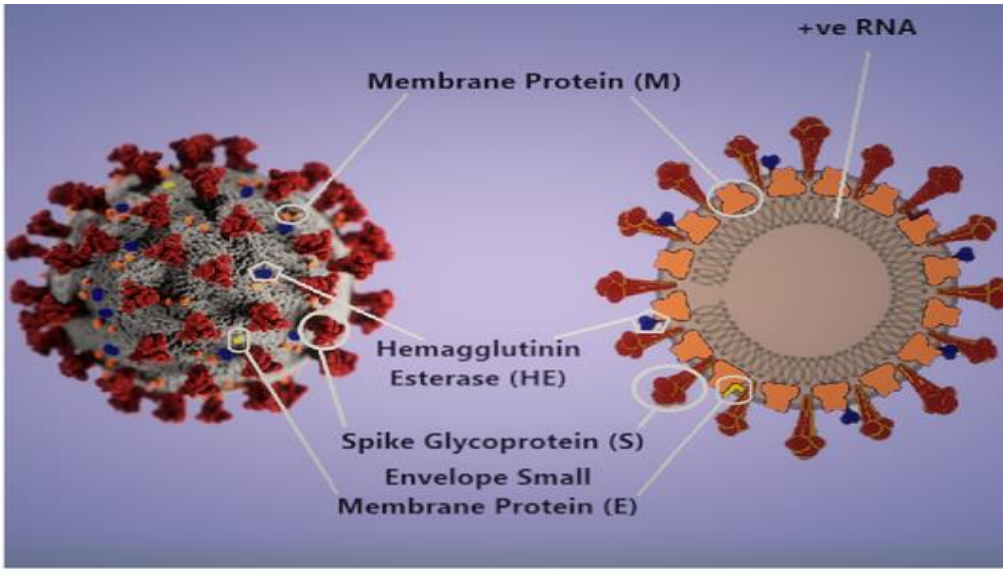

Figure 1:D model of the SARS-COV-2 virion and a schematic diagram and its structural proteins and genome [5 ,41].

PATHOPHYSIOLOGY

SARS-COV-2 enter into the type II pneumocyte through endocytosis process, then it increases its number in the cytoplasm. The high protein manufacturing stress exert on type II pneumocyte it leads to apoptosis. Additionally, the RNA released from the SARS-COV-2 acts as a pathogen-associated-molecular pattern [PAMP] and it will be identified by the pattern recognition receptor or toll-like receptor. This leads to chemokine surge it causes migration of neutrophil and activation. It causes destruction of alveolar-capillary walls. At a microscopic level, the interface can be lost between the intra-alveolar space and the surrounding stroma it leads to fluid leakage and fills into the alveolar sacs [10].

MODE OF TRANSMISSION

COVID-19 can be transmitted through droplet transmission, oral route, conjunctiva and fomites [31]. Additionally, it can be transmitted through patient's body fluids such as respiratory droplets, saliva, feces and urine [32]. Virion can stabilize at low temperatures that is 4°c, at this temperature virion have higher survivability than high temperatures. Covid-19 can spread the infection prior to symptom presentation, during symptomatic course and clinical recovery period [7].

SARS-COV-2: VIROLOGY AND DRUG TARGETS

SARS-COV-2 bind to the receptor then the virus particle uses host cell receptor and endosome enter into the cells. Type 2 transmembrane serine protease, TMPRSS2, these enzymes facilitate the cell entry through the S protein [22]. Once enter into the inside the cell, viral polyproteins are synthesized then viruses are encoded and form the replicase-transcriptase complex then RNA synthesized by using RNA-dependent RNA polymerase enzyme. Structural proteins are synthesized leading to completion of assembly and release of viral particles [7]. These viral lifecycle steps provide potential targets for drug therapy. Promising drug targets include viral entry and immune regulation pathways, non-structural

proteins [eg,3-chymotrypsin-like protease, papain like proteasomal dependent RNA polymerase] [27,50].

CURRENT ANTI-COVID-19 MEDICATIONS

ANTI-VIRAL DRUGS

REMDESIVIR

Remdesivir is an RNA-dependent RNA polymerase inhibitor, we recommend remdesivir for hospitalized patients with mild -moderate covid-19 at high risk of progression to severe disease, those who are not on mechanical ventilation based on some data it may reduce the patient recovery time and also risk of mechanical ventilation [2,17]. Dosing for remdesivir in mild to moderate covid-19 patients is 200 mg on first day and followed by 100mg daily for 5 days total [with extension up to 10 days, there is no clinical improvement][16]. if patient discharge before the completion of course, remdesivir can be discontinued [19]. Remdesivir is not recommended in patient with estimated glomerular filtration rate [< 30 ml/per min 1.73meter square] because remdesivir prepared in cyclodextrin vehicle it may accumulate in kidney cause renal toxicity [3,9]. liver enzymes should be monitored before and during remdesivir administration, alanine aminotransferase >10 times the upper limit of normal should quick consideration of remdesivir discontinuation [18].

MOLNUPIRAVIR

Molnupiravir is a broadspectrum antiviral drug, it is the direct acting oral antiviral agent [39]. it acts on the RNA dependent RNA polymerase enzyme and by competing with uridine and cytidine triphosphate substrate finally leads to incorporation of A and G forming stable complexes in the active site of the RNA dependent RNA polymerase enzyme, it leads to mutagenesis then stop viral replication .it mutates the virus to kill itself [25] Molnupiravir should be given up to 5 days through oral route.it is given to mild -moderate covid-19 patients within <5 days of symptoms. It can drastically decrease the disease progression by reducing the hospitalization. It has safety and tolerability profile [14]. Molnupiravir should be provided only to non-severe covid -19 patient who had higher risk of hospital admission, older people, unvaccinated and those patients having immunodeficiencies or who have chronic diseases according to national institutes health guidelines. It should not be given to the children, pregnant women and breastfeeding people. Those who want to take molnupiravir, they should have contraceptive plan as per the WHO guidelines.

FAVIPIRAVIR

It is a nucleoside analogue. It inhibits the RNA dependent RNA polymerase complex of SARS-COV-2 by binding to its catalytic domain and stops the inclusion of nucleotides for viral RNA replication, it leads to increasing the mutation frequency and perhaps lethal mutagenesis [36]. Favipiravir does not affect the human DNA or proteins because RNA dependent RNA polymerase heterologous to host cell [57]. The viral clearance of favipiravir was also reported, it is directly related to the early initiation of antiviral therapy and before the peak of viremia. It leads to some practitioners start antiviral therapy in pre-symptomatic phase of covid-19. Total of 2 dosage regimens with a dissimilar duration [differs from 2-14 days] found in the most of the studies, they all are started with a loading dose of 1600 or 1800 mg doses twice a day on the first day and continued by 600 or 800 mg twice daily. However,2 included observational studies suggested that higher doses than these could improve the efficacy of favipiravir [35].

ADVERSE EVENTS:

Adverse events of the favipiravir include hyperuricemia with increasing the dose of drug [35]. when administrating of favipiravir to pregnant or lactating women owing to its probable teratogenic effects as well as in men seeking conception [2].

LOPINAVIR-RITONAVIR

Lopinavir-Ritonavir are protease inhibitor for treating HIV infections. Both these drugs are suggested to treat covid -19 by inhibiting the SARS-COV 3C like protease enzyme, it is the key enzyme for protein processing. In vitro efficacy of lopinavir -ritonavir against covid-19 was not effective in clinical studies. This could be because of lack of reaching enough minimum effective plasma concentration of lopinavir-ritonavir against covid-19 patients [26]. Lopinavir- ritonavir tested dose [400mg +100mg] would not achieve minimum effective concentration in 50% of patients [14]. Lopinavir-Ritonavir compared with various treatment modalities, the control interventions include supportive care, umifenovir, novaferon, novaferon+lopinavir-ritonavir and lopinavir-ritonavir + interferon beta 1b+ribavirin, all of these are repurposed drugs or their combinations [49]. They are used in the treatment of covid-19 based on invitro data or on the previous experience with the out-break of influenza, their clinical evidence is limited in covid-19 [29].

ADJUNCTIVE IMMUNOMODULATORS

JAK PATHWAY INHIBITORS

BARICITINIB

Baricitinib is an IL-6 antibody, it is an FDA's emergency use authorization for supply in hospitalized patients require supplemental oxygen. Baricitinib is a suppressor of the JAK-STAT pathway, it is used to blockage of releasing of pro inflammatory cytokine release and systemic inflammation in interferonopathies. It is also NAK inhibitor [numb associated kinase], it has high affinity for AP2-associated protein kinase1[AAK1] a main member in NAK family, it binds with the clathrin, regulate clathrin-mediated endocytosis, it leads to suppression, it indicates the reduction infectivity of a large number of viruses [15]. Blockage of JAK-STAT pathway leads to inhibition of IFN production which may cause impairment of antiviral activity. Therefore, baricitinib may have both anti-viral and anti-inflammatory activity. Covid-19 patients treated with baricitinib leads to reduction of cytokines level in serum and also increase the level of antibodies against the SARS-COV-2 spike protein [13]. Combination therapy of baricitinib with remdesivir did not reveal mortality benefit when compared to remdesivir treatment alone as per the IDSA guideline 2022. Baricitinib used along with the dexamethasone or with IL-6 inhibitor shows greater effect with favourable evidence on reduced mortality, time to discharge, and progression in mechanical ventilation as per the WHO 2021 guideline.

IL-6 PATHWAY INHIBITOR [TOCILIZUMAB]

TOCILIZUMAB

Tocilizumab is a competitive inhibitor of both membrane -bound and soluble IL-6 receptor, it leads to prevention of downstream signal transduction of IL-6[15]. Thus, the inhibition of IL-6 is hypothesized to be a promising therapeutic strategy to interfere with covid-19 induced cytokine storm and also alter the disease progression [19]. We recommend tocilizumab [8mg/kg as a single intravenous dose] as a choice for individuals who require high flow oxygen or more intensive respiratory support. Generally, tocilizumab given to those who are within 96 hours of hospitalization or within the 24 to 48 hours of initiation of ICU-level care [19]. We use only tocilizumab in patients who are also taking dexamethasone and it is limited to single dose [48]. we do not use tocilizumab in patients who are receiving baricitinib. Tocilizumab should be avoided in individual with hypersensitivity to tocilizumab.

DEXAMETHASONE AND OTHER GLUCOCORTICOIDS

Dexamethasone for severely ill patients with covid-19 who are on supplemental oxygen or ventilatory support, the dose of the dexamethasone at a dose of 6mg daily for 10 days or up to discharge [26]. Alternatives for dexamethasone [hydrocortisone 150mg, methylprednisolone 32mg, prednisone 40mg] [12], the usage of alternatives are more limited than those of dexamethasone, patients on low supplemental oxygen, we suggest low dose of dexamethasone with remdesivir [1].

RIBAVIRIN AND INTERFERON

Ribavirin, a guanosine analogue which has distinct antiviral mechanisms, including both direct and indirect mechanisms, indirect mechanisms include inosine monophosphate dehydrogenase inhibition and immunomodulatory effects where as in direct mechanisms interference with RNA capping, polymerase inhibition and lethal mutagenesis [20,29]. Generally, the combination of ribavirin with interferon, demonstrated no discernible effect on clinical outcomes or viral clearance [42]

SERINE PROTEASE INHIBITORS

CAMOSTAT, NAFAMOSTAT

The host cell protease TMPRSS2 is necessary for SARS-CoV2 spike protein receptor priming for its effective attachment to the ACE2 receptor [22] Given this observation, inhibition of TMPRSS2 with serine protease inhibitors like camostat and nafamostat can be a potential option to prevent SARS-CoV-2 viral entry in host cells [22].

ADJUVANT THERAPIES

CONVALESCENT PLASMA

Transfusion of convalescent plasma with a SARS-CoV-2-specific antibody (IgG) binding titer greater than 1:1,000 and a neutralization titer greater than 40 resulted in negative viral load within 12 days [23]. ARDS resolved in four out of five patients on day 12 of transfusion. This indicates convalescent plasma may be useful in clinical recovery of critically ill patients [49,4]. The exploratory COVID-19 convalescent plasma use guidelines were released by the US Food and Drug Administration. The following requirements must be met in order to be eligible for the use of convalescent plasma: laboratory confirmation of a severe COVID-19 (dyspnea, O2 saturation 93%, respiratory rate 30 per minute, partial pressure of arterial oxygen to fraction of inspired oxygen ratio 300, and lung infiltrates >50% within 24-48 hours) or a life-threatening COVID-19 (respiratory failure, septic shock, multi-organ dysfunction) disease The PLACID Trial (CTRI/2020/04/024775)[49], a phase II trial to evaluate the safety and effectiveness of convalescent plasma to reduce COVID-19 associated complications, has been started by the Indian Council of Medical Research (ICMR) [8].

VITAMIN C

The expert consensus from the Shanghai medical association recommends that 100–200 mg/kg intravenous (IV) vitamin C daily can lead to an improvement in the oxygenation index [11]. By virtue of its actions on oxidative stress and inflammation and immunological function, vitamin C has shown efficacy in patients of sepsis with ARDS. In the CITRIS-ALI trial involving patients of sepsis and ARDS (n = 167), IV infusion of vitamin C (50 mg/kg in dextrose 5% in water over 96 hours) resulted in significantly lower 28-day mortality (29.8% vs 46.3%, p = 0.03).

VACCINATION

As of 8 April 2022, WHO has evaluated that the following vaccines against covid-19 have met the necessary criteria for safety and efficacy.

1.AstraZeneca/oxford vaccine 2. Johnson and Johnson 3. Moderna 4. Pfizer/BionTech 5. Sinopharm 6. Sinovac 7. Covaxin 8. Covovax 9. Nuvaxovid 10. Casino

VARIOUS PLATFORMS FOR COVID-19 VACCINE DEVELOPMENT

Various platforms bring looked at for the development of covid-19 vaccines. These include RNA, DNA, non -replicating viral vector and inactivated vaccines. The potential vaccine targets are, SARS-COV-2 is a single standard RNA, it has a lipid bilayer and consists of spike S protein along with membrane and envelope proteins. DNA and RNA based vaccines are made from the viral sequence of the virus. Viral vector vaccines use another virus, for example an adenovirus and incorporate genetic material from SARS-COV-2 into its genome. Inactivated vaccines contain SARS-COV-2 that has been killed using physical or physical agents [13].

IMMUNOLOGICAL EFFICACY OF COVID-19 VACCINES CURRENTLY USED IN INDIA

COVAXIN

Bharat Biotech created the Covaxin, India's first locally produced COVID-19 vaccine, in cooperation with the Indian Council of Medical Research, a biomedical research organisation financed by the Indian government [44]. In June 2020, Covaxin received DCGI approval for human clinical studies, and the first stage of the trials was finished by July. The vaccine does not require sub-zero storage or reconstitution, and it is available in multidose vials that are stable at temperatures ranging from 2 to 6 °C [44]. An adjuvant is used to boost up the specific immune response against the particular antigen, which is present in vaccine formulation. In the case of Covaxin, an aluminium hydroxide-based adjuvant is formed to generate a crystalline aluminum oxyhydroxide by the addition of alkali to the solution of aluminum salt [45].

COVISHIELD

The Serum Institute of India (SII), Pune is now developing Covishield (ChAdOx1-nCOV or AZD1222), a recombinant, replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 S glycoprotein vaccine, based on the AstraZeneca-Oxford model [40]. The UK and India both granted licences for the AZD1222 COVID-19 vaccine on December 30, 2020 and January 2, 2021, respectively [60]. This includes genetically modified and attenuated SARS-CoV-2 and adenovirus strains (causes of the common cold) that are nonreplicating. The preliminary research indicates that AZD1222 is 70.4% effective in preventing COVID-19 with no noticeable negative effects [28]. One dosage contains approximately 5 1010 ChAdOx1-S (recombinant) viral particles (0.5 ml). In addition to ChAdOx1-S (recombinant), this vaccine also contains the recipients' magnesium chloride hexahydrate, L-histidine, L-histidine hydrochloride monohydrate, sodium chloride, ethanol, sucrose, polysorbate 80, and water for injection [37]. The two vaccinations are kept and transported between 2 and 8 °C [38]. Covaxin and Covishield have a two-dose immunisation schedule; for Covaxin, the doses should be spaced 28 days apart; intramuscular injections are suggested, and for Covishield (0.5 ml in each dosage), the doses should be spaced 4-6 weeks apart [43].

MODERNA/MRNA-1273

This vaccine uses nucleoside-modified messenger RNA (mRNA) that is encapsulated in lipid nanoparticles. It encodes the SARS-CoV-2 full-length spike protein that has been prefusion stabilized. This spike glycoprotein moderates host cell attachments. Hence, it is essential for viral entry and thus the primary vaccine target. The vaccine gives rise to a vigorous binding and neutralising antibody response [21]. This also includes CD4+ T-cell and CD8+ cytotoxicT-cell response to eliminate the virus [6]. Following the first and second dosages in the same research, adverse events occurred more frequently in the Moderna group. Pain at the injection site was typically mild and persisted for three days. The erythema, soreness, and induration associated with delayed injection-site reactions, on the other hand, were uncommon and often went away in 4 to 5 days. Following the second dose of the Moderna vaccine, symptoms of fatigue, myalgia, arthralgia, and headaches worsened and lasted for about three days. Age had no bearing on the Moderna group's incidence of unfavourable events, and there were no effects Another study found systemic rash and localised axillary oedema or pain ipsilateral to the injection location as side effects [6]. Half of the 40 individuals in a different trial extension were between the ages of 56 and 70. The remaining half was more than 71 years of age. In both age groups, the adverse events were mainly mild or moderate [6]. Hypersensitivity reactions were reported in both groups but were slightly higher in the Moderna vaccine (1.5%) compare with the placebo (1.1%) [6].

AD26.COV2. S AND THE JANSSEN VACCINATION

This is a full-length SARS-CoV-2 S protein-containing, non-replicating human adenovirus type 26 that triggers an immune response to the SARS-CoV-2 infection. SARS-CoV-2 virus invasion in type 2 alveolar cells of the lungs is prevented by an antibody directed against the S protein, which l the severity and morbidity of the infection [35]. Adenoviral vectors have the benefits of adjuvant properties, scalability, and broad tissue tropism [44].

BNT162B2/ PFIZER

This vaccine protects against the S protein of the SARS-CoV-2 virus and is made up of nucleoside-modified RNA and lipid nanoparticles. With the help of this vaccine, the body can produce antibodies that will attack and neutralise the virus, which requires the S protein to enter the body through type 2 alveolar cells' ACE2 receptor [33].

CONCLUSION

most of the drugs currently available for COVID-19 are not designed specifically against SARS-CoV-2. The search for effective antiviral agents specific to SARS-CoV-2 is still ongoing. Inhibiting viral growth in the early stages of COVID-19 can avoid serious consequences. anti-inflammation therapy combined with antiviral medications is beneficial in severe COVID-19 patients since in the late stages, cytokine release syndrome is the primary cause of multi-organ failure and even death. Remdesivir may hasten the recovery from advanced COVID-19 pneumonia, according to clinical data. Due to the limitations of trial designs, there is debate concerning the therapeutic effectiveness and safety of alternative medicines for emergency usage. The clinical result of COVID-19 serious cases may be improved by IL-6 inhibitors, which reduce severe inflammation brought on by cytokine release following viral infection. Convalescent plasma therapy can be potentially lifesaving in critical ill patients. Low molecular heparin should be initiated in all hospitalized covid-19 patients and dose based on the coagulation profile and risk of thromboembolism. Steroids must be used in low doses and for short period. Pfizer/BNT162b2, Moderna vaccine/mRNA1273, AstraZeneca/AZD122/ChAdOx1 n-CoV-19, and Janssen vaccine/Ad26.COV2.S are the vaccines. It can be noted that the vaccines had efficacies of 95% for Pfizer, 94.1% for Moderna, 70.4% for AstraZeneca, and 66.9% for Janssen, demonstrating their effectiveness in lowering the incidence and severity of SARS-CoV-2 infection in the study populations.

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A Review of Health-Promoting Properties of *Cassia occidentalis*

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Cassia occidentalis, Pharmacological Activities

Abstract

Cassia occidentalis is a widespread and widely utilized plant. It grows in the southern parts of India. It belongs to the Sakavarga family of medicinal plants, including spices, vegetables, and herbs. This herb, known as Kasamarda in Sanskrit, is effective for treating skin disorders, relieving constipation, and curing worm-related diseases. It has an antiallergic, antibacterial, antidote for poison, blood purifier, antifungal, antidiabetic, anti-inflammatory, antimutagenic, psoriasis, melanoblast cell line leprosy, and hepatoprotective properties. The chemicals include Achrosin, aloe emodin, cassia occidental I, Cassia occidental II, emodin, anthraquinones, anthrones, apigenin, guaranty obtain, campesterol, cassiollin, chrysophanic acid, chrysarobin, chrysophanol, chrysoberyl. Its aerial portions have favorable physicochemical qualities with high nutritional value, such as increased energy, crude fibers, and vitamin levels. The data from X-ray fluorescence spectrophotometry revealed that the sample is rich in minerals, particularly Fe, Ca, K, and Mn. Furthermore, minerals such as Mg, Zn, Cu, Na, P, and S are abundant and demonstrate the nutritional value of the chosen material. Flavonoids, alkaloids, lignin, tannins, and phenols are abundant in the plant sample. The present study will highlight all the properties and therapeutic uses, toxicological studies, nutrition value, and mainly the pharmacological activities of *C. occidentalis*.

