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Exploring the Potential of Guava (Psidium guajava L.) Leaf through Network Analysis for Novel Drug Repurposing in Polycystic Ovarian Syndrome Treatment.

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Abstract

Polycystic Ovarian Syndrome (PCOS) is a prevalent endocrine disorder affecting women of reproductive age, characterized by hormonal imbalances, insulin resistance, and ovarian dysfunction. Despite its high prevalence, current treatment options for PCOS are limited and often focus on symptom management rather than addressing the underlying causes. This study aims to explore the therapeutic potential of Guava (Psidium guajava L.) leaf, a medicinal plant known for its anti-inflammatory and antioxidant properties, through network analysis to identify potential drug repurposing opportunities for PCOS treatment. Using a network pharmacology approach, bioactive compounds present in Guava leaf were identified and their interactions with key proteins involved in PCOS were mapped. The network analysis revealed several critical pathways and gene targets that are modulated by Guava leaf compounds, particularly those associated with insulin resistance, androgen production, and inflammation— central factors in the pathophysiology of PCOS. Additionally, the study highlighted the potential for repurposing existing drugs that target similar pathways, thereby offering new therapeutic strategies for PCOS management. Docking studies were carried out to predict the molecular mechanism between the target and actives. The findings suggest that Guava leaf could be a promising candidate for further investigation as a natural adjunct therapy in PCOS treatment, potentially improving outcomes for patients through a multi-targeted approach. This research underscores the importance of integrating traditional medicinal knowledge with modern computational tools to uncover novel applications of natural products in the treatment of complex diseases like PCOS.

Keywords: *Psidium guajava L., network analysis, PCOS*

1. INTRODUCTION

Network pharmacology is a holistic approach that integrates systems biology and pharmacology to understand the complex interactions between drugs, targets, and diseases at a network level. When applied to natural products like guava leaf, network pharmacology can offer insights into the multi-target and multi-pathway mechanisms through which its bioactive compounds exert therapeutic effects. [1-3]

Guava leaf (from Psidium guajava) has been widely recognized in traditional medicine for its diverse therapeutic properties, including antimicrobial, anti-inflammatory, antioxidant, and antidiabetic effects. Modern research attributes these benefits to various bioactive compounds found in guava leaves, such as flavonoids (e.g., quercetin), tannins, and triterpenoids. [4-8]

Network pharmacology allows for the systematic analysis of these bioactive compounds to explore how they interact with multiple molecular targets and biological pathways related to various diseases. [9-12] This approach considers the complex, synergistic effects of guava leaf components, making it possible to map out the molecular mechanisms underlying its medicinal properties. Through techniques like molecular docking, pathway enrichment analysis, and network construction, network pharmacology provides a more comprehensive understanding of how guava leaf can influence biological systems.

By leveraging network pharmacology, researchers can identify potential therapeutic targets, understand the polypharmacological effects of guava leaf compounds, and discover new avenues for drug development. This method not only validates traditional uses of guava leaf but also paves the way for its application in modern medicine.

2. MATERIALS AND METHODS

2.1. Data preparation

Polycystic Ovarian Syndrome Treatment (PCOS) human genes were obtained from Gene card, human database (https://www.genecards.org/) which provides information related to all the annotated and predicted human genes. Information about the actives i.e., α- pinene, Limonene, Menthol, Terphenyl Acetate, Caryophyllene, Oleanolic Acid, and Longicyclene were obtained through PubChem (https://pubchem.ncbi.nlm.nih.gov/) and Swiss Target Prediction (http://www.swisstargetprediction.ch/).

2.1. Construction of PPI of selected genes

Protein- protein interaction (PPI) is an important aspect in order to study the involvement of proteins in various biochemical processes as well as to understand the cellular organization, bioprocess, and functions. The genes of the selected active components were uploaded to STING (https://string-db.org/cgi/) to get the detailed information about the PPIs. The setting for generating the PPI network was in accordance with 'Homo Sapiens' and the confidence in the interaction between the target protein was set to the highest confidence data >0.9.

2.3.Construction of compound–target–pathway–disease network and enrichment analysis of PCOS targets and gene ontology

The compound–target network helps to understand and analyse the mechanism of the components with target as well as the pathways involved. Basically, networks of important active of Psidium guajava L was constructed to understand the in–depth of the genes involved in treatment regime which helps us to find the correlation of the drug and hormonal disorder. In order to find the ontology terms associated with molecular, cellular, biological, and KEGG pathway enrichment analysis was carried out. The analysis was performed by Cytoscape plug–in called ClueGO and Cluepedia. Pathways and networks were ranked according to the amounts of the molecules participating in pathways and networks, respectively. Pathways and networks shared by targets related to PCOS and the potential actives as α- pinene, Limonene, Menthol, Terphenyl Acetate, Caryophyllene, Oleanolic Acid, and Longicyclene targets were identified.