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INTRODUCTION

In Ayurveda, In ayurveda and Unani, Cassia occidentalis is known as Kasamarda and Kasaondi (Kasundi), respectively. In Sanskrit, the words Kasa and Mardan both indicate to demolish or cough out mucus. Therefore, the one who destroys Kasa is known as Kasamarda [1]. A Siddha is known as Paeyaavarai, Thagarai [Figure 1].

Cassia occidentalis L. (Leguminosae) A "famine food" or "edible weed of agriculture." The nighandus rajnighantu, Dhanwantari, Bhavaprakasa, Rajballaba, etc., all reference *C. occidentalis*, commonly known as "kasamarda." Locals frequently use this plant as a replacement for coffee. It is an erect, stinky, annual herb or under a shrub, 60-150cm in height, found throughout India up to an altitude of 1500 m. Leaves are 15-20 cm long and lanceolate or ovate-lanceolate, leaflets three pairs, membranous, glaucous, ovate, or lanceolate (Plate 5.1 II d); flowers

yellow, in short racemes, pods recurved, seeds are dark olive green and elliptical, complex and smooth shining [2].

The presence of the primary phytochemicals in *C. occidentalis* includes acrosin, aloe-emodin, emodin, islanding, kaempferol, obtusifolin, obtain, and physcion, as well as anthraquinones, apigenin, aurantiobtusin, campesterol, cassiollin, chrysoobtusin, chrysaccording to the temperature and soil characteristics of the growing region, the kind and quantity of phytochemicals in *C. occidentalis* vary. For instance, the plant's stems, leaves, and root bark from the Ivory Coast, Africa, do not contain any alkaloids. Yet, many alkaloids were discovered in the samples from Ethiopia. *C. occidentalis* L. is used extensively in traditional medicine and ethnic cuisine, but little is known about its physicochemical makeup and nutritional value. As a result, efforts were made in the current research to investigate the physicochemical characteristics and nutritional profile of *C. occidentalis* L [3].

Botanical Description

It occurs in West Bengal, South India, Burma, and Ceylon. All tropics and subtropics, including the eastern United States from Texas to Iowa, Africa, Asia, and Australia, support the growth of coffee senna. On wastelands, right after the rains, prolific growth. The plant Cassia occidentalis a common weed with subglabrous branches, a diffuse pungent under shrub, and leaflets. Yellow flowers. This species may be found across India, up to a height of 1.500 mt, from the Himalayas to the -5 pairs, cylindrical or compressed fruits, and transversally septate, glabrous pods with 20–30 seeds. The entire plant includes leaves, flowers, and fruit [4] [Figure 2].

The plant is an annual tropical herb with leathery compound leaves that can reach heights of 6 feet. The olive-brown seeds are flattened on both ends and have dark brown seed pads that bend slightly upward. Long pods containing the seeds may be roasted and turned into a beverage like coffee. Each 4-6 pairs of almost sessile, opposite leaflets on an alternating, even pinnately compound leaf is 4-6 cm long, 1.5–2.5 cm broad, ovate or oblong, lanceolate with a pointy tip, and delicate white hairs on the border. When crushed, the leaflets emit a foul odor. A large, ovoid, shiny, dark purple gland is at the base of the rachis. The 5–10 mm long stipules frequently leave an oblique scar on the skin. An inflorescence is a combination of axillary and terminal racemes [5]. The flower is flawless, measuring 2 cm long, and has five yellow petals and five yellowish-green sepals with pronounced red veins. The fruit is a sickle-shaped legume (pod), dry, dehiscent, transversely partitioned, hardly recurved, laterally compressed, with a rounded tip, and carrying 25–50 seeds. Oval-shaped, 3.5-4.5 mm broad, flattened seeds have a round, pointy end, are smooth, pale to dark brown, and can be glossy.

In Thailand, the *Cassia occidentalis* [6] is referred to by various common names depending on the region, language, or district. One such name is Chumhettet. Nepalese words like Barkichakor, Chilmile, Panwar, and Tulotapre.

Negro coffee in English, Kasaumdi in Hindi, Ponnavirm in Malayalam, Kasamardah in Sanskrit, and Kasinda in Telugu.

Taxonomy

Plantae - Kingdom of Plants

Subkingdom: Vascular plants (Tracheobionta)

Spermatophyta: A subclass of seed plants

Magnoliophyta: The order of flowering plants

Magnoliopsida: Dicotyledons as the class

subclass: Rose family

Family: Leguminosae

Family: Leguminosae

Caesalpinia L. nicker species *Cassia occidentalis* is part of the genus.

Figure 1: Image of *Cassia occidentalis* L. (*Leguminosae*)

Uses

Due to its numerous medical benefits, *Cassia occidentalis* has historically been utilized as a febrifuge, tonic, diuretic, anthelminthic, and purgative. It is a folk remedy for sexual illnesses, fevers, piles, colic discomfort, dropsy, and rheumatism. It is applied topically to treat eczema, ringworm, and other skin conditions. It is also known as Coffee Senna or Negro Coffee because the tree seeds are roasted and ground to make strong coffee. The seeds are also used to treat heart conditions and whooping cough. Roots lose their ability to purge when they

Figure 2: Plant of *Cassia occidentalis* L. (*Leguminosae*)

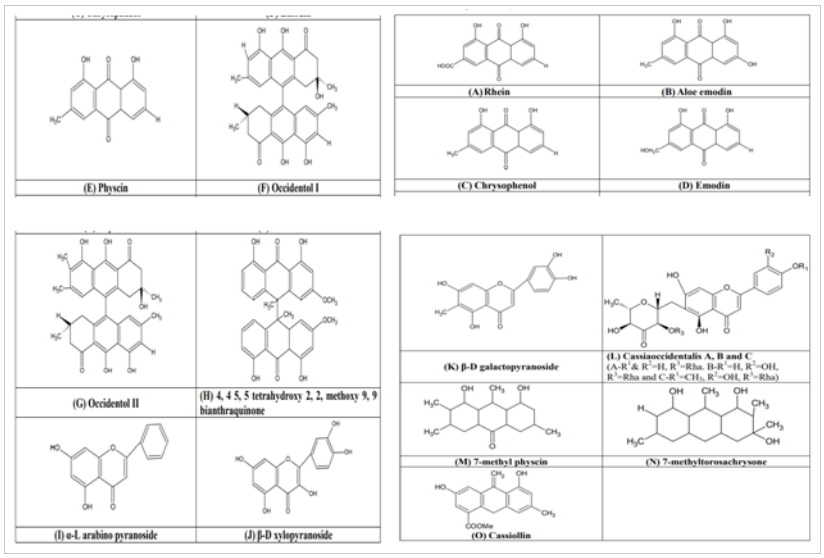

Figure 3: Chemical Structures of Flavanoids, Carotenoids and Phenolics

are burned. This coffee is prescribed as a tonic, a replacement for coffee, and a treatment for asthma, convulsions, and hysteria [7]. *C. occidentalis* L. is used extensively in traditional medicine and ethnic cuisine, but little is known about its physicochemical makeup and nutritional value. Therefore, in the current article, efforts were made to use modern methodologies to investigate the physicochemical characteristics and nutritional profile of *C. occidentalis* L [8] [Table 1].

Chemical Constituents

Crude lipid 14.9%, natural fiber 20.8%, crude protein 2.3%, and carbs 48.1% were all present in the plant. Anthraquinone, proteins, phlorotannins, steroids, tannins, flavonoids, anthraquinones, saponins, terpenes, resins, balsams, amino acids, carbohydrates, sugars, and cardiac glycosides were among the chemical groups found in the various plant parts, according to the phytochemical analysis [9].

Numerous substances were discovered in Cassia occidentalis, including achrosin, aloe-emodin, emodin, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol, rhein, aloe [10].

From the seeds of Cassia occidentalis Linn, three novel chemicals were extracted. These substances have been identified as 5-O-dxylopyranosyl-7-O-l-rhamnopyranosyl-5, 7-dihydroxyflavone (1 3)

Table 1: Ethanol Medicinal Uses

Regions	Ethano Medicinal Use
India	Abscesses, bites (scorpion), constipation, diabetes, edema, fever, inflammation, itch, liver diseases, liver support, rheumatism, ringworm, scabies, skin diseases, snake bite, wounds
Africa	Abscesses, bile complaints, birth control, bronchitis, bruises, cataracts, childbirth, constipation, dysentery, edema, erysipelas, eye infections, fainting, fever, gonorrhea, guinea worms, headache, hematuria, hemorrhages (pregnancy), hernia, increasing perspiration, inflammation, itch, jaundice, kidney infectios, leprosy, malaria,pain (kidney), menstrual disorders, rheumatism, ringworms, scabies, skin diseases, skin parasites, soret hroat, stomach ulcers, stomach ache, swelling, syphilis, tetanus, worms, water retention, wounds
Brazil	Anemia, constipation, edema, fatigue, fever, gonorrhea, liver disorders, malaria, Menstrual disorders, skin problems, tuberculosis, urinary disorders, water retention, weakness
CentralAmerica	Abortions, antifungal, athlete's foot, birth control, constipation, diarrhea, fungal infections, headache, menstrual disorders, menstrual pain, pain, respiratory infections, ringworm, spasms, uterine pain, urinary tract infections, urinary insufficiency, worms
Mexjco	Chills, digestive sluggishness, dyspepsia, ear ache, eczema, edema, fatigue, fever, headache, inflammation (skin), laxative, leprosy, nausea, pain, rash, rheumatism, ringworms, skin problems, sores, stomachache, swelling, tumors, ulcers, venerealdisease,waterretention,worms,yellowfever
Panama	Colic, inflammation, spasms, stomach problems, worms, and as an antiseptic
Haiti	Acne, asthma, burns, colic, constipation, dropsy, eye infections, gonorrhea, headache, malaria, rheumatism, skin rashes and infections, and to increase perspiration
Amazorua	For abdominal pain, birth control, bile insufficiency and malaria
Peru	For asthma, bronchitis, fever and urinary insufficiency

5, 7, 3', 4'-tetrahydroxy-6-methoxyflavone-5-O-l-arabinopyranoside, 3, 5, 7, 3', 4'-pentahydroxy flavone-3-O- -l-rhamnopyranosyl-7-O- -d glucopyranosyl-(1 3)-O- -d xylopyranoside, and 5-O-l-arabinopyr (1 4) O-lrhamnopyranosyl-(1 3)-D-galactopyranoside. There are two novel anthraquinone glycosides: 1,3-dihydroxy6,7,8-trimethoxy and -2-methylanthraquinone 1-hydroxy-3,6, 7,8-tetramethoxy and 3-O rhamnopyranosyl(1-6)-glucopyranosyl (1-6)-galactopyranoside 2-methyl anthraquinone From the leaves of Cassia occidentalis, 1-O-rhamnopyranosyl (1,6)-glucopyranosyl (1,6)-galactopyranoside has been extracted [11]. Glycosides 3,2'-dihydroxy-7,8,4'-trimethoxyflavone-5-O-Dglucopyranosyl (1 2)-D-galactopyranoside.From Cassia occidentalis, apigenin-7-O-D-allopyranoside has been isolated [12].

Total flavonoids were 3.24 g/g, carotenoids were 2.9 g/g, and total phenolics were 6.7 g in the extract of Cassia occidentalis. The total yield of Cassia occidentalis oils was 1.0%, the iodine value was 114.5, the thiocyanogen value was 74.0, the saponification equivalent was 283.0, the yield percentages for liquid fatty acids were 74.9, the iodine value was 151.3, the saponification equivalent was 280.5, the yield percentage for solid fatty acids was 25.1, the iodine value was 3.8, and the yield percentage for saturated acids was 24.0. Defatted Cassia occidentalis flour's mineral makeup contained Ca 3.81, Na 0.11, K 1.86, and Mg 0 [Figure 3].

Pharmacological Activity

Antimicrobial Activity

At concentrations between 900 and 1000 mg, leaf extracts of Cassia occidentalis produced in vari-

ous solvents demonstrated vigorous antibacterial activity against E. coli. No antimicrobial activity was reported against the other tested microorganisms (Pseudomonas multocida, Salmonella typhi, Salmonella typhimurium, Salmonella pyogenes, and Salmonella pneumonia). Still, it was discovered that The concentration ranges of a hexane extract where E. coli was most sensitive were between 500 and 1000 mg. Additionally, another study examined the leaf, seed, and pod for the antifungal activity of the fung, except for the leaf extract's antiaspergilla efficacy, nystatin, and Griseofulvin was investigated [13].

Different organic and aqueous extracts of Cassia occidentalis L leaves were tested for antimicrobial activity by disc diffusion assay against seven human pathogenic bacterial strains and two fungal strains, including P. aeruginosa, K. pneumoniae, P. mirabilis, E. coli, S. aureus, S. epidermidis, and fungus C. albicans. The most susceptible microorganisms were Candida albicans (8 mm inhibition zone in methanol extract), P. mirabilis (15 mm zone of inhibition), as well as P. aeruginosa (18 mm zone of inhibition in aqueous extract). Methanol and aqueous extracts demonstrated significant antimicrobial activity against most of these microorganisms [14].

Using the agar well diffusion technique at a range of doses (80-360 mg/ml), the antibacterial effects of Cassia occidentalis were examined against Escherichia coli, Klebsiella pneumoniae, Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella typhi.

The minimal bactericidal concentration (MBC) was obtained by plating different extract dilutions, whereas The serial dilution technique was used to establish the least inhibitory concentration (MIC). The extract showed that S. aureus and P. aeruginosa were the most sensitive organisms, while K. pneumonia showed some resistance compared to the other tested organisms [15]. Substantial antibacterial activity was observed with MIC values between 160 and 280 mg/ml, and MBC values between 160 and 320 mg/ml were reported.

Central Nervous System Depressant

The leaf ethanol extract of Senna occidentalis possesses a CNS depressant effect was showed [11].

Anti-Inflammatory Activity

Another study examined the anti-inflammatory properties of a methanolic extract of Cassia italica leaves in animal models. For pharmacological testing, the leaves of Cassia occidentalis Linn have been collected through cold extraction with a mixture of equal parts petroleum ether, ethyl acetate, and methanol. The extract demonstrated effective anti-inflammatory action at various dosages, which was significantly done by edema development brought on by carrageenan and formulation. The section at 400 mg/kg dose level demonstrated 36.68% (p0.001) suppression of edema volume at the end of 4 hours in the rat paw edema model caused by carrageenan [6].

Anticonvulsant Activity

According to research, the Cassia occidentalis Linn plant's chloroform extract has exceptional anticonvulsant effectiveness against MES and PTZ-induced convulsion [16].

Anti-Diabetics' Activity

In additional research, it was found that the root extract of Cassia occidentalis, 400 mg/kg, did not significantly differ from that of the animal group treated with aqueous extract, which significantly decreased Fasting blood sugar levels in diabetic as well as healthy rats produced by alloxan and mice, respectively. The leaf extract also significantly reduced blood glucose levels to normal in diabetic mice. According to the current study, the section has the ability to lower blood sugar levels and treat dyslipidemia brought on by hyperglycemia. The methanol fraction of plant leaves (COLMF) was tested against streptozotocin-induced diabetic rats, and this discovery scientifically supports its application in conventional medicine for the treatment of diabetes [4].

Nephroprotective Activity

The 70% hydroalcoholic Cassia occidentalis Linn extract was investigated for its ability to protect rats' kidneys against gentamicin-induced nephrotoxicity.

Calculations of urine Blood urea, serum creatinine levels, urine glucose, urinary sodium, urinary potassium, and animal body weight were used to assess the amount of protection.

GSH, SOD, catalase, and lipid peroxidation tissue levels were evaluated to determine the in-vivo antioxidant activity. The findings indicated that HACO had nephroprotective action against kidney injury from gentamicin [12].

Antitrypanosomal Activity

A related investigation examined the ethanol extract of Senna occidentalis leaf in vitro and in vivo antitrypanosomal properties.

The motility of the parasites was entirely eradicated within 10 minutes of the end of the incubation period when the crude extract, which had an adequate concentration of 6.66 mg/ml, was used [3].

Myostimulant Effect

It has been shown that the biostimulant action of Cassia occidentalis leaves is characterized by an increase in the rhythm and amplitude of isolated intestinal muscle [14].

Cytotoxicity Activity

In a subsequent investigation, alcoholic, hydroalcoholic, and aqueous extracts of plants were used to test their in vitro cytotoxicity and antibacterial effects against eight human cancer cell lines from six distinct tissues and four bacterial strains. Concerning Human cancer, cell lines HCT-15, SW-620, PC-3, MCF-7, SiHa, and OVCAR-5 were tested. Aqueous extract was discovered to perform better than hydroalcoholic and alcoholic extracts at concentrations of 100, 30, and 10 g. In a separate study, the hydroalcoholic section of seeds on clonidine-induced mast cell degradation was investigated and compared with the effect of oral administration of sodium cromoglycate as the gold standard on rats. Mast cell degeneration was the outcome of clonidine [5].

Antipyretic and Antioxidant Activity

Additionally, evaluation based on the antipyretic and antioxidant properties of the methanolic seed extract of the plant using several in-vitro models. The antioxidant potency of Several successive models in vitro systems, including nitric oxide scavenging (NOS) activity, the carotene-linoleic acid model system, hydroxyl radical scavenging (HRS) activity, reducing power, and metal chelating activity, were used to study organic and aqueous leaf extracts of Cassia occidentalis (MCA). Lastly, antioxidant and antipyretic properties (DPPH and Hydrogen Peroxide Method) [17].

Toxicological Studies

A few studies examined the effects of subacute oral administration of the plant during pregnancy in female Wistar rats.

In a related study, male and female Wistar rats were used in a pre-clinical safety evaluation of the hydroalcoholic extract of the plant's stem and leaves. Both groups of rats showed no toxicity, indicating a safe use in humans [18].

Effect on Smooth Muscles Contraction

When the endothelium is intact or not in rat aortic rings, the relaxing effects of an aqueous Cassia occidentalis leaf extract were studied. Noradrenaline (NA) and potassium chloride (KCI) both suppressed contraction in the section dose-dependent manner.

Aortic rings precontracted with 10-7 M NA and 50m M KCI were likewise relaxed. The prostacyclin inhibitor indomethacin and the dye methylene blue did not affect the induced relaxation, which was not dependent on the existence of an intact vascular endothelium [9].

Toxicology/Pharmacokinetics

The clinical and histological results of a horse poisoning epidemic caused by *C. occidentalis* were investigated. After ingesting ground corn that contained 8%.

occidentalis seeds, twenty mares became ill. Of the 20 animals afflicted, 12 passed away. Of the two deaths that occurred within 6 hours of the beginning of clinical indications consistent with hepatic encephalopathy, other animals were put to death 12 hours after the onset of the clinical signs. On the other hand, the remaining eight mares showed signs of moderate sadness and decreased appetite, but they got well with therapy, and no clinical consequences were seen. An increased hepatic lobular pattern and many seeds were seen in the large intestines of 6 animals that underwent necropsies [19]. The primary histopathological findings have been cerebral edema and hepatocellular pericentrolobular necrosis. There has been minor multifocal in one foal semi-membrane abdomyocytic necrosis and haemorrhage. The seeds removed from the guilty feed ingredient and sorted from digestive contents were inspected. Analysis of the resulting plant's leaves, flowers, fruits, and seeds revealed C. occidentalis. Horses poisoned with C. occidentalis seeds exhibit hepatoencephalopathy-related clinical symptoms or frequently pass away abruptly. The liver is affected most often, followed by the CNS [7].

Fresh or dry beans are poisonous, as shown by several animal experiments. Large quantities being consumed by grazing animals have resulted in fatal illnesses. Several mechanisms are engaged depending on the animal type and the amount of beans ingested. The skeletal muscles, liver, kidney, and heart are the organs most affected by toxicity in big animals, rodents, and fowl. Frequently, brain functions were impacted. Skeletal muscle fiber necrosis and hepatic centrilobular necrosis are the two most common gross lesions found after necropsy; renal tubular necrosis was less common. Biochemical abnormalities were reflected in the necrosis of muscle and liver cells. The median fatal dosage (LD50) for mice and rats is 1 g/kg. Numerous anthraquinones, their derivatives, and alkaloids have been linked to toxicity [13].

Toxic and Adverse Effects

The clinical and histological results of a horse poi-

soning epidemic caused by *C. occidentalis* were investigated. After ingesting ground corn that contained 8% *C. occidentalis* seeds, twenty mares became ill. Of the 20 animals afflicted, 12 passed away. Of the two deaths that occurred within 6 hours of the beginning of clinical indications consistent with hepatic encephalopathy, other animals were put to death 12 hours following the onset of the clinical symptoms [20]. On the other hand, the remaining eight mares showed signs of moderate sadness and decreased appetite, but they got well with therapy, and there were also no clinical consequences. An increased hepatic lobular pattern and many seeds were seen in the large intestines of 6 animals that underwent necropsies. The primary histopathological findings were cerebral edema and hepatocellular pericentrolobular necrosis. Mild multifocal semi-membranous abdomyocytic necrosis and haemorrhage were present in one mare. The seeds removed from the guilty feedstuff and sorted from digestive contents were inspected. Examination of the resulting plant's leaves, flowers, fruits, and seeds revealed C. occidentalis. Horses poisoned with C. occidentalis seeds exhibit hepatoencephalopathy-related clinical symptoms and commonly pass away abruptly. A liver and CNS are affected most frequently by lesions [21].

CONCLUSION

This investigation showed that the chosen plant has significant energy value, crude fibres, vitamins, minerals, particularly iron and zinc, and antioxidant enzymes. As a result, C. occidentalis is a safe, wholesome, and functional dietary supplement. The sample possesses several advantageous physicochemical properties, including a high ash concentration, solubility in water, and extract yield. The physicochemical characteristics discovered in this study may be utilized as quality control measures and will help identify and authenticate this plant material.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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MODIFIERS OF IRON ABSORPTION IN SOUTH INDIAN IRON RICH MILLET DIET

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Abstract :

Iron is an essential mineral that the body needs to produce haemoglobin, which transports oxygen from the lungs to all of the body's tissues. Iron is primarily stored as ferritin in the liver, spleen, and bone marrow. Foxtail millet (korra), finger millet (ragi/ragulu), barnyard millet (odalu), little millet (sama), and kodo millet are the staple millets of South India. In addition to these 5, South Indians also consume pearl and jowar millet. Millets Contain modifiers (Inhibitors and Enhancers) of iron absorption. By consuming Millets with appropriate cooking techniques and food combinations , bioavailability of iron can be increased .

Introduction :

An important mineral the body needs to make hemoglobin, a substance in the body that carries oxygen from the lungs to tissues throught out the body.

•The total body iron content of normal adults is 4.3 and 2.3gms in men and women respectively

•Iron is primarily stored in the liver,spleen,and bone marrow in the form of ferritin

— Dietary iron has two main forms: heme and nonheme [1] . Only nonheme iron can be found in plants and iron-fortified meals, whereas both heme and nonheme iron can be found in meat, seafood, and poultry[2]. In western populations, heme iron, which is formed when iron combines with protoporphyrin IX, accounts for 10% to 15% of total iron intake [3-5]

Why do we need iron _

*Iron is a mineral found in every cell in the body

*It is vital for both physical health and mental well being

Source of iron :

Food

*Lean meat and seafood are the best sources of heme iron in the diet [6]. Nonheme iron is found in nuts, beans, vegetables, and fortified grain products. Bread, cereal, and other grain products provide approximately half of the dietary iron in the United States [2,3,5].

*Breast milk contains highly bioavailable iron, but not in sufficient quantities to meet the needs of infants aged 4 to 6 months [2,7].

*Other dietary factors have less of an impact on the bioavailability of heme than nonheme iron, and heme iron has a better bioavailability than nonheme iron [3,4].

*The bioavailability of iron ranges from 5% to 12% in vegetarian diets and between 14% and 18% in mixed diets that contain significant amounts of meat, seafood, and vitamin C (ascorbic acid, which increases the bioavailability of nonheme iron) [2,4].

_ In Table 1, a number of iron-rich foods are included. Because they include substances that prevent iron from being absorbed, such as polyphenols, several plant-based foods that are good sources of iron, such spinach, have low iron bioavailability [8,9].

Table 1: Iron Content of Selected Foods [10]

Food	**Milligrams per serving**	**Percent DV***
Breakfast cereals, fortified with 100% of the DV for iron, 1 serving	18	100
Oysters, eastern, cooked with moist heat, 3 ounces	8	44
White beans, canned, 1 cup	8	44
Beef liver, pan fried, 3 ounces	5	28
Lentils, boiled and drained, ½ cup	3	17
Spinach, boiled and drained, ½ cup	3	17

Tofu, firm, ½ cup	3	17
Chocolate, dark, 45%–69% cacao solids, 1 ounce	2	11
Kidney beans, canned, ½ cup	2	11
Sardines, Atlantic, canned in oil, drained solids with bone, 3 ounces	2	11
Chickpeas, boiled and drained, ½ cup	2	11
Tomatoes, canned, stewed, ½ cup	2	11
Beef, braised bottom round, trimmed to 1/8" fat, 3 ounces	2	11
Potato, baked, flesh and skin, 1 medium potato	2	11
Cashew nuts, oil roasted, 1 ounce (18 nuts)	2	11
Green peas, boiled, ½ cup	1	6
Chicken, roasted, meat and skin, 3 ounces	1	6
Rice, white, long grain, enriched, parboiled, drained, ½ cup	1	6
Bread, whole wheat, 1 slice	1	6
Bread, white, 1 slice	1	6
Raisins, seedless, ¼ cup	1	6
Spaghetti, whole wheat, cooked, 1 cup	1	6
Tuna, light, canned in water, 3 ounces	1	6
Turkey, roasted, breast meat and skin, 3 ounces	1	6
Nuts, pistachio, dry roasted, 1 ounce (49 nuts)	1	6
Broccoli, boiled and drained, ½ cup	1	6
Egg, hard boiled, 1 large	1	6

*DV stands for Daily Value. The U.S. Food and Drug Administration (FDA) created DVs to assist customers in comparing the nutritional content of foods and dietary supplements in the context of a complete

diet. For adults and kids who are 4 years old and older, the DV for iron is 18 mg [11]. The FDA requires iron content to be listed on food labels. Foods with 20% or more DV are considered high sources of a nutrient, but foods with lower percentages of DV also contribute to a healthy diet.

Daily requirements of iron diet :*Dietary Reference Intakes (DRIs), created by the Food and Nutrition Board (FNB) of the Institute of Medicine (IOM) of the National Academies, offer intake guidelines for iron and other minerals (formerly National Academy of Sciences) [5].

According to age and gender, these variables include :

• Recommended Dietary Allowance (RDA) : It is frequently used to create individualised diet plans that are nutritionally adequate for people. The average daily intake is sufficient to meet the nutrient needs of nearly all healthy people (97%–98%).

• Adequate Intake (AI): When there is not enough data to calculate an RDA, intake at this level is presumed to guarantee nutritional adequacy.

•Estimated Average Requirement (EAR): It is typically used to evaluate the nutrient intakes of groups of people and to develop diets that are nutritionally appropriate for them. However, it can also be used to evaluate the nutrient intakes of individuals. The average daily consumption is thought to meet the needs of 50% of healthy individuals.

•Tolerable Upper Intake Level (UL): The recommended daily intake is unlikely to have a negative impact on health.

Table 2_ lists the current iron RDAs for nonvegetarians. Vegetarian RDAs are 1.8 times greater than those for meat eaters. This is due to the fact that heme iron from meat is more accessible than nonheme iron from plant-based meals, and that nonheme iron is more readily absorbed when consumed with meat, poultry, and seafood [5].

Table 2: Recommended Dietary Allowances (RDAs) for Iron [5]

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.27mg*	0.27mg*		
7-12 years	11mg	11mg		
1-3years	7mg	7mg		
4-8 years	10mg	10mg		
9-13years	8mg	8mg		
14-18years	11mg	15mg		
19-50years	8mg	18mg	27mg	10mg
51+years	8mg	8mg	27mg	9mg

Iron deficiency : In the United States, an iron deficiency is not unusual, particularly in young children, women who are fertile, and pregnant women. People with iron deficiency usually have other nutrient deficiencies because it is associated with poor diet, malabsorptive disorders, and blood loss [2]. According to the World Health Organization (WHO), iron deficiency is thought to be the cause of over half of the 1.62 billion instances of that exist worldwide [20]. Iron deficiency is frequently caused by enteropathies and blood loss brought on by gastrointestinal parasites in poor nations [2].

Table 3-

	Systemic iron overload	Iron misdistribution/local iron deposition*
Genetic	• Hereditary hemochromatosis (HFE-, TfR2-, HJV-, HAMP-, or FPN-related) • Ferroprotein disease • Aceruloplasminemia • Atransferrinemia • DMT-1 deficiency • Private/sporadic iron overload diseases (e.g. H-ferritin related iron overload) • Hereditary iron-loading anaemias due to inefficient erythropoiesis	•Congenital sideroblastic anemias • Friederich ataxia • Neuroferritinopathy
Acquired	• Post-transfusion • Parenteral • Oral • Alloimmune neonatal hemochromatosis	• Chronic liver diseases • Neurodegenerative disorders (including NBIA unrelated to iron-genes) • Anemia of chronic diseases

Iron overload diseases:

*sideroblastic anaemias

*thalassemia's

*sickle cell disease

*rare anaemias

Kinetics of iron :

Absorption-

Iron is absorbed mainly from the small intestine, predominantly in the duodenum and upper jejunum.

Heme and non-heme iron are the two varieties of dietary iron that are absorbable.

- Heme iron is the most easily absorbed form of iron (15% to 35%) and accounts for 10% or more of the total iron we absorb. Heme iron is produced from hemoglobin and myoglobin of animal dietary sources (meat, fish, poultry).
- Non-heme iron, which comes from plants and meals with added iron, is less readily absorbed [27].

Some dietary components inhibit or enhance the duodenal pH-dependent process of iron absorption[27].

→ Phytate, a substance present in diets based on plants and showing a dose-dependent effect on iron absorption, is one of the inhibitors of iron absorption. Black and herbal tea, coffee, wine, legumes, grains, fruit, and vegetables all contain polyphenols, which have been shown to prevent the absorption of iron. Calcium inhibits both heme and non-heme iron at the point of first uptake into enterocytes, in contrast to other inhibitors such polyphenols and phytates that solely impede non-heme iron absorption. Human iron absorption has been demonstrated to be inhibited by animal proteins such casein, whey, egg whites, and proteins from plants (soy protein). Oxalic acid, which is present in spinach, chard, beans, and almonds, binds to iron and prevents it from being absorbed.

Distribution :

Total body iron

3500 to 3700 mg in a 70kg man

2000 to 2500 mg in female.

Body iron content of an adult is 3-5 g (~ 45 mg / kg woman, ~ 55 mg / kg for men). The majority of the body's iron (60–70%) is contained in the hemoglobin of circulating erythrocytes. Hepatocytes and RES macrophages store ferritin and hemosiderin, which make up about 20–30% of the body's total iron supply. Although only about 3 mg of iron is bound to transferrin, every day, about 20 mg of iron passes through the plasma transferrin compartment. When there is an excess of iron, NTBI can manifest in the plasma. The bone marrow is the main consumer of circulating iron. Every day, 200 billion new erythrocytes are formed, and each one contains 18–20 mg of iron, most of which is recycled. Iron loss is made up for by the 1-2 mg of iron that healthy persons absorb each day. NTBI - non-transferin bound iron; RBCs - red blood cells; Tf – transferrin[30].

Iron transport and storage : The primary iron transport protein is called transferrin (transports iron through blood). The Fe2+ transported by ferroprotein needs to be converted to Fe3+ because this is the form of iron that binds to transferrin. Hephaestin and ceruloplasmin are two copper-containing proteins that catalyse this oxidation of Fe2+. While ceruloplasmin is the main copper transport protein in blood, hephaestin is present in the membrane of enterocytes. The main protein that carries out this function in a coupled (necessarily simultaneous) manner with transport via ferroprotein is hephaestin. This indicates that for the Fe2+ to be transported through ferroportin, it must be oxidised. Evidence suggests that when iron status is low, ceruloplasmin is involved in oxidising Fe2+. Fe3+ is oxidised and then binds to transferrin before being delivered to a tissue cell with a transferrin receptor. As shown below, transferrin binds to the transferrin receptor and is then endocytosed.[66]

As a result, because red blood cells only live for 120 days, iron recycling is important. The liver, spleen, and bone marrow break down red blood cells, and the iron can be used for the same things as previously mentioned: cellular use, storage, or transportation to another tissue on transferrin. The creation of heme and ultimately red blood cells will use the majority of this iron.[66]

Among minerals, iron is special in that our body can only excrete a small amount of it. Hepcidin, a hormone, thus controls absorption. The liver has an iron sensor, which signals the release of hepcidin when iron levels rise. Ferroportin is degraded by hepcidin. As a result, the iron cannot be moved into circulation.[66]

The enterocyte, which eventually removes off and is excreted in faeces, is now holding the iron captive. As a result, hepcidin reduces the absorption of iron.

Modifiers of iron absorption in south Indian iron rich millet diet

The term "millets" refers to a wide range of annual cereal crops that are known for their tendency to yield tiny seeds. Several grasses that are used as food, fodder, and biofuel are included in this group, including foxtail millet (Setaria italica), finger millet (Elucine coracana), pearl millet (Pennisetum glaucum), proso millet (Panicum miliaceum), kodo millet (Paspalum scorbiculatum), barnyard millet (Echinocloa sp,)etc. [54]

A diverse collection of small-seeded grasses known as millets are widely cultivated as cereal grains for use as fodder and human sustenance all over the world. The majority of the species that are commonly referred to as millets are members of the Paniceae tribe, but some millets are also members of other taxa.[31]

TYPES OF IRON RICH MILLETS-

1.Barnyard millet(sanwa)

2. sorghum millet (jowar)

3.pearl millet (bajra)

4.kodo millet

5. finger millet (Ragi, Nachni)

6.little millet (kutki)

7.foxtail millet (korra)

8.proso millet (chena)

Barnyard millet : Echinochloa spp., also known as barnyard millet, is one of Asia's most important minor millet crops. The two most widely grown and well-known Echinochloa spices are Echinochloa esculenta (barnyard millet, which has a Japanese origin) and Echinochloa frumentacea (Indian based barnyard millet).Barnyard millet is highly adaptable despite having a brief life cycle, and current research has demonstrated that this crop is a functional food due to its high nutritional and antioxidant value (see table 4 for details)[32].

Table 4; [32]. **source-** Barnyard millet: The underutilized nutraceutical minor millet crop Anjali Singh, Munnangi Bharath, Apurva Kotiyal, Lipakshi Rana and Devanshi Rajpa

Domain	**Eukaryotes**
Kingdom	plantae
Phylum	spermatophyte
Sub phylum	Angiospermae
class	monocotyledonae
order	cyperales
family	poaceae
genus	echinochloa
species	Echinochloa species

- The grains of barnyard millet are used as food and can be cooked like rice to be consumed. They can also be used as a functional food for those with atopic dermatitis and allergic illnesses.[32]
- Echinochloa esculenta (barnyard millet) is a minor plant. millet variety grown in India, Japan, and China in the world and is used as a food and animal feed.[34]

It has the following nutrients per 100 g, which correspond to: [33].

- Calories: 300 kcal
- Fat: 3.6 g
- Dietary Fiber: 13.6 g
- Carbohydrate: 55 g
- Calcium: 22 mg
- Vitamin B1: 0.33 mg
- **Iron: 18.6 mg**
- Vitamin B3: 4.2 mg

Enhancers of iron absorption in barnyard millet -[34].Barnyard millet has a sufficient amount of nutrients, high iron content, and low glucose index (Amadou et al., 2013). The fermentation of L.'s barnyard millet plantarum has a beneficial effect on the nutritional content and antioxidant activity of millet and also extends the shelf life of millet-based products probiotic supplements

Inhibitors of iron absorption in barnyard millet -Natural protein glycation inhibitors derived from barnyard millet may be a more efficient way to manage protein glycoxidation and AGE formation and offer advantages without the negative side effects of synthetic drugs. On the other hand, the antiglycation properties of barnyard millet have not been thoroughly studied.[67]Amadori products are thickened, dehydrated, and oxidised in the last stages to provide premium glycation products (Vistoli et al., 2013) . This protein glycation is connected to glycoxidation, which results in the creation of oxygen and active carbonyl intermediates.

Additionally, several particular Glycation End (AGE) products such pentosidine, pyrraline, crossline, argpyrimidine, and pentolysine cause oxidative damage in cells and change their regular biological processes[32].

5 Major Diseases of Barnyard Millet (With Management) | Plant Diseases[35].

1.Head Smut

2. Grain Smut

3. Kernel Smut

4. Leaf Spot or Blight

Health Benefits of Barnyard Millet;[36]

1. Low in Calories

2.Rich in Fiber

3.Low Glycemic Index

4. Gluten-Free Food

5.Good Source of Iron

Pearl millet : Pearl millet, also known as bulrush millet (Pennisetum glaucum (L.) R. Br.), is a tropical cereal grass with small grains. It is also known as P. typhoides, P. Americanum, or P. spicatum. There are other slang terms, including "bajra" (in India), "Gero" (in Nigeria's Hausa language), "hegni" (in Niger's Djerma language), "sanyo" (in Mali), "dukhon" (in Sudan's Arabic), and "mahangu" (Namibia).With an annual production of over 14 million tones worldwide, pearl millet is the most significant millet in terms of quantity (Mt) [37]. In the last two decades, the world's production has only marginally increased, primarily as a result of disregard and preference for maize. However, due to the crop's specialized weh adaptation to heat and aridity, pearl millet yield may increase as the world becomes hotter and drier. Around 200 years ago, pearl millet farming is thought to have spread to East and Central Africa, India, and other dry regions [31].

Benefits of pearl millet [38].

1.Pearl millet promotes heart health

2.Pearl millet manages diabetes

3.Pearl millet prevents cancer:

4.Pearl millet prevents anemia

Enhancers of iron absorption in pearl millet - Breeding goals for pearl millet include resistance to harmful diseases such downy mildew, ergot, smut, rust, and head Mould of pearl millet as well as improved nutritional quality, saline adaptability, stover feeding value, high tillering, and drought tolerance [39].

Inhibitors of iron absorption in pearl millet -By using chromatographic techniques incorporating CM-sephadex and SP-Sepharose cation exchange columns and ammonium sulphate precipitation, a cysteine protease inhibitor with antifungal activity was extracted from pearl millet seeds and refined to homogeneity. Its molecular mass was determined by molecular characterization to be 24 Kd, and its isoelectric point was 9.8 [40]

Finger millet : It is also known as ragi in India, dagusha in Ethiopia and Eritrea, and wimbi in Swahili in East Africa. In English, finger millet is often referred to as bird's foot, coracana, and African millet. It is a staple diet for millions of people living in the semi-arid tropical regions of Africa and Asia. Finger millet is the fourth most popular millet in the world, following Panicum millaceum, Setaria italica, and pennisetums

(pearl millet) (proso millet). It is projected to make up around 8% of the area and 11% of the global production of millets[31].

Growing finger millet takes a little bit more water than growing the other millets. With a yield of 1150 kg ha-1 in elevated areas, it is the most productive among the millets. The crop can be grown in regions with annual precipitation ranging from 500 to over 1000 mm in elevations up to 2000 m. Finger millet annual world production is at least 4.5 million tones, of which Africa produces about 2 million tones.[31]

Health benefits of Finger Millet[44].

1. Controls Diabetes

2. Reverts Skin Aging

4. Bone health

5. Anti-cancer potential.

Nutrients per Serving [45].

- Calories: 189
- **Protein**: 6 grams
- **Fat**: 2 grams
- **Carbohydrates**: 36 grams
- **Fiber**: 4 grams

Enhancers of iron absorption in finger millet:millets provide a variety of nutrients to the diet, they are a fantastic way to increase the iron density of the diet. The daily iron and calcium intakes will increase by 50% and 350%, respectively, if finger millet (ragi) is substituted for just 100 gm of the daily cereal (rice) intake.[48]

Inhibitors of iron absorption in finger millet: Ragi also includes other antinutrients, including phytates and polyphenols, which are known to prevent the absorption of iron. White finger millet typically contains 0.04–0.09% polyphenols, whereas brown cultivars have 0.08–3.47%. Ragi is most frequently consumed in its dark variant. The less popular variety is the white ragi[49]

Little millet : Panicum sumatrense, sometimes known as little millet, is an essential minor grains, which are widely grown in the tropics and are a staple food for groups with low incomes in some nations. In addition to providing minerals and vitamins, little millet is a comparable supply of protein, fat, carbs, and crude fibre to other cereals like rice and wheat. Additionally, it contains phytochemicals such tannins, phytate, phenolic acids, and flavonoids [50].

Nutrition-Based Health Benefits Of Little Millet-[51]

1.High in protein

2.Promote healthy digestion

3.Prevents anemia

4.Control diabetes

5.Support Weight loss:

A 100 grams serving of little millet contains:[51]

- **Calories:** 329
- **Protein:** 7.7 grams
- **Fiber:** 7.6 grams
- **Fat:** 4.7 grams
- **Carbs:** 67 grams

Inhibitors of iron absorption in little millet-Four stored grains and four phytophagous insect pests had their gut α amylases tested to see how three proteinaceous inhibitors isolated from tiny and Finger millet affected their ability to digest food.On the other hand, proteinaceous inhibitors from finger millet (FMCO11 and FMCO13) and little millet (LMCO3) inhibited insect-pests' amylases in varying degrees. The two compounds with the highest levels of inhibition against Callosobruchus chinensis -amylase were LMCO3 and FMCO13, with respective inhibition percent's of roughly 70% and 50%.[52].

Enhancers of iron absorption in little millet -As they provide a variety of nutrients to the diet, millets are a fantastic way to increase dietary iron density. When finger millet (ragi) is substituted for just 100 g of daily cereal (rice), daily iron and calcium intakes increase by 50% and 350%, respectively. Although these are great advantages, they may be countered by the ragi grain's high inherent phytate content, which may inhibit iron absorption [53].

Foxtail millet: The annual grass foxtail millet, scientifically known as Setaria italica (also known as Panicum italicum L.), is cultivated for human consumption. It is Asia's most frequently grown millet species and the second-most widely planted millet species overall [55].

Foxtail millet has the conventional architectural form of a domesticated plant, which consists of a single stalk or a few tillers with enormous inflorescences that mature essentially simultaneously. A fully developed foxtail millet plant has thin, upright, and leafy stems that range in height from 120 to 200 cm (3.9 to 6.6 feet) [56].

Benefits of Foxtail Millets: [56]

1.**Proper functioning of the nervous system**

2. **Protects Bone health and muscle health**

3. **Good for Cardiac Health**4. **Regulates blood sugar level**.