2.4. Molecular docking of selected genes

Docking was carried out to find the binding affinity as well as orientation of the selected active components by docking the compounds of the selected class. Docking was performed on using

Glide v_7.6 program interfaced with Maestro v_11.3 of Schrodinger ¨ (2017) (Schrodinger, LLC, New York, NY, USA) of actives with their respective targets which are screened down relating to PCOS. The structures of compounds were built using Maestro build panel and optimized to lower energy conformers using Ligprep v_3.3. Molecular docking helps us to narrow down the search of target proteins to which our actives has more binding affinity which can prove the potential targets for the disease.

3. RESULTS

1.1. Data mining

A total of 5,935 human genes associated with PCOS were identified in the Gene Bank database. Genes obtained from Swiss target prediction was found to be 100 for α- pinene, Menthol, Terphenyl Acetate, Oleanolic Acid; whereas 93 genes for Limonene, 81 genes for Caryophyllene and 18 genes for Longicyclene. After filtering out the common genes between the compounds and disease, 30 genes were used for constructing the network of α- pinene, Limonene, Menthol,

TABLE 1: COMBINED COMMON GENE LIST OF COMPOUNDS AND DISEASE

Sr. No	Gene	Gene Description	UniProt ID
1	ACHE	Acetylcholinesterase	P22302
2	ACP1	Acyl carrier protein	P24666
3	ADORA1	Adenosine receptor A1	P30542
4	ADRA2B	Alpha-2B adrenergic receptor	P18089
5	BACE1	Beta-secretase 1	P56817
6	BCHE	Cholinesterase	P06276
7	CCR5	C-C chemokine receptor type 5	P51681
8	CDC25A	M-phase inducer phosphatase 1	P30304
9	HTR2B	5-hydroxytryptamine receptor 2B	P41595
10	ESR1	Estrogen receptor	P03372
1	GPBAR1	G-protein coupled bile acid receptor 1	Q8TDU6
12	HSD11B1	Corticosteroid 11-beta-dehydrogenase isozyme 1	P28845
13	NR1H4	Bile acid receptor	Q96RI1
14	AKR1C3	Aldo-keto reductase family 1 member C3	P42330
15	CYSLTR1	Cysteinyl leukotriene receptor 1	Q9Y271
16	EDNRA	Endothelin-1 receptor	P25101
17	TERT	Telomerase reverse transcriptase	O14746
18	TOP2A	DNA topoisomerase 2-alpha	P11388
19	TRPV1	Transient receptor potential cation channel V	Q8NER1
20	ABL1	Tyrosine-protein kinase ABL1	P00519
21	APOB	Apolipoprotein B	P04114
22	AURKA	Aurora kinase A	O14965
23	CDK1	Cyclin-dependent kinase 1	P06493
24	CES1	Liver carboxylesterase 1	P23141
25	CLK1	Dual specificity protein kinase	P49759
26	EGFR	Epidermal growth factor receptor	P00533
27	EIF2AK3	Eukaryotic translation initiation factor 2-alpha kinase 3	Q9NZJ5
28	PTGS2, COX2	Prostaglandin-endoperoxide synthase 2	Q68DD2
29	SCARB1, 2	Scavenger Receptor Class B Member 2	Q14108
30	PLA2G4	Phospholipase A2 Group IVA	P47712

Terphenyl Acetate, Caryophyllene, Oleanolic Acid, and Longicyclene with respect to PCOS (Table 1).

3.2. Protein–protein interaction

PPI network was conducted in order to analyse and understand the mechanisms of PCOS based on the study of protein–protein interactions by using STRING software. A total of 55 interrelations, as well as 29 related targets, are obtained in PPI network after setting the confidence level greater than 0.9 and rejecting the target protein independent of the network. The importance prioritization of key proteins is analysed according to the degree of the node exported from STRING database. Among them, the AKR1C3 value (degree = 21), PTGS2 value (degree= 22), SCARB1 value (degree= 25) and PLA2G4 value (degree = 28) is much higher than that of other protein nodes, which indicates that this protein might play a role of bridge to connect other nodes in PPI network. So, PPI network analysis and pathway analysis of novel genes were carried out for the recognition of critical genes related to the PCOS.