4. **Lowering Blood Cholesterol**

Table 5-Nutritional Value of Foxtail Millets[56]

Proteins	12.3 grams
Fat	4.3 grams
Carbohydrates	60.9 grams
Crude Fibre	8 grams
Minerals	3.3 grams
Calcium	31 mg
Phosphorous	290 mg
Iron	**2.8 mg**
Energy	331 kcal

Enhancers of iron absorption in foxtail millet -Nitrogen application in soils with low available nitrogen is beneficial for enhancing (Increasing) productivity. Nitrogen in combination with phosphorus, or the recommended dose of balanced fertilizer, can increase foxtail millet yield [59].

Inhibitors of iron absorption in foxtail millet-CO7 cultivar of foxtail millet (IC50, 22.37 and 57.26 g/ml) and CO4 cultivar of little millet (IC50, 18.97 and 55.69 g/ml) soluble and bound fractions inhibited -glucosidase strongly [60]

Proso millet : Proso millet (Panicum miliaceum L.) is an annual cereal crop that was domesticated around 10,000 years ago in China's semiarid regions. It is grown primarily in India, Nigeria, Niger, and China. Despite its high nutritional and health benefits, proso millet is used as fodder and bird seed in Europe and North America.[61]

Proso millet is also known as common millet, hog millet, broom corn, yellow hog millet, Hershey millet, and white millet. Proso millet is a warm-season grass that can produce seed within 60 to 100 days of planting. It has a low moisture requirement due to its relatively short growing season and is capable of producing food or feed where other grain crops would fail. Proso millet was introduced to the United States' eastern Atlantic Coast by European immigrants in 1875, but it is now mostly grown in the Great Plains.[62]

Health benefits of Proso Millet [63]

1.Beneficial in anti-ageing.

2.Beneficial for nervous system

3.Beneficial in preventing pellagra and other Niacin dependent conditions

4.Useful for strengthening bones

Mineral elements- source : Millet, proso | Tables of composition and nutritional values of feed

Table 6

Parameter		As fed	On DM	Unit	Other	Unit
Calcium		0.4	0.4	g/kg		-
Phosphorus		2.7	3	g/kg		-
Phytate phosphorus		1.8	1.9	g/kg	65	% P
Magnesium		1.2	1.3	g/kg		-
Potassium		3.7	4.1	g/kg		-
Sodium		0.08	0.09	g/kg		-
Chlorine		0.04	0.05	g/kg		-
Sulfur		1.1	1.2	g/kg		-
Dietary cation-anion difference		30	34	mEq/kg		-
Electrolyte balance		98	109	mEq/kg		-
Manganese		12	13	mg/kg		-
Zinc		29	32	mg/kg		-
Copper		6	7	mg/kg		-
Iron		53	58	mg/kg		-
Selenium		0.2	0.2	mg/kg		-
Iodine		0.05	0.05	mg/kg		-

*The iron content of millets was 2.60g, 7.0g, in little millet, proso millet.[64]

Inhibitors of iron absorption in proso millet : Like oleic acid from olive oil and polyunsaturated fatty acids (PUFAs) like-Ghama linoleic acid, proso and Japanese millet containing LA, which was an HDAC inhibitor, could be useful in preventing cancer. Other fatty acids like oleic and Ghama linoleic acids were also present in proso and Japanese millet, with the amount of LA in each being inversely correlated with the inhibitory activity against HDAC and cancer cell growth. Even though there is conflicting evidence regarding the anti-tumor activity of PUFAs, including LA, in vivo, our findings provide an intriguing explanation: the anti-tumor effect of PUFAs may depend on this HDAC inhibitory activity and/or CYP17 inhibitory activity.[65]

CONCLUSION :

Iron is an important mineral the body needs to make hemoglobin, a substance in the body that carries oxygen from the lungs to tissues throught out the body. These studies saying that some constituents are modifying the iron absorption in the millets. Millets are an excellent approach to boost dietary iron density since they offer a variety of nutrients to the diet. Just 100 g of daily cereal (rice) can be replaced with finger millet (ragi), increasing daily iron and calcium intakes by 50% and 350%, respectively. The soluble and binding fractions of the CO7 cultivar of foxtail millet (IC50, 22.37 and 57.26 g/ml) and CO4 cultivar of small millet (IC50, 18.97 and 55.69 g/ml) both strongly inhibited -glucosidase. It is advantageous to apply nitrogen to soils with low levels of accessible nitrogen to increase productivity. The recommended amount of balanced fertilizer or the addition of nitrogen and phosphorus can enhance foxtail millet yield. Traditional food processing and preparation techniques that improve the bioavailability of micronutrients in diets based on cereals are available at the household level for pearl millet. The techniques include germination, fermentation, hydrothermal processing, blanching, decortication, and soaking. These techniques reduces the levels of phytic acid and polyphenols, which improves iron bioavailability. Lactobacillus plantarum 299v is used to increase iron absorption in pearl millet. The bioavailability of iron was increased in proso millet by fermenting raw grains of rice and black gram dal. Because millets contain a lot of dietary phytates and fiber, their iron bioavailability is low. Processing methods like soaking, germination, and fermentation can increase iron absorption. Barnyard millet is treated with a combination of Lactobacillus rhamnoses GG, which enhances iron absorption. So iron absorption in millets can be improved by combining them with vitamin C.

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Formulation and evaluation of biocompounds of berberis asiatica compressed tablets in hyperlipidemia treatment

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ABSTRACT

The objective of the research is to design and evaluate the bio compounds of Berberis asiatica (BCBA) compressed tablets in the management of hyperlipidemia. BCBA of dose 500 mg was compressed into tablets by employing microcrystalline cellulose (MCC) and sodium starch glycolate (SSG) as super disintegrant and necessary excipients by employing direct compression method. The fourier transform infrared (FTIR) results depicts no incompatibility among the drug and excipients. The study results of pre-compression parameters has excellent flow qualities and compressibility.The post-compression parameters show that the results are within the specified standard deviations. SSG exhibit better disintegrating property than MCC. Better disintegration properties and in vitro drug release tests were exhibited by the optimized formulation F6.

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INTRODUCTION

A high amount of lipids in your blood, such as cholesterol and triglycerides, is referred to as hyperlipidemia. It is thought to be a Coronary heart disease (CHD) risk factor if the onset can happen at any age, with males experiencing it at >45 years of age and women experiencing it at >55 years of age [1]. Hyperlipidemia is a key source of increased atherogenic risk; both hereditary diseases and lifestyle (sedentary behavior and high-calorie, saturated-fat, and cholesterol-rich meals) contribute to dyslipidemias prevalent in advanced countries worldwide [2, 3].

There are four treatment lines available for hyperlipidemic patients including Statins, Bile acid sequestrants, Nicotinic acid and Fibric acid derivatives as explained, yet no treatment is effective enough to properly manage Hyperlipidemia due to the lack of compliance with patients and adherence to the therapy. Medical plants have played a significant part in ancient traditional medical systems.

Till now, plants remain one of the most affordable sources of medication for the vast majority of the population with secure and negligible side effects. The World Health Organization has endorsed the utilization of natural medications and traditional medicines for the benefit of the world's population owing to cost-effectiveness and fewer side effects.

Berberis asiatica is reported in having antihyperlipidemic activity at a dose of 500mg [4]. BCBA was formulated into tablet dosage form by direct compression method.

MATERIALS AND METHODS

Chemicals

The study made use of analytical grade chemicals (Sigma Aldrich, Hi-media, and Merck India Ltd).

Collection and Preparation of aqueous Extract of B. asiatica (BCBA)

The B. asiatica heartwood was obtained from the Sri Srinivasa ayurvedic pharmacy in Tirupati. The heartwood had been dried and roughly ground. At room temperature, 200 g of heartwood powder was macerated with 1L distilled water for 24 hours. The extract was concentrated, and the resulting semisolid mass of 20 g was housed in an airtight container devoid of excess heat, moisture, and air, and labelled BCBA.

UV spectral analysis of BCBA

The calibration curve is the primary basis for estimating rate of drug release in in vitro dissolution studies. To determine the maximum absorption wavelength of selected candidates, UV spectral analysis of BCBA was undertaken using a UV-Visible spectrophotometer between 200 and 400 nm.

Compatibility studies

Chemical compatibility between the BCBA and excipients was studied by FT-IR Spectroscopy. About 2% test sample was combined with potassium bromide (KBR) to obtain fine powder by grinding using small glass mortar and then crushed into KBR pellets by a hydraulic press at a pressure of 10000 psi and waited for 1 minute collecting the pellet. Each sample was screened for 32 single scans at the range of 400- 4000 cm-1.

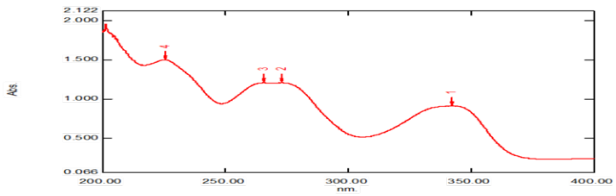

Figure 1: UV-Visible absorption spectra of BCBA

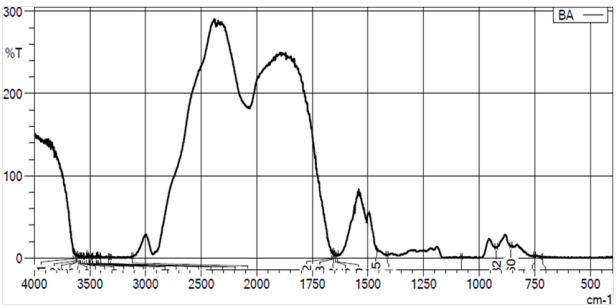

Figure 2: FTIR of BCBA

Preparation of BCBA compressed tablets

The BCBA tablets were manufactured adopting the direct compression approach. The calculated amount of drug & excipients was weighed individually, mix homogeneously and compressed [Table 1].

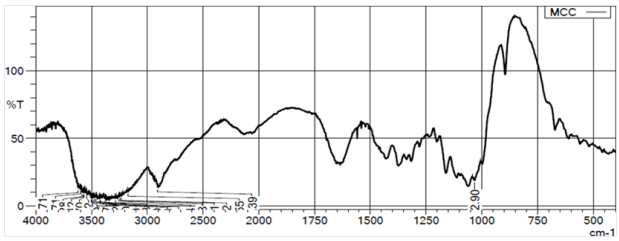

Figure 3: FTIR of MCC

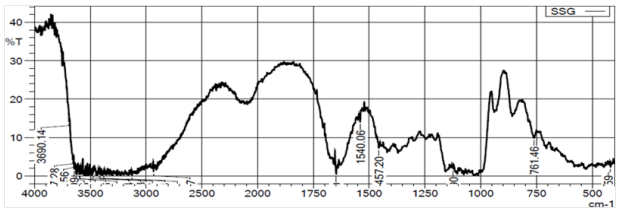

Figure 4: FT-IR of SSG

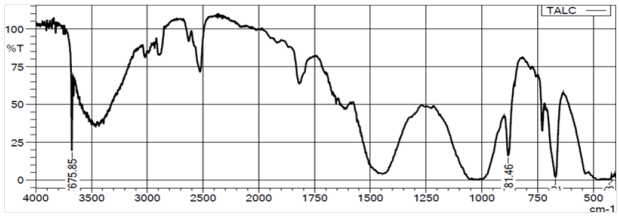

Figure 5: FT-IR of Talc

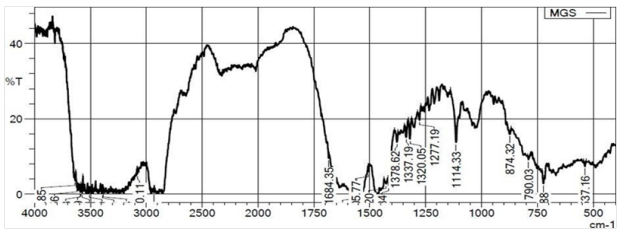

Figure 6: FT-IR of Magnesium stearate

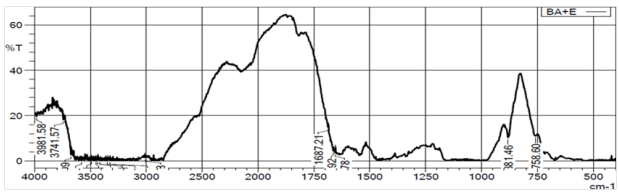

Figure 7: FT-IR of BCBA and excipients

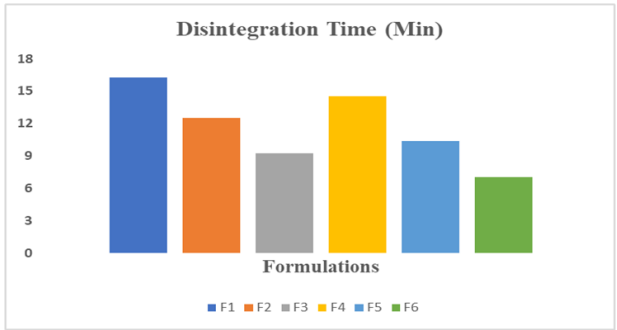

Figure 8: Disintegration time of Formulations F1-F6

Table 1: Formula for the Preparation of BCBA tablets

Ingredients	F1	F2	F3	F4	F5	F6
BCBA	500	500	500	500	500	500
MCC	12..5	25	37.5	-	-	-
SSG	-	-	-	10	20	40
Talc	37.5	37.5	37.5	37.5	37.5	37.5
Magnesium stearate	25	25	25	25	25	25

Table 2: Pre-compression parameters of formulation F1-F6

S.No	Formulation	Angle of repose	Bulk density	Tapped density	Carr's index	Hausner'sratio
1.	F1	21.38	0.86	0.87	1.14	1.01
2.	F2	23.49	0.89	0.91	2.19	1.02
3.	F3	24.07	0.86	0.89	3.37	1.03
4.	F4	22.09	0.87	0.88	1.13	1.01
5.	F5	23.02	0.88	0.90	2.22	1.02
6.	F6	21.63	0.87	0.92	5.43	1.05

Table 3: Post compression parameters of formulation F1-F6

S.No	Formulation	Thickness& Diameter (Limit:<5%)	Hardness(Limit 5 Kg/cm^2)	Friability (Limit:<1%)	% Weight variation
1.	F1	Within limits	4.5	0.68	Pass
2.	F2	Within limits	3	0.79	Pass
3.	F3	Within limits	4	0.67	Pass
4.	F4	Within limits	4	0.93	Pass
5.	F5	Within limits	3	0.46	Pass
6.	F6	Within limits	3.5	0.54	Pass

Table 4: Release kinetics of formulations F1-F6

Formulati	Zero Order (r)	First Order (r)	Higuchi(r)	HixsonCrowell (r)	Korsmeyer Peppas	
					(r)	(n)
F1	0.9465	0.9913	0.9723	0.9154	0.8785	0.31
F2	0.9531	0.9937	0.9134	0.9274	0.8683	0.29
F3	0.9559	0.9957	0.9265	0.9156	0.8753	0.36
F4	0.9642	0.9991	0.8923	0.9356	0.8469	0.35
F5	0.9102	0.9902	0.9014	0.9216	0.8961	0.28
F6	0.9546	0.9992	0.9112	0.9057	0.8521	0.27

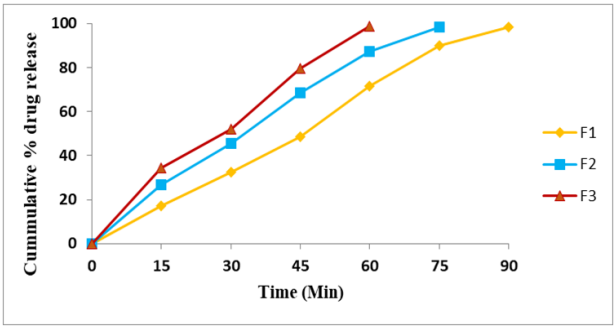

Figure 9: Dissolution studies of formulations F1-F3

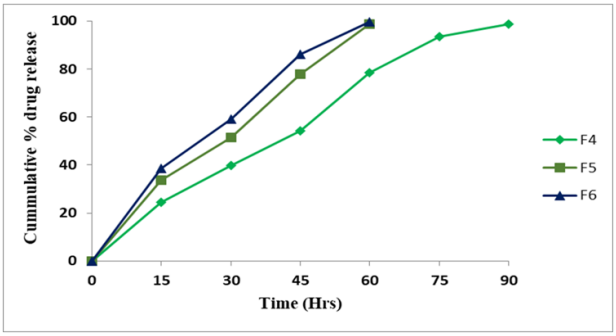

Figure 10: Dissolution studies of formulations F4-F6

Pre compression parameters

Bulk density (Db)

A bulk density apparatus was used to determine the bulk density of the powder mixture. It is the ratio of total powder mass to total powder volume. It was calculated by pouring the weighted powder into a measuring cylinder and recording the volume. It is presented in g/ml and is represented as

D_b= M/V_b

Tapped density (D_t)

It is the ratio of total powder mass to tapped powder volume. Tapping the powder to constant volume yielded the tapped volume. It is measured in g/ml and is provided by [5].

D_t = M/V_t

Compressibility index (I) and Hausner's ratio

Carr's index and Hausner's ratio measure the compressibility and the flow nature of powder mixture. It was calculated employing following formula [6].

I= D_t - D_b/D_t x 100

Hauseners ratio = Dt / Db

Angle of repose

The angle of repose is frequently used to determine the frictional forces in a loose powder. This is the maximum angle that formed between the surface of powder pile and the plane. A weighed powder were delivered via a funnel from a specific height (2 cm) onto a level surface, forming a heap. The heap's height and radius were recorded. The formula used to determine the angle of repose [7].

Angle of repose ø = tan-1(h/r)

Post compression parameters Dimensions

The tablets thickness and diameter evaluated using digital vernier callipers or screw guaze. The permitted difference is ±5% [8]

The percent difference in thickness and diameter can be calculated by using the formula:

Percent difference = Average – Individual reading /AverageX100

Hardness

It was performed with a hardness tester and stated in kg/cm^2.

Friability (F)

Friability was determined using the Roche friabilator and represented as a percentage. Randomly collect 20 tablets and weighed collectively and record as W1 and placed in the friability chamber, which was rotated at 25 rpm for 4 minutes. Then collect the tablets and weigh record as W2. The % friability computed using the formula below [9].

%F = W1-W2/W1 X 100

Weight variation test

Collect randomly 20 tablets, weighed individually. Determine the average weight for 20 tablets. Calculate the % weight variation for 20 tablets individually. Not more than two individual weights of tablets should fall out of the limits i.e.,±5%.

% Weight variation = Average weight – Individual tablet weight / Average weight x 100

Disintegration test

Disintegration test can be done by using tablet disintegration tester which contains 6 glass tubes with mesh of size 10 at bottom of each glass tube. Tablets are placed in glass tubes and dip in a one litre beaker of water maintained at body temperature. The basket operated at a speed of 28 to 32 cycles per minute by employing a motor. The time required to tablet to completely disintegrate into pieces is noted which gives disintegration time [10].

Dissolution studies

Rotating paddle apparatus (USP type II) employed. Tablet was placed in 900 ml of Phosphate Buffer Saline 7.4 dissolution medium. The dissolution medium was stirred at a rate of 50 rpm and maintained at a temperature equivalent to that of body

temperature. The sample of 1 ml pipette out, diluted to 10 ml at every 15 min interval upto 90 min. The amount of drug release at every interval was calculated by using UV-spectrophotometer Wavelength: 340 nm.

Release kinetics

Data obtained from the in vitro release studies were fitted to various kinetic equations such as zero order, first order, Higuchi model and Korsmeyer-Peppas model [11].

RESULTS AND DISCUSSION

UV Spectral Analysis of BCBA

The BCBA solution was examined between 200 and 400 nm showed in the Figure 1. The absorption maximum was found to be 340 nm used for further studies showed in Figure 1.

FTIR study

The FTIR studies reported compatibility of BCBA with excipients and no significant interactions were observed [Figures 2, 3, 4, 5, 6 and 7].

Precompression parameters

Pursuant to the study results of pre compression parameters include angle of repose, cars index, and hausners ratio, the powder mixture of all formulations (F1-F6) has excellent flow qualities and compressibility [Table 2].

Postcompression parameters

The observation of results of post-compression parameters depicts that thickness and diameter of the compressed tablets are within the limits (i.e<5%) states that no variation in the tablet weight. Hardness and friability reveals the tablets of all formulations (F1-F6) are having sufficient strength and surface strength. The results of percentage weight variation of formulations F1-F6 reveals variation of tablet weight are within the prescribed limits [Table 3].

Disintegration time

The disintegration time of formulations F1-F6 was in the range of 16 min 25 sec to 7 min 2 sec which falls within in the prescribed limits. Disintegration time of the tablets was highly influenced by the super-disintegrating agents and its concentration. The increased concentration of microcrystalline cellulose in formulations F1-F3 the disintegration time decreased from 16 min 25 seconds to 9 min 27 sec. The increased concentration of sodium starch glycolate in formulations F4-F6 the disintegration time decreased from 14 min 55 sec to 7 min 2 sec. In comparison to microcrystalline cellulose sodium starch glycolate exhibits better disintegrating property. The results show that the disintegration time of the tablets lowers as the concentration of super disintegrating agent increases. The F6 formulation with sodium starch glycolate was optimized which disintegrates in less time than the formulations with microcrystalline cellulose as a super disintegrant [Figure 8].

Dissolution studies

The rate of drug release is high in formulations contain SSG as super disintegrant in comparison to formulations prepared with MCC. The F6 formulation prepared with 8 % SSG releases the complete drug at the end of 60 min in comparison with the formulations prepared with 4% and 6% SSG. The rate of drug release is high in F6 formulation, hence it was optimized [Figures 9 and 10].

Mathematical Model Fitting of Obtained Drug Release Data

The obtained drug release profiles data from all 6 runs of BCBA compressed tablets at different time intervals was fitted to various drug release kinetic models. As mentioned in the table the correlation coefficient value (r) was found to be maximum for first order model. The maximum r value for first order model confirmed that the diffusion of drug into dissolution medium is dose-dependent [Table 4].

CONCLUSION

Bioactive compounds of Berberis asiatica compressed tablets were successfully processed by direct compression method. The FTIR studies reported compatibility of BCBA with excipients. Pre-compression parameters depicts the powder mixture of all formulations (F1-F6) has excellent flow qualities and compressibility. The post-compression parameters depicts that the tablets are having sufficient hardness, surface strength, thickness and diameter are within the limits, passes the percentage weight variation test. Sodium starch glycolate exhibit better disintegrating property than microcrystalline cellulose. The optimized formulation F6 showed better disintegrating property and In vitro drug release studies.

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Nil.

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ORIGINAL ARTICLE

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Formulation and evaluation of bio compounds of berberis aristata compressed tablets in psoriasis treatment

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ABSTRACT

The objective of the research is to design and evaluate the bio compounds of Berberis aristata (BCBA) compressed tablets in psoriasis treatment. BCBA of dose 300 mg was compressed into tablets by employing microcrystalline cellulose (MCC) and sodium starch glycolate (SSG) as superdisintegrant and necessary excipients by employing direct compression method. The fourier transform infrared (FTIR) results depicts no incompatibility among the drug and excipients. The study results of pre compression parameters have excellent flow qualities and compressibility. The post-compression parameters show that the results are within the specified standard deviations. SSG exhibit better disintegrating property than MCC. The optimized formulation F6 showed better disintegrating property and In vitro drug release studies in comparison with marketed formulation.

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INTRODUCTION

Psoriasis is an inflammatory disease that affects 1-3% of the world's population and is persistent, painful, recurrence and remitting. Although the disease can strike at any age, many individuals are diagnosed in their twenties. Some authors have recognized two types of psoriasis: type I, which appears before the age of 40, and type II, which appears later and peaks between the ages of 55 and 60. The condition affects both men and women indiscriminately. The severity varies, spanning from one or more cutaneous patches to severe peeling of the entire skin. Up to 30% of individuals may experience joint pain [1–3].

Despite the fact that there are four treatment lines accessible for psoriatic patients, including topical, systemic, phototherapy, and biological treatment, no medication is successful enough to appropriately control psoriasis owing to patient compliance and adherence to therapy. Medical plants have played a significant part in ancient traditional medical systems.

Till now, plants remain one of the most affordable sources of medication for the vast majority of the population with secure and negligible side effects.

The World Health Organization has endorsed the utilization of natural medications and traditional medicines for the benefit of the world's population owing to cost-effectiveness and fewer side-effects.

Berberis aristata is reported in having anti-psoriatic activity at a dose of 350mg [4]. BCBA was formulated into tablet dosage form by direct compression method.

MATERIALS AND METHODS

Chemicals

The study made use of analytical grade chemicals (Sigma Aldrich, Hi-media, and Merck India Ltd).

UV spectral analysis of BCBA

The calibration curve is the primary basis for estimating rate of drug release in in vitro drug dissolution studies. To determine the maximum absorption wavelength of selected candidates, UV spectral analysis of BCBA was undertaken using a UV-Visible spectrophotometer between 200 and 400 nm.

Compatibility studies

Chemical compatibility between the BCBA and excipients was studied by FT-IR Spectroscopy. About 2% test sample was combined with potassium bromide (KBR) to obtain fine powder by grinding using small glass mortar and then crushed into KBR pellets by a hydraulic press at a pressure of 10000 psi and waited for 1 minute collecting the pellet. Each sample was screened for 32 single scans at the range of 400- 4000 cm-1.

Preparation of BCBA compressed tablets

The BCBA tablets were manufactured adopting the direct compression approach. The calculated amount of drug & excipients was weighed individually, mix homogeneously and compressed [Table 1].

Pre compression parameters

Bulk density (Db)

A bulk density apparatus was used to determine the bulk density of the powder mixture. It is the ratio of total powder mass to total powder volume. It was calculated by pouring the weighted powder into a measuring cylinder and recording the volume. It is presented in gm/ml and is represented as

D_b= M/V_b

Tapped density (D_t)

It is the ratio of total powder mass to tapped powder volume. Tapping the powder to constant volume yielded the tapped volume. It is measured in g/ml and is provided by [5].

D_t = M/V_t

Compressibility index (I) and Hausner's ratio

Carr's index and Hausner's ratio measure the compressibility and the flow nature of powder mixture. It was calculated employing following formula [6].

I= D_t - D_b / D_t x 100

Hauseners ratio = Dt / Db

Angle of repose

The angle of repose is frequently used to determine the frictional forces in a loose powder. This is the maximum angle that formed between the surface of powder pile and the plane. A weighed powder were delivered via a funnel from a specific height (2 cm) onto a level surface, forming a heap. The heap's height and radius were recorded. The formula used to determine the angle of repose [7].

Angle of repose ø = tan-1(h/r)

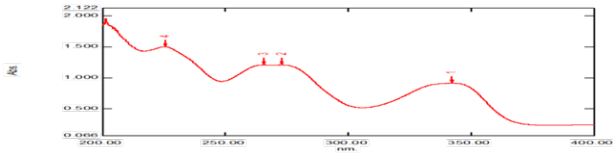

Figure 1: UV-Vis absorption spectra of BCBA

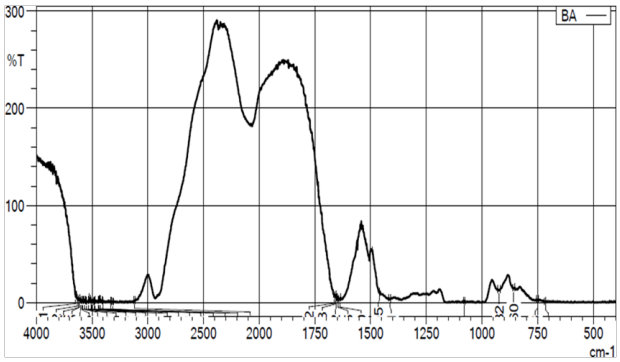

Figure 2: FTIR spectrum of BCBA

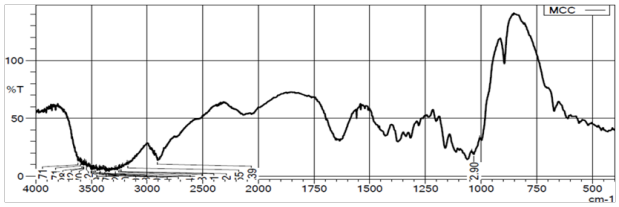

Figure 3: FTIR of MCC

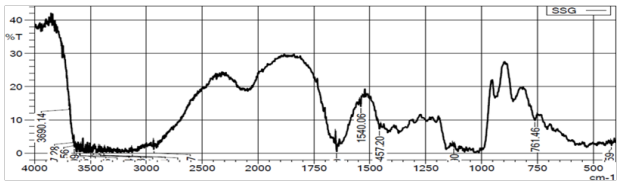

Figure 4: FT-IR spectrum of SSG

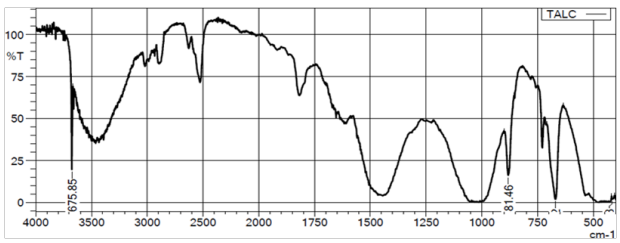

Figure 5: FT-IR of Talc

Post compression parameters Dimensions

The tablets thickness and diameter evaluated using digital vernier callipers or screw guaze. The permitted difference is ±5% [8].

Table 1: Formula for the Preparation of BCBA tablets

Ingredients	F1	F2	F3	F4	F5	F6	F7
BCBA	350	350	350	350		350	350
Microcrystalline cellulose	-	17.5	26.5	35	-	-	-
Sodium starch glycolate	-	-	-	-	14	21	28
Talc	-	17.5	17.5	17.5	17.5	17.5	17.5
Magnesium stearate	-	17.5	17.5	17.5	17.5	17.5	17.5

Table 2: Precompression parameters of formulation F1-F7

S.No	Formulation	Angle of repose	Bulk density	Tapped density	Carr's index	Hausner's ratio
1	F1	25	0.83	0.96	13.54	1.15
2	F2	24	0.72	0.80	10	1.11
3	F3	23	0.69	0.77	9.7	1.10
4	F4	21	0.69	0.76	9.0	1.10
5	F5	18	0.71	0.80	11.2	1.13
6	F6	24	0.73	0.86	15	1.17
7	F7	23	0.66	0.72	8.0	1.09

Table 3: Postcompression parameters of formulation F1-F7 & MP

S.No	Formulation	Thickness & Diameter	Hardness(Limit 3-5 Kg/cm^2)	Friability (Limit:<1%)	%Weight variation
1.	F1	Within limits	3.5	0.6	Pass
2.	F2	Within limits	3.5	0.47	Pass
3.	F3	Within limits	3.5	0.42	Pass
4.	F4	Within limits	3.5	0.93	Pass
5.	F5	Within limits	3.5	0.79	Pass
6.	F6	Within limits	3.5	0.67	Pass
7.	F7	Within limits	3.5	0.92	Pass
8.	Marketed Tablet	Within limits	3	0.57	Pass

Table 4: Release kinetics of formulations F1-F7 & MP

Formulation	Zero Order (r)	First Order(r)	Higuchi(r)	Hixson Crowell (r)	Korsm Pepp (r)
F1	0.9593	0.9803	0.9822	0.9259	0.8785
F2	0.9453	0.9908	0.9032	0.9127	0.8683
F3	0.9674	0.9957	0.8963	0.9245	0.8753
F4	0.9651	0.9996	0.9371	0.9637	0.8469
F5	0.9002	0.9918	0.9572	0.9511	0.8961
F6	0.9456	0.9901	0.9127	0.9782	0.8521
F7	0.9217	0.9946	0.8631	0.9745	0.9041
MP	0.9743	0.9995	0.8564	0.9785	0.8524

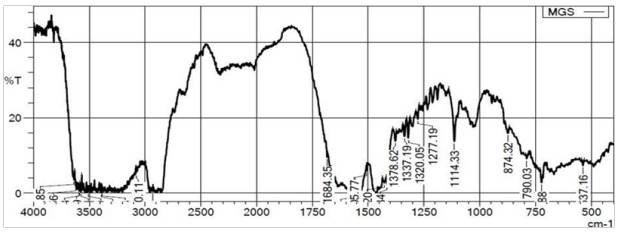

Figure 6: FT-IR of Magnesium stearate

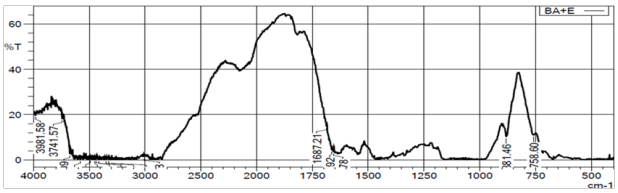

Figure 7: FT-IR of BCBA and excipients

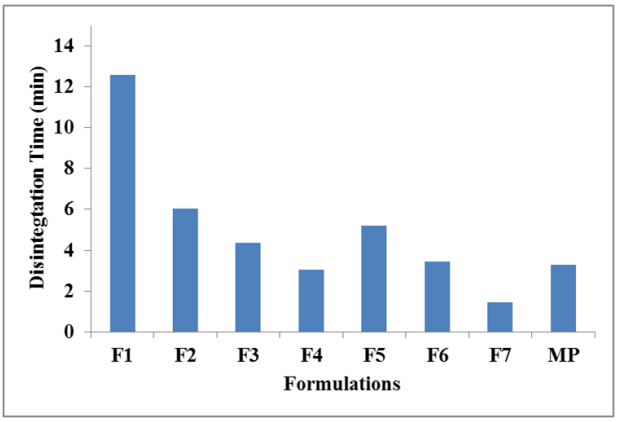

Figure 8: Disintegration time of Formulations F1-F7 & Marketed Product

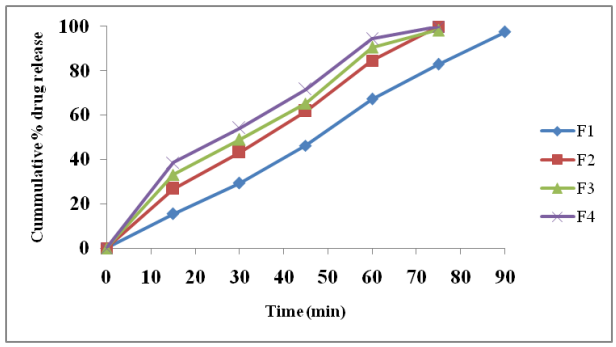

Figure 9: Dissolution studies of formulations F1-F4

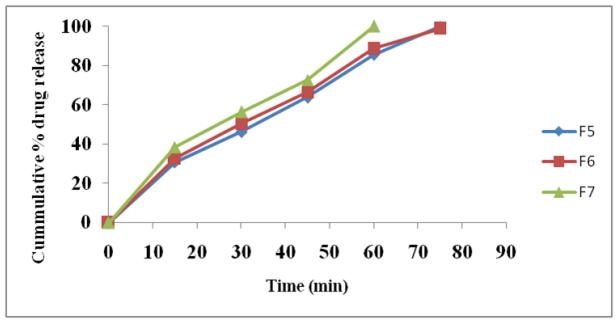

Figure 10: Dissolution studies of formulations F5-F7

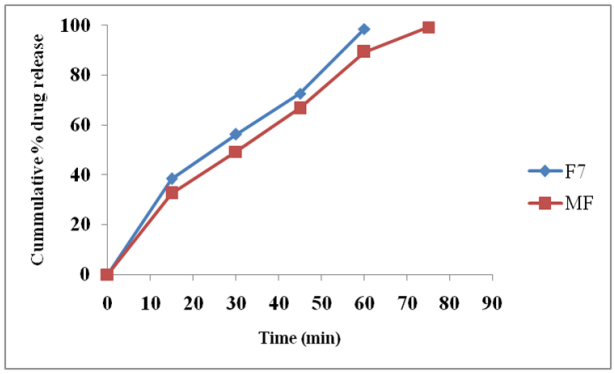

Figure 11: Dissolution studies of formulations F7 and Marketed Product

The percent difference in thickness and diameter can be calculated by using the formula:

Percent difference = Average – Individual reading /AverageX100

Hardness

It was performed with a hardness tester and stated in kg/cm^2.

Friability (F)

Friability was determined using the Roche friabilator and represented as a percentage. Randomly collect 20 tablets and weighed collectively and record as W1 and placed in the friability chamber, which was rotated at 25 rpm for 4 minutes. Then collect the tablets and weigh record as W2. The % friability computed using the formula below [9].

%F = W1-W2/W1 X 100

Weight variation test

Collect randomly 20 tablets, weighed individually. Determine the average weight for 20 tablets. Calculate the % weight variation for 20 tablets individually. Not more than two individual weights of tablets should fall out of the limits i.e.,±5%.

% Weight variation = Average weight – Individual tablet weight / Average weight x 100

Disintegration test

Disintegration test can be done by using tablet disintegration tester which contains 6 glass tubes with mesh of size 10 at bottom of each glass tube. Tablets are placed in glass tubes and dip in a one litre beaker of water maintained at body temperature. The basket operated at a speed of 28 to 32 cycles per minute by employing a motor. The time required to tablet to completely disintegrate into pieces is noted which gives disintegration time [10].

Dissolution studies

Rotating paddle apparatus (USP type II) employed. Tablet was placed in 900 ml of Phosphate Buffer

Saline 7.4 dissolution medium. The dissolution medium was stirred at a rate of 50 rpm and maintained at a temperature equivalent to that of body temperature. The sample of 1 ml pipette out, diluted to 10 ml at every 15 min interval upto 90 min. The amount of drug release at every interval was calculated by using UV-spectrophotometer Wave length: 340 nm.

Release kinetics

Data obtained from the in vitro release studies were fitted to various kinetic equations such as zero order, first order, Higuchi model and Korsmeyer-Peppas model [11].

RESULTS AND DISCUSSION

UV Spectral Analysis of BCBA

The BCBA solution was examined between 200 and 400 nm as showed in the Figure 1. The absorption maximum was found to be 340 nm used for further studies showed in Figure 1.

FTIR study

FTIR of BCBA

The FTIR studies reported compatibility of BCBA with excipients and no significant interactions were observed (Figures 2, 3, 4, 5, 6 and 7).

Precompression parameters

Pursuant to the study results of pre compression parameters include angle of repose, cars index,and hausners ratio, the powder mixture of all formulations (F1-F7) has excelentf low qualities and compressibility [Table 2].

Postcompression parameters

The observation of results of post compression parameters depicts that thickness and diameter of the compressed tablets are within the limits (i.e <5%) states that no variation in the tablet weight.

Hardness and friability reveals the tablets of all formulations (F1-F7) are having sufficient strength and surface strength.

The results of percentage weight variation of formulations F1-F7 including marketed product reveals variation of tablet weight are within the prescribed limits [Table 3].

Disintegration time

The disintegration time of formulations F1-F7 and marketed product was in the range of 1mim 45 sec to 7 min 57 sec which falls within in the prescribed limits. Disintegration time of the tablets was highly influenced by the super disintegrating agents and its concentration. The increased concentration of microcrystalline cellulose in formulations F2-F4 the disintegration time decreased from 6 min 2 seconds to 3 min 6 sec. The increased concentration of sodium starch glycolate in formulations F5-F7 the disintegration time decreased from 5 min 18 sec to 1 min 45 sec. In comparison to microcrystalline cellulose the sodium starch glycolate exhibits better disintegrating property.The results show that the disintegration time of the tablets lowers as the concentration of super disintegrating agent increases. The F7 formulation with sodium starch glycolate was optimized which disintegrates in less time than the marketed product (Figure 8).

Dissolution studies

The rate of drug release is high in formulations contain SSG as superdisintegrant in comparison to formulations prepared with MCC. The F7 formulation prepared with 8 % SSG releases the complete drug at the end of 60 min in comparison with the formulations prepared with 4% and 6% SSG. The rate of drug release is high in F7 formulation comparison to marketed product, hence it was optimized (Figures 9, 10 and 11).

Mathematical Model Fitting of Obtained Drug Release Data

The obtained drug release profiles data from all the 7 runs of BCBA compressed tablets and marketed product at different time intervals was fitted to various drug release kinetic models. As mentioned in the table the correlation coefficient value (r) was found to be maximum for first order model. The maximum r value for first order model confirmed that the diffusion of drug into dissolution medium is dose dependent [Table 4].

CONCLUSION

The bio components of Berberis aristata compressed tablets were effectively processed using the direct compression technique. The FTIR analysis showed that BCBA is compatible with excipients. According to the research findings of pre compression parameters the powder mixture of all formulations (F1-F7) has acceptable flow qualities and compressibility. The post compression parameters show that the tablets have adequate hardness, surface strength, thickness, and diameter, and also it pass the % weight variation test. Sodium starch glycolate disintegrates faster than microcrystalline cellulose. In compared to the marketed product, the optimised formulation F7 exhibited improved disintegration properties and in vitro drug release studies.

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Conflict

Nil.

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Review Article

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Review on Pregnancy Testing Kit Analysis

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Abstract

Healthcare professionals and the general public both often use pregnancy tests. The measurement of human chorionic gonadotrophin (HCG) in urine is a prerequisite for all tests. None of the other pregnancy-specific agents that have been suggesting as pregnancy tests can match the sensitivity and practicality of the immunoassay of HCG. Additionally, ultrasound detection is less sensitive than HCG analysis. The latest generation of tests is based on monoclonal antibodies to the beta-subunit of HCG; this feature allows for outstanding sensitivity because it essentially precludes the chance of cross-reaction with pituitary luteinizing hormone (L.H.).It is crucial to understand that the 'beta-subunit' antibody reacts with both intact HCG, which constitutes the majority of pregnancy serum, and beta-subunit fragments (beta-core), which include the majority form in the urine. Non-pregnant persons' blood and urine both have trace quantities of HCG. Around 6-8 days after fertilization, HCG from the implantation blastocyst first manifests in the mother's blood; levels quickly increase to peak at 7-10 weeks. Urine may show positive findings with most modern pregnancy test kits (sensitivity 25 units per liter) 3–4 days after implantation; by seven days (the time of the anticipated period), 98% of results will be positive. One week following the missed period, a negative test almost always indicates that the lady is not pregnant. False positive test results brought on by interfering elements are highly improbable with today's test kit generation. It is unlikely that new tests or alternative technology will be able to achieve the degree of sensitivity and specificity that pregnancy tests have currently attained.