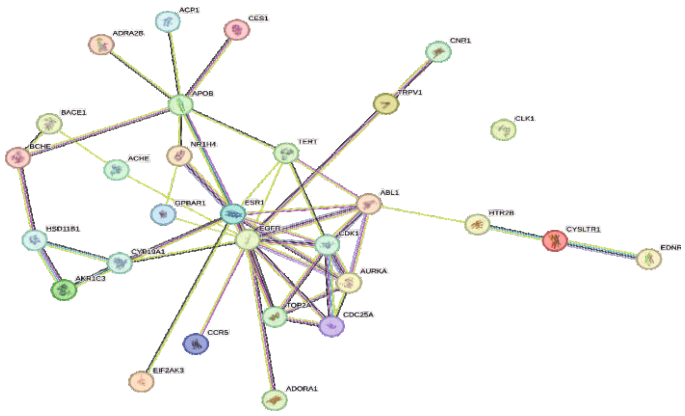


Fig 1: Protein- Protein interaction of genes related to PCOS

3.3. Compound–target network and network-based drug re–purposing for PCOS

3.3.1. Construction of compound–target network

The actives and disorder interaction network were constructed which elucidate the mechanisms of action of actives in the treatment of PCOS. The connectivity of a drug in the networks represents the active's effects, either therapeutic, in the context of the pathway networks, or toxic, in the case of the ADR network. As far as active–target network is considered the important genes which correlates with PCOS are AKR1C3,PTGS2, EGFR, SCARB1, and PLA2G4. Inhibition of these targets may turn out to be an important aspect of network regulation of PCOS as it involves regulation, inhibition and modulation of the proteins which are important to overcome

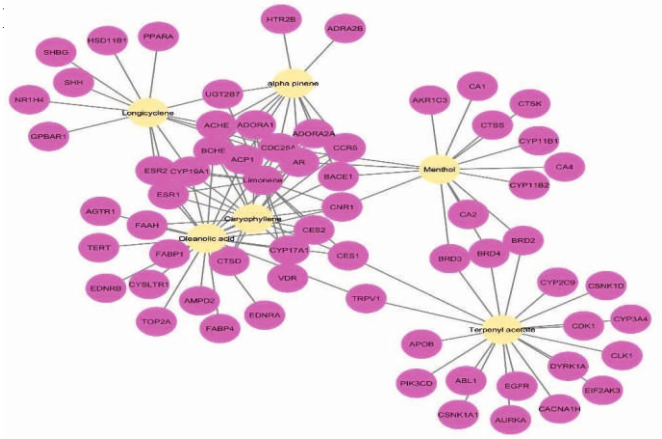


Fig. 2: Compound- target network (the network of α- pinene, Limonene,

Menthol, Terphenyl Acetate, Caryophyllene, Oleanolic Acid, and Longicyclene with respect to PCOS)

1.1.1. Gene ontology and KEGG pathway enrichment analysis

KEGG pathway enrichment analysis revealed multiple significant biological pathways (adjusted P value < 0.05), including PPAR signaling, RNA transport, GnRH secretion, regulation of blood pressure, chemical carcinogenesis, and influenza. Gene ontology (GO) biological process enrichment analysis further confirmed multiple viral infection–related processes (adjusted P value < 0.001), steroid hormone biosynthesis, regulation of cholesterol storage, and prostate gland development.