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INTRODUCTION

The first was a kit for a quick and accurate pregnancy test. After that, the dual-specific non-competitive sandwich assays and the in-vitro test for allergy diagnosis were performed. Then, the solid phase radioimmunoassay and a solid phase binding assay for vitamin B12 were performed. At the time, the university didn't demonstrate much interest in supporting ideas that could lead to commercial products. I even heard scathing criticism from a few university friends. The last 20 years have seen a complete shift in this mentality. Today, the government encourages colleges to promote inno-

vations and offer the necessary tools to assist with their preservation and commercialization. But each of the inventions of the 1960s began with a few original discoveries that led to technical advancement. The development of assay techniques with distinctive properties and the ensuing hunt for suitable applications led to the invention of pregnancy and allergy tests. The diagnostic test kit industries were pioneered by Organon in Holland with its pregnancy test kit and Pharmacia in Sweden with its radioimmunoassay test kits. Pharmacia Diagnostics rose to prominence as one of the world's top manufacturers of diagnostic test kits, and it has remained so in the area of allergy diagnostics [1].

Definition

A testing kit, often known as a test kit, is an invitro diagnostic tool made up of reagents, articles, or any combination of these, and it is designed to be used to carry out a specific test for reagent testing, pregnancy testing, or soil testing.

Types of Testing Kits

The types of testing kits shown in Figure 1.

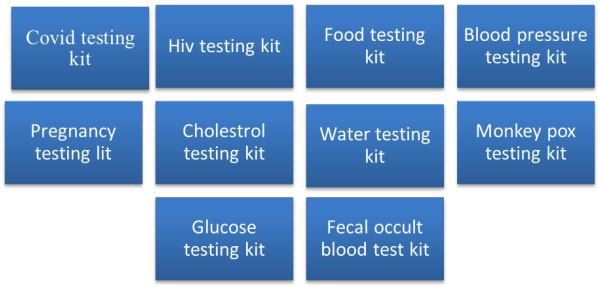

Figure 1: Types of Testing Kits

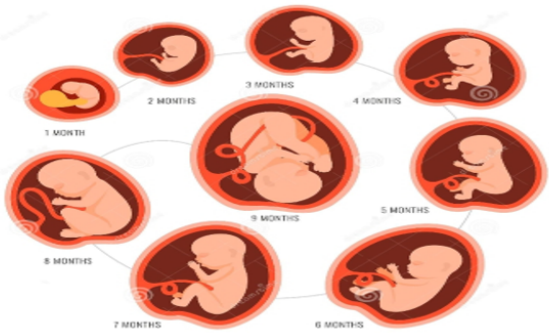

Figure 2: Stages of Pregnancy

Pregnancy

The period of a woman's life during which one or more infants develop inside her is known as pregnancy, also known as gravidity or gestation. Multiple pregnancies entail having many children, such as twins. A sexual encounter or assisted reproductive technologies can result in pregnancy. It culminates in childbirth and typically lasts 40 weeks from the Last Menstrual Period (LMP), a date the patient, ideally, is aware of. This is roughly nine lunar months, with a month lasting about 291-292 days. About 38 weeks have passed since conception. A fetus is a word used until birth to describe the growing child during the first eight weeks after the beginning. The period of a woman's life during which one or more infants develop inside her is known as pregnancy, also known as gravidity or gestation. Patients frequently refer to the fetus as a "baby." Missed periods, sensitive breasts, nausea and vomiting, hunger, and frequent urination are just a few early pregnancy symptoms. A urine pregnancy test can be used to confirm pregnancy; most frequently, the patient does the test at home, followed by a series of blood tests in the doctor's office. The period during which one or more kids develop is known as pregnancy, gravidity, or gestation. A hormone called Human Chorionic Gonadotropin is found in urine and blood tests. During the first few days of pregnancy, your body quickly accumulates this hormone, created by the placenta, shortly after the embryo connects to the uterine lining. Most of your pregnancy symptoms are brought on by this abrupt change in hormone levels [2].

Definition

The period when the fertilized egg develops in the uterus between conception (the fertilization of an egg by a sperm) and birth. The average human pregnancy lasts 288 days. The zygote begins to divide, and after 5-7 days of division and growth, it adheres to the uterine wall, developing into an embryo and the placenta [3].

Stages of Pregnancy

Approximately >280 days, >9 months, >40 weeks, or >3 trimesters make up a typical pregnancy.

First Trimester [Week 1-12]

Almost every organ system in the body experiences hormonal changes during the first trimester of pregnancy. The infant proliferates. In this trimester, the primary organs begin to develop, and menstruation has halted.

Mood swings, headaches, and morning sickness are among the symptoms and indicators of pregnancy.

1. The baby's brain, heart, and spinal cord have started to develop.
2. The eighth week is when the baby's heart begins to beat.

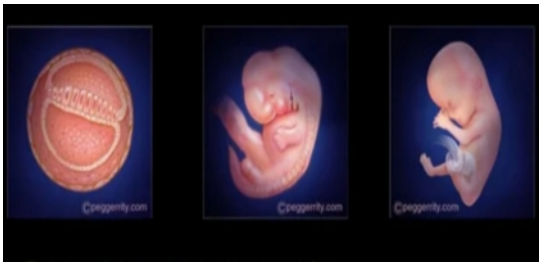

Figure 3: Development of an Embryo in the First Trimester

3. The development of the sex organs.
4. The Face begins to take shape.
5. After eight weeks, the fetus is around 2.5 cm long and more human-like in appearance.
6. We can determine if he is a boy or a girl at 12 weeks, and the infant can first.

Second Trimester [Week 13 -28]

The majority of early pregnancy symptoms will eventually go away. Additional symptoms include heartburn, limb swelling, and back pain. Breast and abdominal stretch marks. The size of the uterus will increase quickly. Blow and heart rate is increased to meet the fetus's needs. Bone and muscle tissue are still forming.

1. In the sixteenth week, skin starts to form.
2. At 20 weeks, the infant can hear and swallow.
3. In the 24th week, your baby's head starts to sprout natural hair.
4. The lungs develop but do not function.
5. At the end of the third trimester, the baby is currently 30 cm long.

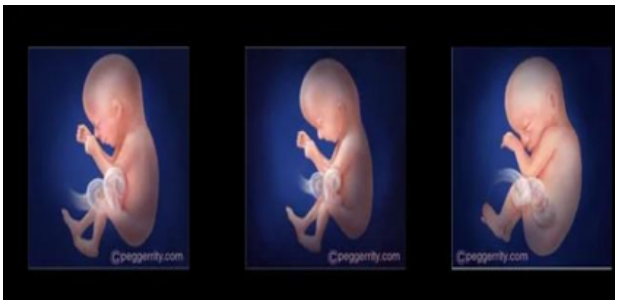

Figure 4: Development of the Baby in the Second Trimester

Third Trimester [Week 29-40]

Your fetus expands, and the body organs develop during this third trimester. The fetus moves around a lot. particularly during the 27th and 32nd weeks. In the uterus, a fetus is typically in a head-down position. Mother's breath is short. The mother's belly button can spit out. The breast first produces colostrum [Rich yellow milk].

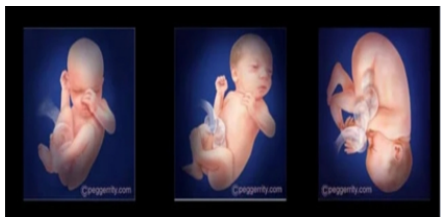

Figure 5: Development of Baby in the Third Trimester

At 34 weeks, the baby's bones have finished developing.

1. The ability to notice changes in light and to open and close the eyes. By the conclusion of the 37th week, the baby is deemed full-term, and the organs are prepared for independent function.
2. The baby may become head down for delivery as the due date approaches.

Diet and Source

Month 1

1. Green leafy vegetables, such as spinach, rocket, and parsley, are high in folate.
2. Legumes [lentils, beans, chickpeas] and whole grains. Natural remedies for minimizing nausea and vomiting in the early stages of pregnancy. It has been demonstrated that giving 40 mg of vitamin B6 twice daily is helpful.

Month 2

1. Ginger can help with nausea. Try adding two tablespoons of grated ginger to boiling water for tea, eating crystallized ginger candies, or cooking with ginger powder.
2. Good sources of vitamin E to minimize the risk of miscarriage include almonds, olive oil, hazelnuts, and egg yolk.

Month 3

1. Water: To keep your infant hydrated, consume at least ten glasses of water daily in addition to fruits and vegetables that are high in water content.

2. Ginger, keep taking ginger if you're still feeling queasy this month.

Month 4

1. Eating more high-quality proteins, such as eggs, improves your consumption of iron-rich foods.
2. Eat foods strong in iron, such as leafy greens, with every meal and a supply of vitamin C to boost the body's absorption of iron from non-animal sources (lemon juice, capsicum, or pepper).

Month 5

1. Calcium is essential for a baby's bones, teeth, heart, and muscles.

Suitable sources

1. Small fish with palatable bones
2. Tahini
3. Leafy green vegetables
4. Dairy items

Your body cannot store vitamin C, so it's crucial to get it regularly through foods like tomatoes, broccoli, and oranges.

Month 6

1. To avoid constipation, eat whole grains, fruits, vegetables, and legumes.
2. Need 25 to 30 g of fiber daily, about the same as five large apples and 2 cups of lentils.
3. As a home cure for constipation, take 1 tbsp of psyllium dissolved in a glass of water before night to promote a healthy bowel movement the next day.

Month 7

An adequate protein intake is essential for the fetus' development during pregnancy. For a healthy pregnancy, most women require 80 grams or more of protein per day.

Suitable sources

1. Red meat and poultry that is free-range.
2. Natural-raised eggs
3. Unprocessed soy products like tempeh, beans, and peas.
4. Seeds and nuts.

Month 8

The third trimester is when a baby's brain grows and develops quickly, thanks to omega-3 fatty acids.

1. Oily fish, such as salmon and sardines, are good providers of omega-3.
2. Hazelnuts, pecans, and walnuts
3. crushed flax seeds
4. It has been demonstrated that sour cherries encourage sound sleep.
5. Before going to bed, try consuming a glass of unsweetened cherry juice.

Month 9

1. Consuming one clove of garlic every week, garlic has been shown to lower the risk of premature labor.
2. Dates, six dates per day throughout the last four weeks of pregnancy.
3. Eating two handfuls of raisins a week reduces the likelihood of early work [4].

Foods Avoid During Pregnancy

1. Uncooked or raw meats
2. Cold-cured meat slices
3. Raw (sashimi/sushi) fish
4. Creamy cheeses
5. Snack salads
6. The raw egg
7. Fresh produce that hasn't been washed
8. Caffeine Level

Signs and Symptoms

1. Minimal spotting and cramps
2. Period missed
3. Fatigue
4. Nausea

5. Aching or tingling breasts
6. A lot of urinating
7. Bloating
8. Travel sickness
9. Mood changes
10. Temperature variations

Other Signs Include

1. Their blood pressure is high.
2. Profound exhaustion and heartburn
3. Rapid heartbeat
4. Changes to the breasts and nipples Weight gain

Pregnancy Testing Kit

Introduction

The earliest known pregnancy test was developed around 1350 BC and stated, "If the veins in her arm beat against thy hand, thou shalt says he is pregnant." This proves how long medicine has been interested in developing reliable methods to determine whether a woman is pregnant. Hippocrates described a technique to identify pregnancy about 400 BC when he said, "Ingest honey in water: if pregnant, uncomfortable stomach distention will follow.

In the Middle Ages, "piss-prophets" used uroscopy to detect pregnancy. A pregnant woman's urine was supposed to float on top of milk around 1200 AD. Black streaks on an iron needle that had been dipped in a woman's urine were thought to be an indication of pregnancy around 1500 AD. In France in the 19th century, a "cysteine pellicle" material was identified in urine and used to determine pregnancy. The AZ test, the first pregnancy test with a scientific basis, was first described by Ascheim and Zondek in 1928. Between 1929 and 1950, the bioassay and subsequent trials were developed based on the detection of hCG in a pregnant woman's urine. For these studies, weight changes in the uterus, prostate, or seminal vesicles of rabbits, rats, or mice, as well as changes in gamete production in toads, were used. Beginning around 1960, immunological methods were developed to identify hCG and determine pregnancy. The early antibody-based hCG assays used heme or latex agglutination to find immunoactivehCG in a pregnant woman's urine. In 1971 and 1974, the radio immune test for hCG and the radioreceptorhCG assay were first made available. The latest generation of at-home pregnancy tests was introduced in the 1990s. Monoclonal antibodies and visual markers are used in an immunochromatographic manner in these tests. It can identify pregnancy as early as 15 days after ovulation or one day after a missed period, and results are available in just five minutes [5].

Modern Pregnancy Tests

After scientists started injecting urine into mice and rabbits, the first accurate pregnancy test became available in the 1920s. Similar to how it did in humans, the hormone hCG, only found in pregnant women's urine, made a mammal's ovaries expand and produce an egg. The animal is pregnant if it ovulates. By the 1940s, scientists had switched to African clawed frogs from rabbits. Except that frogs lay their eggs externally, the test functioned similarly. Instead of slaughtering and dissecting rabbits for research, ovulation in frogs might be studied. The newest immune chromatographic or lateral flow tests need the woman to dip a test stick in her urine and wait for the results, which usually take a few minutes. If there is any hCG in the blood, it will bind to a specific antibody that is colored labeled and move along a test strip due to capillary action. Complex responsible for a line or positive symbol appearing. This line or cross would not show up without hCG, suggesting a negative result [6].

Human chorionic gonadotropin [hCG] detection is the foundation of pregnancy testing. The purpose of a pregnancy test is to determine if a woman suspected of being pregnant has human chorionic gonadotropin in her urine. It is possible to do biological and immunological tests to determine whether hCG is present in a pregnant woman's urine.

Pregnancy tests are C.D.s that patients use most frequently, whether at home or in a clinical setting. To deliver precise findings in less than 5 minutes, the latest generation of pregnancy tests based on monoclonal antibodies was created in the 1990s. 99% of home pregnancy tests are accurate, on average [7].

The reasons for erroneous findings in home pregnancy tests include testing before there is enough human chorionic gonadotrophin (hCG) in the urine, trouble comprehending product instructions, and difficulties appropriately interpreting test results.

Definition

Pregnancy tests, which determine whether or not a female is pregnant, detect the amount of hCG in the body. Using a pregnancy test kit to look for the female pregnancy hormone, human chorionic gonadotropin (hCG), in blood or urine, and ultrasound imaging are the two basic methods. A blood test for hCG can be used to determine the earliest pregnancy [8].

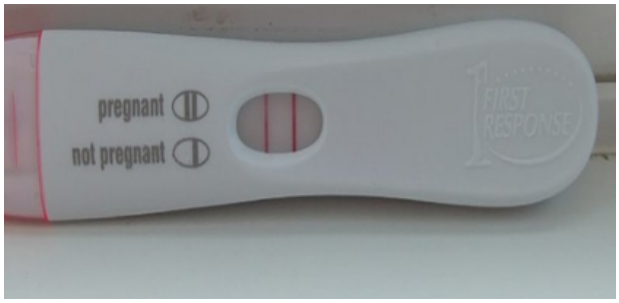

Figure 6: Modern Hormone Pregnancy Test, showing a Positive Result

Pregnancy Testing Methods

Pregnancy testing methods are two types.

Human Chorionic Gonadotropin[hCG]

hCG, a glycoprotein hormone discovered in the early 20th century, rises swiftly in the first few weeks of pregnancy and usually peaks between 8 and 10 weeks gestation. What will become the placenta produces hCG. Urine or blood samples can be used for hCG testing in a medical facility. Generally, the tests used to find hCG in blood or urine are trustworthy and affordable. The earliest hCG can be identified in a blood sample is as early as six days after ovulation and, on average, 8–10 days after ovulation. Blood and urine both have higher concentrations of hCG. Consequently, a urine test may still be harmful even after a positive blood test. A glycoprotein called human chorionic gonadotropin, which has 91 amino acids, comprises two distinct, noncovalently connected subunits. Serum and urine both contain diverse hCG metabolites in different forms. The most important documents include intact hCG nicked intact hCG (where there is a nick in the -polypeptide chain, typically between amino acid positions 40 and 50 from the -subunit's N-terminal end free hCG), and free nicked hCG, WHO International Standards have been produced. Later in pregnancy, free-core, a distinct type detected in urine, becomes the predominant form. As early as 7 to 10 days after conception, hCG can be seen in urine and serum during a healthy pregnancy. After the first missed period, hCG levels frequently reach 100 mIU/mL,2,3,4 continue to increase, culminating at 100,000–200,000 mIU/mL around 10–12 weeks into pregnancy [9].

The rapid rise in hCG concentration during early fetal growth and its subsequent presentation in the urine and serum shortly after conception makes it an ideal diagnostic for early pregnancy identification. The Card Pregnancy Test is a quick test with a sensitivity of 25 mIU/mL that qualitatively identifies the presence of hCG in urine samples [10].

Qualitative Tests

Qualitative tests yield yes/no or positive/negative results based on the presence of the hCG beta subunit in blood or urine. On a qualitative level, the criteria for a positive test are frequently based on an hCG cut-off where at least 95% of pregnant women would have a result on the day of their first missing period. Qualitative urine pregnancy tests have varying degrees of sensitivity. High-sensitivity tests may detect hCG levels between 20 and 50 mill international units/mL (mIU/mL). Low-sensitivity assays can identify hCG groups between 1500 and 2000 mIU/mL and establish the efficacy of a medication-assisted abortion. Qualitative urine tests for home use are commonly made as lateral flow assays [11].

Quantitative Tests

Quantitative analyses determine the precise concentration of hCG in the sample. Blood tests can identify hCG levels as low as one mIU/mL, and doctors routinely declare a pregnancy test positive at five mIU/mL [12].

Urine Pregnancy Test

One of the most widely used methods of determining pregnancy is the urine pregnancy test. It operates by determining hCG concentrations in the urine. Home pregnancy tests are urine examinations that can be carried out in solitude. Today's market is filled with a range of urine pregnancy tests. The sensitivity of each test varies, with some urine tests able to identify hCG levels as low as 15 ml/u.You typically deposit one to several drops of pee on a chemical test strip that has been prepared or insert the strip into the urine stream when performing an at-home pregnancy test. The ribbon is made specifically to find hCG.For several of these tests, hCG can be detected in your urine around ten days after conception. However, taking it after your missing period reduces the risk of a false-negative test [13].

Performing a Home Pregnancy Urine Test

Although the process differs from test to test, performing a pregnancy test at home is simple. Before using the exam, make sure to read the instructions that come with it. Additionally, make sure the expiration date hasn't already passed. Most urine tests include a diagnostic strip that may identify hCG when exposed to urine. Although you should always read the instructions on the container, the following guidelines usually work well for home pregnancy urine tests:

1. Take the test strip out of its packaging.
2. Dip the test strip into a cup of urine or insert it into your urine stream.

3. Permit the given time to pass.

The test strip should have changed colors or shown a pregnancy indicator if you are pregnant.

Mechanism of Pregnancy Tests

It operates on the immune chromatography tenet. The sandwich ELISA technique and the immunochromatographic test share the same fundamental principles. The sole distinction is that capillary action causes an immunological reaction on the chromatographic paper. This technique applies two types of targeted antibodies to the antigen [14]. A different antibody is labeled with colloidal gold and inserted into a sample pad, while one of the antibodies is fixed on the chromatographic paper. The sample pad is linked to the membrane's end to complete an immunochromatographic unit. On the sample pad, the liquid sample is dropped. The sample's antigen forms an immunocomplex with the colloidally gold-labeled antibody. When an antibody is immobilized on a membrane, its complex comes into touch with it as it travels with the liquid sample. Next, the antibody that has been immobilized forms an immuno-complex with it. This produces a red-purple line that is colored. The membrane will display a reddish-purple line if an attractive antigen is present [15].

Due to the urine's rapid membrane migration, it is possible to determine if the hCG antigen is present or absent within 15 minutes.

Figure 7: Week of Pregnancy vs hCG Concentration

Components of pregnancy testing kit device: The immunoassay strip/pad and the housing are the two main parts of a home pregnancy test.

A monoclonal antibody (MAb), a protein that reacts with any hCG found in the urine, is coated on the immunoassay strip. If the hCG level is consistent with recognized pregnancy levels, this reaction changes color.

Immunoassay Strip

Nonwoven fibers are compressed into a thin strip and coated with reactive antibodies to create the immunoassay strip. A color change results from the antibodies' interactions with the pregnancy hormone.

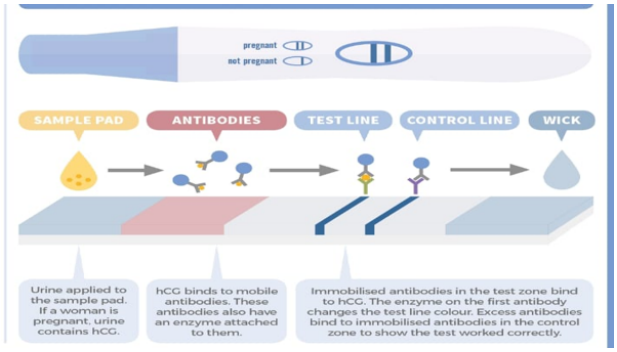

Figure 8: Mechanism of Urine Pregnancy Test

Absorbent Pad

The absorbent pad used to contact the urine stream in the direct application type of test extends from the test chamber. The immunoassay strip touches the liquid after the place has absorbed it.

Plastic Housing

The test strip and the absorbent pad are housed in a two-piece container that shields them from environmental toxins and makes the device portable.

The test and control zones of the strip may be seen through a leak-proof, clear plastic glass on the side of the housing, which also prevents urine from accidentally splashing over the test strip [16].