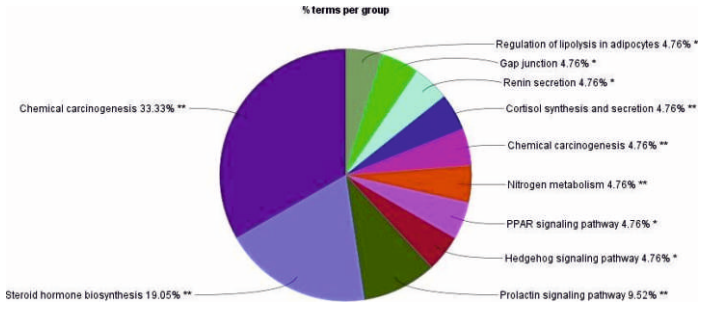


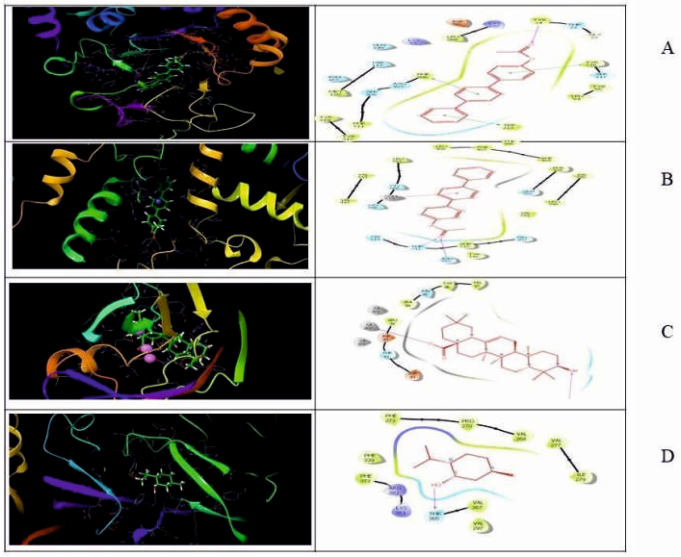
Fig. 3: KEGG pathway enrichment analysis

3.4. Molecular docking analysis

The mechanism of the selected actives were studied by the interaction of the actives and the target through molecular docking. The crystal structures were taken from RCSB protein data bank. The docking score is as mentioned in the table 2; whereas 3D and 2D interactions are given in the figure 4.

Table 2: Molecular Docking Score of compounds against screened targets.

Sr. no	Tyrosine kinase	Actives	Docking score
1	AKR1C3	Terpinyl acetate	-7.273
2	PTGS2	Terpinyl acetate	-6.404
3	SCARB	Oleanolic acid	-5.379
4	PLA2GH	Menthol	-6.399



4. DISCUSSION

The origin for the proposed network–based drug repurposing methodologies is based on the concepts that the proteins associated functionally govern the hormonal disorder which are localized in the within the comprehensive protein–protein interaction network in human cells. For actives with multiple targets to be effective against PCOS, its target proteins should be within or in the corresponding subnetwork in the human protein–protein interactome, as we demonstrated using natural source i.e., through network–based strategy. To improve the quality and completeness of the human protein interactome network, we integrated PPIs and further constructed out the active– target– pathway–disease network. In this study, we presented a network–based methodology for systematic identification of repurposable actives in order to predict the molecular mechanism for potential treatment of PCOS. Integration of compound–target–pathway–disease networks, and human protein–protein network is essential for such identification. Based on comprehensive evaluation, we prioritized 7 candidate repurposable actives for targeting PCOS. According to the stated output, AKR1C3, PTGS2, COX2, SCARB1, and PLA2G4 are found to be essentially related and involvement in PCOS. AKR1C3 plays a dual role in PCOS: it contributes to hyperandrogenism by promoting the formation of potent androgens in adipocytes, and it induces FASN by stabilizing the androgen receptor (AR) even in the absence of androgens. Targeting AKR1C3 with bifunctional inhibitors may offer a therapeutic approach to reduce cardiometabolic risks in women with PCOS. Prostaglandin-endoperoxide synthase (PTGS), commonly referred to as cyclooxygenase, is the crucial enzyme responsible for prostaglandin biosynthesis, functioning both as a dioxygenase and a peroxidase. PTGS exists in two forms: PTGS1, which is constitutively expressed, and PTGS2, which is inducible. This gene encodes the inducible isozyme, PTGS2. Its expression is triggered by specific stimulatory events, indicating its key role in driving prostanoid production related to inflammation and cell growth. The SR-B1 (SCARB1) gene encodes a membrane protein responsible for extracting cholesterol from spherical HDL particles. According to the Reverse Cholesterol Transport (RCT) hypothesis, SR- B1 is crucial in facilitating the transfer of cholesterol from HDL to the liver for excretion. Mutations in this receptor would theoretically lead to elevated serum HDL cholesterol levels and a reduced cholesterol flux through RCT, potentially increasing the risk of Atherosclerotic cardiovascular disease (ASCVD). However, a family with a functional mutation in SR-B1, despite having higher serum HDL cholesterol, exhibitedaltered platelet function, and reduced adrenal steroidogenesis without an associated increase in ASCVD risk.

5. CONCLUSION

In conclusion, this study offers a powerful, integrative network–based systems pharmacology methodology for rapid identification of repurposable drugs and drug combinations for the potential treatment of PCOS. Our approach can minimize the translational gap between preclinical testing results and clinical outcomes, which is a significant problem in the rapid development of efficient treatment strategies for the emerging PCOS outbreak. From the current outlook, if broadly

applied, the network tools developed here could help develop effective treatment strategies for other emerging PCOS and other human complex diseases and disorders as well.

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GENOTOXIC AND CHIRAL IMPURITIES IN PHARMACEUTICALS: PRACTICAL ASPECTS AND REGULATORY CONSIDERATIONS

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ABSTRACT

Impurity profiling involves identifying and characterizing known and unknown contaminants in drug products to enhance drug safety. Regulatory organizations such as the ICH, EMEA, and USFDA have established guidelines to limit impurity levels in drug substances and products. Among the most concerning categories are genotoxic and chiral impurities, which can potentially induce human carcinogenesis even at low concentrations. This has led to increased scrutiny from regulatory bodies and the pharmaceutical industry, necessitating more rigorous analysis and control by manufacturers. The reactive and unstable nature of these impurities presents significant analytical challenges, especially in identifying and managing them at trace levels in accordance with toxicological thresholds and daily dose requirements. This review paper offers a comprehensive overview of the current challenges in chiral and genotoxic profiling, along with the regulatory landscape and their significance in the pharmaceutical industry. It also explores various methods for impurity profiling, including chromatographic and spectroscopic techniques, as well as emerging methods like mass spectrometry imaging.

KEYWORDS: Impurity profiling; Chiral impurity; Genotoxic impurity; Regulatory guidelines

1. INTRODUCTION

An impurity is any substance that compromises the integrity of materials, such as drug substances or active pharmaceutical ingredients (APIs), impacting their safety, efficacy, and potency. Impurities can vary in potency and may even have genotoxic effects. Therefore, utilizing hyphenated analytical techniques for their separation is essential, and according to International Conference on Harmonization (ICH) guidelines, these methods must be developed and validated. Impurity profiling provides detailed quantitative and qualitative reports on the types of known and unknown impurities present in APIs manufactured through specific production techniques. The relevance of impurity profiling in the pharmaceutical industry is underscored by several key factors: understanding impurity structures is vital for new drug development; synthesized impurities are confirmed using spectroscopic techniques; regulatory agencies increasingly scrutinize APIs for both purity and impurity profiles; synthesized materials can serve as "impurity standards" for quantifying impurities; and impurity profiling informs toxicological studies essential for ensuring medication safety. Additionally, it acts as an indicator of manufacturing processes. Research and development in pharmaceuticals rely on sensitive analytical methods to monitor genotoxic impurity levels, ensuring safety during drug manufacturing. Establishing limits for chiral impurities is critical for the safety and efficacy of clinical trials. This review addresses the challenges, regulatory

status, and significance of enantiomeric and regioisomeric impurities in pharmaceuticals, consolidating existing literature on chiral and genotoxic impurity profiling.

2. IMPACT OF GENOTOXIC AND CHIRAL IMPURITY ON PHARMACEUTICALS

The presence of genotoxic and chiral impurities significantly affects pharmaceutical safety and efficacy. Genotoxic impurities can induce mutations and cancer, while chiral impurities can alter drug pharmacological properties, necessitating their detection and quantification for drug development and quality control. Effective impurity profiling techniques such as chromatography and spectroscopy are essential for identifying and characterizing these impurities. Genotoxic impurities, even at trace levels, pose a significant health risk, requiring rigorous monitoring during pharmaceutical manufacturing. Chiral impurity profiling is time-consuming but crucial due to potential pharmacological differences between enantiomers[5-9].

3. SOURCES OF GENOTOXIC AND CHIRAL IMPURITIES

3.1. Genotoxic Compounds Used as Reactants

This section lists seven main groups of reactants used in API synthesis, including boronic acids, epoxides, hydrazine, TEMPO, dialkyl sulfates, alkyl halides, and