Urine Collection Cup/Vials and Reagents

A plastic collection cup includes test kits that need separate urine collection. Additionally, they could come with plastic vials containing pre-measured reagent amounts that must be mixed with the urine before smearing on the test strip.

Control: It includes antibodies that detect free latex or colloidal gold (goat polyclonal antibodies) to ensure the test was successful.

Test: It has a particular capture molecule that only binds to particles with an analyte (antigen or antibody molecule) fixed [17].

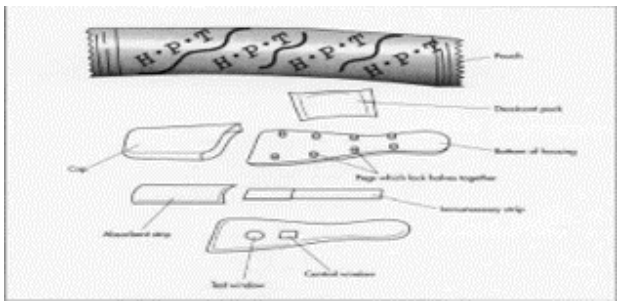

Figure 9: Components of the Testing Kit

Specimen for Urine Pregnancy Test

Any random urine sample is taken and placed in a clean, dry, and detergent-free area. At least 12 days must pass following the first missing menstrual period before it can be collected. If the test is repeated after a week and the findings are negative.

The procedure of the Urine Pregnancy Test

1. Test kits can be kept at room temperature for storage.
2. Take the test equipment out of the pouch and use it immediately.
3. Position the test instrument on a smooth, clean surface.
4. Start the timer while transferring three complete drops of urine to the specimen using the dropper held vertically. Avoid creating air bubbles.
5. Watch for the band to start playing.
6. Review the outcome in 15 minutes.

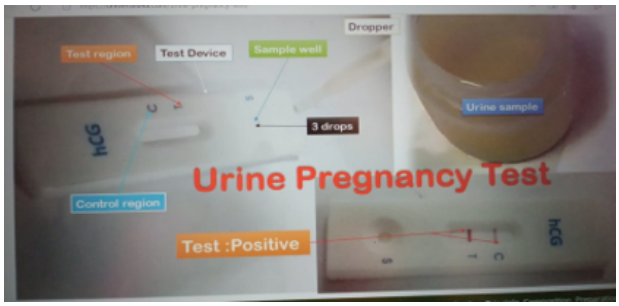

Figure 10: Procedure of Urine Pregnancy Test

Result Interpretation of Urine Pregnancy Test

1. Only the band at the control region tested negative.
2. Both bands in the test (T) control regions are positive.
3. Test invalid if there is no band or a band in the test zone [18].

Blood Pregnancy Test

Pregnancy blood tests are carried out in a medical facility or at your doctor's office. They measure the hCG levels in your blood to identify pregnancy. There are two varieties of blood tests for pregnancy:

Quantitative Blood Test

The quantitative blood test measures the precise concentration of hCG in your bloodstream to identify

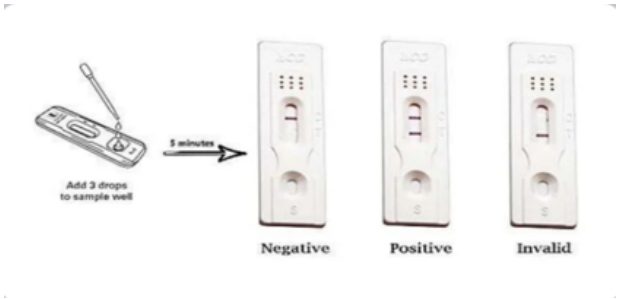

Figure 11: Result Interpretation of Urine Tests

pregnancy. Additionally, it can be used to determine how far along in your pregnancy you are [19].

Qualitative Blood Test

The qualitative blood test identifies the presence of hCG in your blood as a sign that you are pregnant. It merely offers a yes-or-no response as to whether you are pregnant and does not measure your hCG levels [20].

Conducted a Blood Test

1. Professionals in the medical field conduct blood pregnancy tests in a clinic or office setting. It is very similar to a quick blood test.
2. Because they are expensive and frequently yield the same results as a urine test, blood tests are not commonly performed. A little blood sample is used for this pregnancy test, which is then examined at a hospital or doctor's office.
3. This blood test can identify the number of pregnancy hormones in your body and detect whether it is there.
4. In rare cases, such as for women undergoing infertility treatments or when the healthcare provider suspects there may be an issue, a blood test for pregnancy may be performed.
5. For this test, a sample of your blood is taken and sent to a lab for analysis. These blood tests are more sensitive than urine tests because they may pick up on insufficient quantities of hCG. That suggests that they can respond more precisely nine to twelve days after fertilization, which is very early in pregnancy.
6. Results could appear in as little as a few hours or as long as a day [21].

Your healthcare professional may also use a blood test to compare the levels of hCG throughout the pregnancy. During the first several weeks of pregnancy, your hCG levels typically double every two days. The levels can indicate a problem with the

pregnancy if they don't increase. Extremely high hCG levels could indicate twin gestation or a problem with the pregnant woman.

Procedure

Conducted a blood test

Professionals in the medical field conduct blood pregnancy tests in a clinic or office setting. It is very similar to a quick blood test.

1. Alcohol will be used to clean a place on your arm.
2. Your arm gets pricked with a needle.
3. The needle draws blood into a little tube.
4. The tube is delivered to a lab where it will be examined. Results of pregnancy tests usually take one or two days [22].

Accuracy

When used by knowledgeable technicians, home pregnancy test kits are nearly as accurate (97.4%) as professional laboratory testing, according to a systematic evaluation that was published in 1998. Consumer use, however, reduced the accuracy to 75% since many customers either misinterpreted or ignored the kits' instructions, according to the reviewers [23].

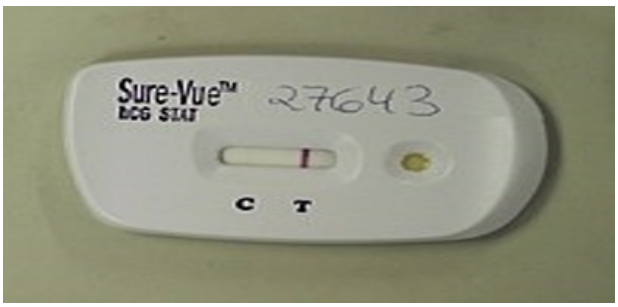

Figure 12: The Control Line of this Pregnancy Test is Blank, Making the Test Invalid

False Positive

Pregnancy test results that are falsely positive are uncommon and can happen for several reasons, including:

Improper test administration and user interpretation Biochemical conception and non-pregnant production of the hCG molecule can occur in certain liver diseases, cancers like choriocarcinoma and other germ cell tumors, IgA deficiencies, heterophile antibodies, enterocystoplasties, gestational trophoblastic diseases (GTD), and gestational

Figure 13: This Pregnancy Test Displays the Control Line on the Left, Demonstrating the Accuracy of the Results, on the Right Side, a Gray-Purple Line, Inaddition to the Test Line, Indicates the Patient is Pregnant

trophoblastic neoplasms, as well as other conditions. Germs and blood in the urine Even if a pregnancy is present, incorrect evaporation lines may appear if a home pregnancy test is examined outside the recommended 3-5 minute window or reaction time. False positive results on tests that have been used after expired are also possible.

'Phantom hCG,' caused by people with human anti-animal or heterophilic antibodies, can result in false positive pregnancy tests. In addition, malignancy, quiescent pregnancy, pituitary sulfated hCG, familial hCG syndrome, and pituitary sulfated hCG can all result in false positives. Asa result of pharmaceutical use, Those who use the risks of the following medication have falsely positive urine tests: chlorpromazine, promethazine, phenothiazines, methadone, aspirin, carbamazepine, and medicines that increase urinary pH [24].

False-Negative

Inadequate testing can result in erroneous negative results. Early in pregnancy, hCG levels rise quickly, and the possibility of false negative test results reduces as the pregnancy progresses [25]. Less sensitive urine tests and qualitative blood tests may not be able to detect pregnancy three to four days after implantation. There is little probability that a late menstrual cycle may lead to a false negative because menstruation typically starts 14 days after ovulation. It's possible that ovulation won't happen when it should in the menstrual cycle. Even in people with a history of regular menstrual cycles, several factors might cause unexpectedly early or late ovulation. False negative results are most frequently caused by the "hook effect," which occurs when a very high hCG concentration is tested without dilution and yields an inaccurate result [26].

The hCG levels are measured semi-quantitatively using a multilayer urine pregnancy test (MLPT). This test can help determine whether a pharmaceutical

abortion was successful. The hCG levels are measured between 25 and 99, 100 and 499, 500 and 1999, 2000 and 9999, and above 10,000 mIU/mL.

Efficacy of Urine vs. Blood Pregnancy Tests

There is a need for blood pregnancy tests because of the complications. Since urine tests are inexpensive, painless, easy to use, and can be performed in the privacy of one's home, they are typically employed for most pregnancies. These tests are also accurate and give doctors speedy results with more specific pregnancy information [27].

Ultrasonography

Pregnancy detection and diagnosis are also possible uses for obstetric ultrasonography. Before getting an ultrasound, a positive urine pregnancy test at home is rather typical. Abdominal and vaginal ultrasounds can be performed, although the vaginal version enables earlier pregnancy visibility [28]. Obstetric ultrasound can detect a fetal pole between 5.5 and 6 weeks of gestation, the yolk sac between 5 and 6 weeks, and the gestational sac (a collection of intrauterine fluid) between 4.5 and 5 weeks. Utilizing ultrasound, multiple gestations can be identified [29].

1. A piezoelectric crystal transducer produces high-frequency U.T. wave pulses.
2. The ultrasound screen shows the echos, or reflected waves, from distinct uterine regions.
3. It is the most accurate way of pregnancy detection.
4. It is possible to see the gestational ring as early as the fifth week of pregnancy.
5. Heartbeat by the tenth week and fetal movement by the eleventh [30].

Examples of Home Pregnancy Testing Kits Brands

1. I-can one-step pregnancy test device
2. Get news hCG pregnancy test kit
3. Prega news hCG pregnancy test kit
4. CiplaMamaxpert Rapid pregnancy detection kit
5. Ovlo plus pregnancy test kit
6. Accurate advanced Hcg pregnancy test kit
7. Plush preg-oh! Pregnancy kit test
8. Pergamon test kit
9. IS IT a Pregnancy test kit
10. Medrol pregnancy urine test strips [31].

CONCLUSION

Home pregnancy tests are the most popular diagnostic tests used at home and in a clinical environment. False negative or positive results could have significant repercussions, such as a young woman becoming pregnant without her consent. This is crucial for medical practitioners since they must be aware of the diagnostic potential, accuracy, and potential limitations of home pregnancy test to counsel patients appropriately. In the USA, stringent standards and definitions are in place to guarantee that all commercially available tests function satisfactorily. Assessment is carried out in Germany and other European nations by a notified organisation that has been granted accreditation by the relevant authorities of E.U. member states. In order to issue C.E. marking in line with the New Approach directives, notified organisations are capable of completing conformity assessments; however, these assessments are not based on standard definitions. Therefore, it would be especially appreciated if a set of standard definitions and testing standards were defined.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Review Article

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Review on Food Analysis by Using Gas Chromatography

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Abstract

Explains gas chromatography (G.C.), a crucial analytical method used in the food business. It makes it possible to swiftly and affordably separate and identify complicated organic compounds. The substances that G.C. will analyse must quickly move into the gas phase and be volatile. An inert carrier gas vaporizes the substance to be examined and transports it through a substantial column. A packing material wrapped in an involatile liquid fills the column. In a mixture, the molecules of each material are divided between the gas and the liquid. A substance will move with the carrier gas longer and escape from the column more quickly the more volatile it is. G.C. must remove certain chemicals from the food analysis.

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INTRODUCTION

Chromatography is the more precise term for color writing. It is a physical process of separation wherein a mixture of substances can be isolated, separated, and purified into various molecules that depend on multiple distribution rates depending on solubility, Affinity, and Interaction. Chromatography is a very efficient technique that separates ingredients' constituent parts according to their properties, structure, size, and other factors. To physically separate components, chromatography uses two stages: a stable phase and a moving phase travels by way of it in a specific direction (called a mobile phase).

Stationary Phase

The stationary phase is always made up of a layer of liquid that has been adsorbed onto the outside of a sturdy endorsement [1].

e.g: Glass, Silica, Alumina

Mobile Phase

A liquid or a gaseous component is always present in this phase.

e.g., N-Hexane, Petroleum ether, Cyclo hexane

Gas Chromatography

Vapor phases Other names of gas chromatography include chromatography (VPC) and gas-liquid partition chromatography (GLPC). Gas chromatography also referred to as fluid chromatography, is a method of sequentially separating components through partitioning stationary and mobile phases within a column. It segregates, organizes, and differentiates members in organic chemical mixtures. This approach is practical in separating compounds with high volatility, Thermal stability, and low molecular weights [2].

History of Gas Chromatography

Both A.J.P. Martin and R.L.M. Synge received Nobel awards in 1952 for their contributions to liquid/soil

chromatography. Martin proposed using vapor as the mobile phase in his award speech.

James and Martins utilized ethyl acetate years ago vapor, a combination with triglycerides which have been absorbed for dissipate attached to an absorbent and placed in a tube. The vapor steam eluting from such a capillary was passed through a mechanical titration apparatus, producing a graph depicting a set of procedures reflecting its progressive insertion of bases when they were individually drained were being used to neutralize through automated titration [3].

In the late 1940s, during World War II, Austrian chemist Erika Cremer developed gas chromatography just at universities in Innsbruck, a time when women, particularly in German-speaking nations, were anticipated to limit their activities to raising children, going to church, and working within the kitchen, according to a 2008 article by Leslie Meter. According to all reports, a bright female scientist, Professor Dr. Cremer, passed away in 1996.

Principles of Gas Chromatography

There are two phases towards the mishmash: a fixed phase and a mobile phase, sometimes known as this mixture, through to the stationary phase using only a carrier gas. Molecules in the mobile phase move through to the stationary phase and interact. A magnitude but every interaction's resonance towards the stationary phase change because of each component's attributes and structural peculiarities. As a result, depending on the driving force, Various elements possess varying columns of residence time and exit it in different sequences [4].

Instrumentation of Gas Chromatography

Components of Gas Chromatography

1. Mobile phase
2. Sample injector
3. The separation column
4. Supports
5. Detectors
6. Recorder [Figure 1].

Mobile Phase

Typically, three different Gas chromatography make use of various categories of gas:

Carrier gas: Transferring the injected sample to the separation column requires carrier gas. Furthermore, these are still in charge of transporting separated components toward the detectors after that. E.g : Helium, Hydrogen, Nitrogen

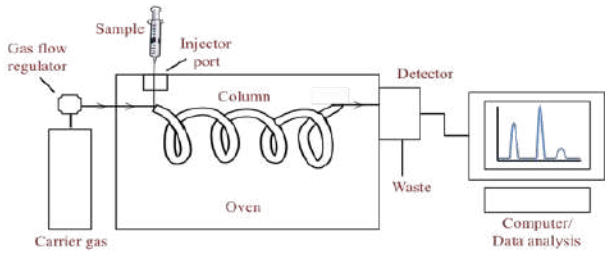

Figure 1: Schematic Diagram of Gas Chromatography

Fuel gas: They support the flame in a hydrogen-based flame ionization detector (FID).

Zero air: This is cleaned air that supports the combustion of the flame in the detector by acting as an oxidant. The abovementioned three are blended in the desired ratio before being introduced to the gas chromatographic apparatus [5].

Sample Injector

In gas chromatography, the sample that needs to be analyzed is injected using a sample injector. A representative has been infused utilizing such a calibrated syringe. A sampler and mobile phase should be in the same physical condition because they must travel together. Since the mobile phase in gas chromatography has been in a gaseous state, a sample must also be in a gaseous condition. A heater built inside the sample injector enables the vaporization of liquid samples. If the model is solid, it is crushed, ground, and transformed into a liquid state [6].

The Column of Separation

A metal column made up of bent metallic into a U shape, wound into an open spiral, or shaped like this fundamental basis in gas chromatography is just a plain pancake. Metals can be utilized up to 2500. Column insertion is made simple using Swege lock fittings, and several sizes of columns are employed based on the needs. Liquid phase: The only liquid-phase restrictions are their thermal stability, moisture-holding capability, and brittleness. Fluid steps No single phase could overcome the separating difficulties at every temperature. Silicone crude oils and apiezon L were nonpolar along with paraffin, squalane, and silicone gum rubber. Such compounds categorize all components according to the sequence in which they boil. Transitional Polarity: Such compounds have a polarity and polarizable component attached to such a protracted nonpolar skeleton, permitting these to absorb either nonpolar or polar solutes. A case study. Diethyl hexyl phthalate is applied to differentiate higher the boiling alcohols. Polarized carbowaxes were liquids phase composed primarily of polar functional groups. Molecules were divided into nonpolar and

opposing groups. Opposite liquid steps with strong hydrogen bonds, like glycol, are those having hydrogen bonds. A chemical reaction with a solute is used in specific purpose phases to achieve separations. Unsaturated hydrocarbons are separated, for instance, by AgNO3 in glycol [7].

Supports

An efficacy established is the level of assistance and the degrees of partitioning, respectively, by the structure and surface properties like such supportive components. Support should remain inert while having the ability to create a thin film that can immobilize a significant amount of liquid phase on its surface.

When diatomaceous earth is heated to 9000 C and treated with Na 2CO3, the particle fusion results in coarser aggregates. The support should be able to be packed into a uniform bed and should be sturdy enough to resist disintegration during handling. Up to 3% of stationary phases can be coated with glass beads with reduced permeability and a small surface area. Furthermore, the use of cross-linked polymeric pellets with varying degrees or sturdiness of styrene-alkyl-vinyl-benzene is employed. Examples: Glass, Silica, Alumina.

Detector

Detectors, which might be concentration-dependent or mass-dependent, detect the individual elements' arrival and emit a signal. The sensors should be near the column's outflow and at a suitable temperature to prevent decomposing. Crucial properties of chromatographic detectors are High reliability, Sensitivity, Good range of temperatures, Preferably Non-destructive, Small peak value to avert peak broadening, Low noise, Linear range, a short reaction period as well as a rate of flow that seem to be independent. A detector interacts well with solvents when they elute from the column. The sensor transforms this interaction into an electronic signal and transmits it to the data system. A chromatogram is produced after the signal's strength is plotted against time (starting from the injection time). While some detectors react to any solution that elutes through such a column, others exclusively react to solutes with particular structures, functional groups, or atoms. Selective sensors respond more favorably to a given class of solute. Most detectors need one or more gases to operate perfectly; ancillary, makeup, reagents, as well as products of combustion are all present. One gas may occasionally have numerous uses. The kind of detector gas depends on the particular detector but is also very standard among G.C. manufacturers. Each type of detector has a different flow rate depending on the G.C. manufacturer. It's crucial to adhere to the advised flow rates to get a detector's optimum sensitivity, selectivity, and linear range [8].

Types of Detectors Used in Gas chromatography

1. Flame ionization detector
2. Nitrogen phosphorus detector
3. Electron capture detector
4. Thermal conductivity detector
5. Flame photometric detector
6. Photoionization detector
7. Electrolytic conductivity detector
8. Mass spectrometer

Flame Ionization Detector

The most popular detector in gas chromatography is the FID. Since practically all organic molecules contain carbon atoms (C), the FID is sensitive to and capable of identifying them. However, double-bonded carbon atoms by oxygenation, such as those in the carboxyl and carbonyl groups, are insensitive to the FID (C.O., CO2, HCHO, HCOOH, CS2, CCl4, etc.)

Mechanism

Chemicals combust inside an amount of hydrogen as such process. Atoms generated via substances that contain co2 were taken towards the collection. A signal has been developed by measuring those electrons, which achieve collectors [9].

Selectivity: C-H bonding compounds. A subpar response for some organic materials without hydrogen.

Sensitivity: 0.1-10 ng

Linear range: 10^5-10^7

Gases: Hydrogen as well as oxygen as combustion; helium or nitrogen for makeup

Temperature: 250–300°C; for high thermal findings, 400–450°C.

The FID produces a hydrogen flame by burning air and hydrogen supplied from below. The hydrogen flame oxidizes the carbon in a sample that was brought into the detector on a carrier gas, which results in an ionization process.

A collector electrode draws the generated ions into an electric field, where the constituents are then measured [10].

Applications: Organic compound analysis.

Nitrogen Phosphorus Detector (NDP)

Mechanism

Chemicals burn within a platelet that is powered via hydrocarbons and air surrounding a rubidium bead. Components possessing phosphorous but also nitrogen create ions that are drawn to the collector. A signal is produced, and the number of ions that hit the collector is counted.

Selectivity: Nitrogen as well as phosphorous consisting compounds

Sensitivity: 1-10 pg

Linear range: 10^{4}-10-^{6}

Gases: Combustion uses hydrogen and air, whereas helium is utilized for makeup.

Temperature: 250-300°C

Electron Capture Detector (ECD)

A highly sensitive, selective detector for electrophilic chemicals is the ECD. Metal-organic compounds, diketone cannabinoids, natural halogen cannabinoids, etc., can all be detected by the ECD. Education, Culture, Sports, Science, and Technological Ministries in Japan must receive notification of usage since the ECD is equipped with a radioactive isotope [11].

The ECD employs the following detecting principle. The voltage value change that keeps the collected ion current at collectors constant is what the ECD uses to detect ions.

Mechanism

Each detector's cell's 63Ni alloy acts as a source of the electron. Electricity gets generated within the cells. Electronegative compounds' capability to capture electrons leads to a decrease in electricity. During indirect indicators of electrical losses, a signal is generated.

Selectivity: Nitrates, conjugated carbonyls, as well as halogens

Sensitivity: 1-100 pg (nitrogen), 0.1–10 pg (halogenated substances), but also 0.1–1 ng (carbonyls)

Linear range: 10^{3}-10^{4}

Gases: Nitrogen or argon/methane

Temperature: 300-400°C.

Applications

1. Environment analysis
2. Environmental organic mercury
3. Chlorinated VOCs in discharge water
4. Residual PCBs and chlorinated insecticides

Thermal Conductivity Detectors (TCD)

Except for the carrier gas, all chemicals are detectable by the TCD. Inorganic gases and other components that the FID is not sensitive to are mainly detected using the TCD [12].

The gas helium is frequently employed as a carrier. (He and H2 are examined using N2 and Ar.)

Mechanism

An applied current heats a filament inside a detector cell. The filament current alters as carrier gas-carrying solutes move through the cell. A reference cell's current and the current change are compared. A signal is produced once the difference is measured.

Selectivity: every combination, excluding the carrier gas

Sensitivity: 5-20 ng

Linear range: 10^{5}-10^{6}

Gases: Makeup - same as the carrier gas

Temperature: 150-250°C

Applications: Water, formaldehyde, formic acid, etc.

Flame Photometric Detectors (FPD)

The FPD is a sensitive, selective detector for organic tin (Sn), sulfur, and phosphorus (P) molecules. The FPD is exceptionally demanding as it only picks up element-specific light generated within a hydrogen flame [13].

Mechanism

Compounds burn in a hydrogen-air flame as the mechanism. Light-emitting species are produced by chemicals that contain sulfur and phosphorus (sulfur at 394 nm and phosphorous at 526 nm). Only one of the wavelengths can pass through a monochromatic filter. A signal is produced after measuring the amount of light with a photomultiplier tube. Each detection mode requires a distinct filter.

Selectivity: Compounds containing phosphorus or sulfur. One at a time only.

Sensitivity: 10-100 pg (sulfur); 1-10 pg (phosphorous)

Linear range: Non-linear (sulfur); 10^{3}-10^{5} (phosphorous)

Gases: Combustion - hydrogen and air; Makeup - nitrogen

Temperature: 250-300°C [14].

Food

Definition

An essential element that the body must have and also be healthy is food. Proper nutrition is crucial for overall well-being all through lifetime and good development throughout infancy, childhood, adolescence, and adulthood [15]. The human diet is not limited to any particular type of food. Man consumes a range of plant and animal-based foods because no single item can satisfy all of our nutritional needs. The adage "We are what we eat" is accurate. Of course, most of us do not turn into bananas if we eat a banana, but whether for good or bad, our bodies must assimilate, modify, and excrete the chemicals we ingest. Food is a necessary component of life, and access to it frequently determines the size of a population. There is no doubting the significance of food, even while there is some debate among friends over whether we "eat to live" or "live to eat" (and some individuals "are dying to eat" or "eat themselves to death"). Chemical analysis is the only way to determine which chemicals are present in food and how many there are. The nutritional requirements for the various substances or their impacts on health can then be determined. Chromatography, in particular, has been crucial for separating numerous organic compounds in food. Analytical chemistry, in general, has played a significant role in human development through its ability to identify and quantify components in food [Table 1] [Figure 2] [16].

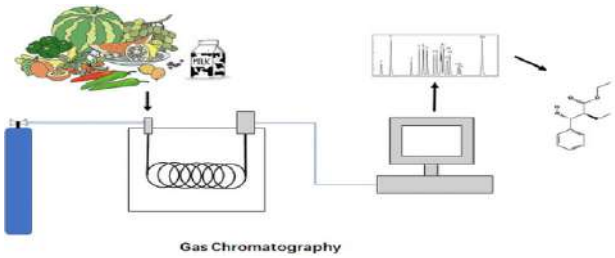

Figure 2: Food Analysis by Using Gas Chromatography

Need for Food Analysis

Food analysis is done to verify the nutritional value and safety of food. Foods and raw materials shouldn't include any toxic ingredients or microbe concentrations that are unsafe. Eating out and purchasing food from the grocery store should be safe. An approved laboratory must analyze the food producers under official food control. Accredited laboratories also carry out numerous additional kinds of food analysis.

Meeting Government and Industry Regulations

Government and business organizations impose strict regulations on the food and beverage industry. For instance, peanut butter in the USA must include at least 90% peanuts, a total fat level of no more than 55%, and a maximum of 10% other components like salt and sugar [17]. Everyone can meet and maintain these requirements with the help of food analysis, from product makers to ingredient suppliers.

Protecting Consumers

Consumer safety is a top priority in the food and beverage industry. Before they are placed on store shelves for customer purchase, analytical procedures are employed to screen materials and completed goods. For instance, foodborne bacteria and viruses can be found in items like lettuce using polymerase chain reaction (PCR) assays. Toxins left behind by pesticides and herbicides, as well as traces of metal, wood, glass, and other pollutants, are also found using laboratory tests.

Preventing Food Fraud

Food fraud is a global problem that impacts customers, product makers, and ingredient suppliers. One of the most well-known instances of food fraud occurred in 2013 when it was discovered that frozen ready meals and burgers purportedly made with beef included horse DNA.

Nutritional Labeling

Consumers can get detailed information about the nature and composition of food through nutritional labeling.

Most nations mandate that food producers display uniform nutritional labels that include information on fat, cholesterol, carbs, salt, sugars, and protein. Consumers can make informed decisions using this information.

Consistency and Quality Control

While consistency can differ for small-batch items, large-scale producers place a high premium on keeping the same general features.

Regular testing enables manufacturers to match consumer expectations, eventually boosting revenues and expanding their market share.

Testing is done to keep track of everything from starch and sugar content to flavor and texture, which might vary in products like potatoes.

Research and Development

Food analysis is essential to corporate research and development. For instance, the American business Beyond Meat uses food analysis methods to create novel plant-based beef products.

The company has become the market leader and formed alliances with well-known companies like KFC, Pizza Hut, and Panda Express [18].

Table 1: Chromatographic Techniques Used for Food Analysis

Components of Food	The Chromatographic Method Used for the Analysis
Carbohydrates	High-performance liquid chromatography (HPLC) Gas chromatography (G.C.) Thin layer chromatography (TLC)
Proteins	Column chromatography High-performance liquid chromatography (HPLC)
Fats	High-performance liquid chromatography (HPLC) Gas chromatography (G.C.) Thin layer chromatography (TLC)
Vitamins & Minerals	High-performance liquid chromatography (HPLC) Gas chromatography (G.C.)
Dietary fibers	High-performance liquid chromatography (HPLC)

Sample Preparation for Gas Chromatography

Generally, a food product cannot be injected straight into a G.C. without undergoing sample preparation. Degradation of non-volatile contents will occur due to the injection port's high temperatures, and several erroneous G.C. peaks will be produced corresponding to the resulting volatile degradation products.

Additionally, it is frequently necessary to isolate the target ingredient from the food matrix to allow concentration to G.C. detectable limits or to segregate it from most food.

As a result, sample pretreatment, component isolation, and concentration are usually required before G.C. analysis. Grinding, homogenization, or other methods of reducing particle size are frequently used in sample preparation.

Many foods have active enzyme systems that change the food product's composition. This is particularly clear in the field of flavor work.

It may be necessary to inactivate enzyme systems using high temperatures, fast thermal processing, sample storage under freezing conditions, sample drying, or homogenization with alcohol.

During the production of the sample, the food may experience microbial growth or chemical reactions. Certain chemicals (such as sodium fluoride), thermal processing, drying, or freezing storage frequently suppress microorganisms [19].

False peaks on the G.C. often occur from chemical interactions. As a result, the model needs to be kept in settings that prevent degradation.

Analysis of Carbohydrates

1. Carbohydrates are a crucial source of energy metabolism for animals and plants that depend upon plants for food.
2. In addition to serving as a crucial component of nourishment, sugars and starches also act as a structural substance (cellulose), one of the three essential elements in RNA and DNA, a constituent of such power component ATP, recognition sites onto cell surface membrane. Saccharides are another name for carbohydrates, or sugars, if they are tiny.
3. Like HPLC, G.C. (gas-liquid chromatography, or GLC) offers a qualitative and quantitative examination of carbohydrates.
4. Sugars must be transformed into volatile derivatives for G.C. The alditol peracetates are the derivatives that are utilized the most frequently. These derivatives are made in the manner described in the D galactose illustration.
5. The most popular type of detector for hyperacetylated carbohydrate derivatives is a flame ionization detector (FID), but mass spectrometers are increasingly being employed.
6. The detection limits are lowered using a mass spectrometric (M.S.) detector and further reduced by an MS/MS detector.
7. The origins and adulterations of food and additives have been identified using gas chromatography combustion-isotope ratio mass spectrometry.
8. The two preparation steps required for G.C. for carbohydrate analysis—reducing aldehydic groups to primary hydroxyl groups and converting reduced sugars (alditol) into volatile peracetate esters—pose the biggest challenge. Of course, each of these steps must be 100% finished for the analysis to be successful.

Neutral Sugars

A surplus of sodium or potassium borohydride dissolved in Balanced diluted glucose from 80% ethanolic extracts or hydrolysis of such polysaccharides over 40 °C was decreased by utilizing ammonia hypochlorite. Inclusion of glacial acetic acid after the process to remove any extra borohydride. The acidified solution is dried by evaporation. There may be an issue: Fructose will be converted to a mixture of d-glucitol (sorbitol) and d-mannitol if it is available, whether it is a naturally occurring sugar, the result of the hydrolysis Reduction to Alditolsof inulin, or an additive [from a high-fructose syrup (HFS), invert sugar, or honey].

Acetylation of Alditols

A dry mixture of alditols is mixed with acetic anhydride and a catalyst. About 10 min at room temperature, add water, then dichloromethane. The alditolperacetate residue is dissolved in a polar organic solvent (often acetone) for chromatography. Following mixing, the dichloromethane layer is cleaned with water before being dried by evaporation.

GC of Alditol Peracetates

If inositol is added as an internal standard before acetylation, alditol peracetates can be chromatographed isothermally and recognized by their retention durations compared to inositol hexaacetate. To assess elution durations and relative responses, it is crucial to run standards of the alditol peracetates of the sugars being measured with inositol hexaacetate as an internal standard [20].

Fatty Acid Composition and Fatty Acid Methyl Esters (FAMEs)

By measuring the types and amounts of fatty acids present, typically, The rich acid concentration, or fatty acid profile, of such packaged foods could be determined by separating those triglycerides as well as evaluating those using capillary G.C. can be established.

Principle

Triacylglycerols are often transesterified to create fatty acid methyl esters to boost volatility before G.C. analysis. Acyl lipids are easily transesterified using a base like sodium hydroxide or methanol. This mixture's sodium methoxide will quickly convert acyl lipids into FAMEs but won't interact with free fatty acids. Acidic substances like methanolic HCL or boron trifluoride react with FFAs quickly but with acyl lipids more slowly. Two-step methylation is used in procedures like the AOCS Method Ce 1b-89, which involves treating the lipid with 0.5 N NaOH and too much BF3/methanol. FFAs, acyl lipids, and phospholipids can all be quickly methylated because of this. The stage using sodium hydroxide is not a saponification process. Direct transmethylation is what it is [21].

Procedure

By homogenizing the meal with a suitable solvent, such as hexane-isopropanol (3:2, vol/vol), and then letting the solvent evaporate, the lipid can be extracted from the food, for instance. The extracted lipid is mixed with sodium hydroxide methanol, an internal standard in isooctane, and then heated at 100 °C for five minutes to create the FAMEs. After the sample has cooled, further BF3-methanol is added, and the mixture is heated at 100 °C for 30 minutes. The upper isooctane solution containing the FAMEs is withdrawn, dried with anhydrous Na2SO4, and then diluted to a concentration of 5–10% for injection onto the G.C. This is done after adding saturated aqueous sodium chloride, more isooctane, and mixing. Several techniques outline steps and circumstances for using G.C. to determine the composition of fatty acids. AOCS Method Ce 1f-96 is most suited for determining trans-isomer fatty acids, while AOCS Method Ce 1b-89 specializes in marine oils.

Cholesterol and Phytosterols

There are numerous techniques for measuring cholesterol and phytosterols in diverse matrices. A review of the scientific literature will provide information on existing procedures and methods that may be simplified or tailored for usage with certain foods.

Principle

It is saponified to remove the fat from the food. Acyl lipids are hydrolyzed during the saponification process to produce water-soluble FFA salts. Following hydrolysis, specific components (unsaponifiable or nonsaponifiable matter) remain soluble in organic solvents because their solubility does not change. Trimethylsilyl (TMS) ethers or acetate esters are created by extracting and derivatizing cholesterol (from the unsaponifiable fraction). This raises their volatility and lessens chromatographic peak tailing issues. Capillary G.C. is used to accomplish quantification.

Procedure

The food's lipids are taken out, saponified, and the extractable portion is taken out. To do this, a part of the chloroform layer is filtered through anhydrous sodium sulfate and dried in a water bath with a stream of nitrogen gas. The mixture is then refluxed while concentrated potassium hydroxide

and ethanol are added. Shaking is done after adding aliquots of benzene and 1 N potassium hydroxide. After removing the aqueous layer, the procedure is repeated using 0.5 N potassium hydroxide. The benzene layer is dried with anhydrous sodium sulfate, and an aliquot is evaporated to dryness on a rotary evaporator after being repeatedly washed with water. The leftovers are absorbed by dimethylformamide. Trimethylchlorosilane and hexamethyldisilazane (HMDS) are added to an aliquot of this sample to be derivatized. After including water (to react with and deactivate excess reagent) and an internal standard in heptane, the mixture is centrifuged. A GC with a nonpolar column receives a partial injection of the heptane layer. The HMDS and TMCS reagents are quickly inactivated by water. Hence the reaction environment must remain anhydrous [22].

Measurement of Vitamin D

Like steroids, vitamin D and its metabolites are relatively non-volatile substances that require G.C. oven temperatures of 200–3500 to separate. When one considers their shared chemical basis, it may not be a surprise that there are numerous similarities between the issues related to the G.C. analysis of steroid hormones and vitamin D. Both categories of steroids can be analyzed by G.C. without the creation of derivatives, even though there is significant peak broadening, which is likely due to adsorption into the column's "inert" support. The selection of suitable products can improve peak shape and separation. Because C2l-corticosteroids with an l7-hydroxyl group are thermally unstable, high oven temperatures can cause side chain cleavage, which results in the formation of C19 steroids unless appropriate derivatives are generated before G.C. Vitamin D and its metabolites also experience temperature changes G.C. The B ring is closed when vitamin D is injected into a G.C. column, resulting in the formation of the isomers pyro- (9acH3' loan) and is pyro- (9SCH3, 10SH) calciferous.

Two peaks are produced when a single vitamin D metabolite is added to a G.C. column; these peaks are typically created in a constant ratio. At all temperatures over 25 °C, similar cyclization occurs in the test tube, and the balance of the isomers to one another remains constant. When a "profile" is required, those interested in G.C. prefer to use methods in which a single compound results in a single peak. A single molecule can produce many mountains, leading to severe interpretational issues. Since one metabolite can only make one peak, much work has been done to discover vitamin D derivatives and its metabolites that would withstand thermal cyclization during G.C. analysis. Isotachysterols are the only derivatives successfully applied in this situation thus far [23].

Monitoring Pesticide Residues in Greenhouse Tomato

One of the most popularly cultivated vegetables in the world is the tomato (Lycopersicum esculentum, also known as Solanum lycopersicum or Lycopersicum lycopersicum), which is a member of the Solanaceae family [Figure 3].

Materials and Reagents

A mixture of pesticides (600 mg/ml of azoxystrobin, difenoconazole, fludioxonil, pirimicarb, as well as tebuconazole; 300 lg/ml of captan, chlorpyrifos, cyprodinil, but rather 100 lg/ml of Sigma-Aldrich provided the internal standard (I.S.) triphenylphosphate (TPP), which was 99.0% pure. TPP individual stock I.S. solution was created in Mecn at a concentration of 20 g/ml. The relevant concentrations of working mixes were made, stored in the dark, and kept chilled at 4^0c [24].

Figure 3: Image of Tomato

Sampling and Sample Preparation

A DLLME approach and a modified Quenchers method were used to remove the pesticides from the tomato. Fresh tomato samples that had not been washed were minced using a Silvercrest SSMS 600 B2 Kompernass hand blender. The following steps make up the extraction process:

Weigh and transfer 10 0.1 g of the sample to a 50 ml centrifuge tube. Add the solution and vortex for one minute to thoroughly mix it with the selection. Add 10 ml of mean to the models, close the lines, and violently shake them by hand for 30 seconds. Add 4 g anhydrous mgso4 and 1 g NaCl to the sample tubes.

Close the tubes, then violently shake them by hand for one minute. Centrifuge the tubes for five minutes at 5000 rpm. Transfer 8 to 15 ml of the upper layer of the men extract into an amber container. Pour 1 ml of the finished extract into the 15 ml conical SarstedttubesAdd 100 ll of carbon tetrachloride and 4 ml of ultrapure water to this extract. Shaking the test tube forcefully by hand for one minute causes tiny droplets of carbon tetrachloride in an aqueous

solution to disperse, forming a hazy solution. Use a pipette to remove the upper aqueous phase from conical tubes centrifuged at 5000 rpm for 4 min (dispersive particles of silt are at the bottom of the centrifuge tube) (A vial containing 100 microlites of the settled sediment is filled with one ll, which is then injected into the GC-ms system [25].

Apparatus and G.C.–M.S. Conditions

A DB-5MS column underwent G.C. separation. The carrier gas was helium, and the pressure was scaled from 150 kpa to 367.1 kpa while increasing at a rate of 2.2 kpa/min. The injector temperature was 280 C, and the injection was performed in the splitless mode for 0.5 minutes. The glass liner was equipped with a Restekcarbofrit plug. The following was the oven temperature program: After holding at 80 C for 2.0 min, the temperature ramped up to 180 C at 20 C/min, then to 230 C at 5 C/min, to 280 C at 20 C/min, and ultimately to 300 C at 40 C/min, where it remained for 3.0 min. The MS transfer line was maintained at a temperature of 280 C. A 23-minute runtime was used. The following mass spectrometric settings were set: electron impact ionization with a 70eV energy, 230 C for the ion source, and 150 C for the M.S. quadrupole. Each molecule was measured based on peak area utilizing one target and one or two qualifier ions, with the M.S. instrument regularly set in selective ion monitoring (SIM) mode [26].

Fatty Acids in Walnuts and Peanuts

Nuts are considered nutrient-rich foods, and eating them has been linked to a lower risk of coronary heart disease. Due in part to their high quantity of unsaturated fatty acids, nuts provide many health benefits. The fatty acid composition of nuts can be ascertained using validated techniques from AOCS® and AOAC®. This work illustrates the application of some of these approaches to determine the fatty acid composition of walnuts and peanuts. We will present data demonstrating analysis on three distinct selectivity G.C. capillary columns: Omegawax®, SPTM-2560, and SLB®-IL111 [Figure 4] [27].

Column Selectivity for Fatty Acid Methyl Esters

The polyethylene glycol (PEG)-based phase creates the moderately polar Omegawax. Fatty acid methyl esters (FAMEs) are eluted according to the degree of unsaturation with little overlap across various carbon chain lengths. Applications requiring the examination of saturated, monounsaturated, and polyunsaturated fatty acids employ it. It cannot, however, offer the best resolution of the cis and trans isomer groups. It is necessary to use a more polar column to study cis/trans-FAMEs. An extremely opposite cyano silicone column is the SP-2560. This phase's selectivity allows for the resolution of cis and trans isomers and positional geometric isomer separations [28]. The polarity of the SLB-IL111, an ionic liquid column, is greater than that of the SP-2560. The SLB-IL111 has shown elution patterns that work well with the SP-2560 for analyzing cis/trans-FAMEs. To profile the fatty acids (including PUFAs) found in peanuts and walnuts, we employed Omegawax. The C18:1 cis/trans isomers were then determined using the SP-2560 and SLB-IL111 columns [29].

Figure 4: Image of Walnuts and Peanuts

Experimental

According to AOCS® Official Method Ce 1k-093, 1 g samples of dry-roasted peanuts and shelled, chopped walnuts were prepared using acid digestion/alkali hydrolysis, followed by methylation. Antioxidant BHT was added before extraction. Before G.C. analysis, all samples were concentrated to 1 mL. The following G.C. columns were used in the analysis:

1. Omegawax®, 30 m x 0.25 mm I.D., 0.25 μM
2. SP™-2560, 100 m x 0.25 mm I.D., 0.20 μM
3. SLB®-IL111, 100 m x 0.25 mm I.D., 0.20 μM [30]

CONCLUSION

Gas chromatography is one of the oldest chromatographic techniques with numerous applications. Gas chromatography provides plenty of contribution to food analysis, gives meaningful results, and may Analysis many other food products in the future. Gas chromatography in food analysis is effectively implemented in the processes of authenticating as well as preventing crime involving a wide range of beverages and food items, including olives as well as other edible veggies, oils, honey as well as other bee products, etc. primarily using gas chromatography through connection with such a mass spectrometer as well as a flame ionization detection was applied.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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