Table 1. Used as Reactants: Genotoxic Compounds

Name of reactant	Examples	Properties
Alkyl Halides	Methyl, ethyl, and propyl halides	They are possible alkylating agents, and DNA cross-linking is thought to be the cause of their toxicity. genotoxic alkyl halide contaminants in APIs' origins given on the basis of source of impurities
Dialkylc Sulfates	methylating agents, DMS	The methyl and ethyl derivatives of dialkyl sulfates are most frequently utilized in the pharmaceutical industry; the latter has been used as a chemical agent.
Epoxides	2,3- epoxypropanol (glycidol), 1-chloro-2,3-epoxypropane (epichlorohydrin), or 1,2-epoxy-3-butene,	Epoxides, which have atoms in three rings, are the most basic cyclic ethers. Due to these compounds' high reactivity, which allows there are two electrophilic carbon atoms to interact with DNA's nucleophilic centres and produce alkylated products, they are genotoxic.
Hydrazines	1,2 dimethylhydrazine ; 1,1 dimethylhydrazine	The formation of oxygen-centered radicals, carbon-centered radicals, and carbocations, all of which are known to be relatively reactive species, is what causes hydrazine and its derivatives to be poisonous.
TEMPO (2,2,6,6-Tetramethylpiperidin-1-oxyl)	-	A frequent process reagent and probable process impurity is TEMPO.
Aromatic Amines	4- Nitroaniline ; 4- amino-2-Nitrophenol	Electrophilic species are created during metabolic activation even though aromatic amines are frequently not genotoxic by nature. The primary metabolic process for aromatic amines is oxidation, which results in the creation of an N-hydroxy molecule that is conjugated as an acetate, sulphate, or glucuronide.
Boronic Acids	Carboxy phenyl boronic acid ; Methyl phenyl boronic acid	Recent research has revealed boronic acids to be a brand-new class of bacterial mutagens.

alkylating agents, as detailed in Table 1[7-11].

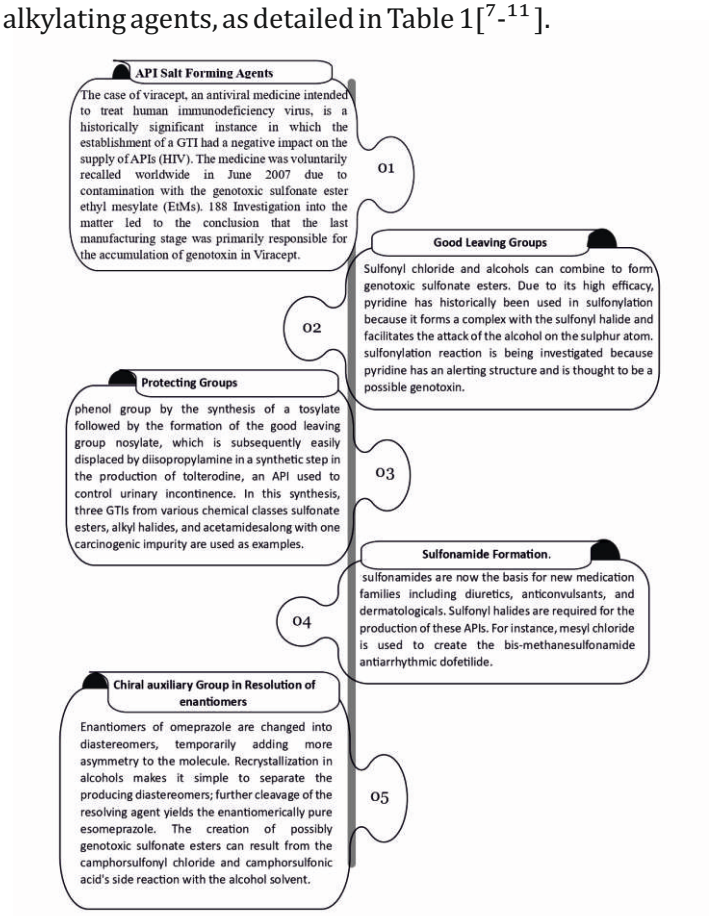

FIGURE 1. CLASSES OF SULFONATE ESTERS AND THEIR PRECURSORS

3.2. Genotoxic impurities generated in side reactions

During the synthesis of an API, genotoxic impurities might develop as side products. Amongst the various genotoxic impurities, the sulfonate esters class has attracted the most interest and has been the subject of the most research. Since this class of compounds is frequently created during side reactions with alcohols, it may initially be difficult to recognize them during API synthesis[12].

3.3 Formation of Sulfonate Esters and Their Predecessors

Alkylating agents like sulfonate esters, potential genotoxic compounds, add alkyl residues to genetic material bases' reactive sites, sourced from side reactions or remaining reactants, as summarized in Figure 1 and Table 2 for stoichiometric and catalytic amounts, respectively [13].

Table 2. Catalytic Amounts of Sulfonate Esters and their Precursors

Cyclizations	A pharmaceutical example of a cyclization where a sulfonic acid is utilized as a catalyst is the TsOH-aided cyclodehydration of a diol to form the chroman framework during the production process of the hypoglycemic medication englitazone.
Migration of Double Bonds	A steroid called etonogestrel is utilized in hormonal contraceptives, and p-toluene sulfonic acid helps in the double bond migration process during its manufacture.
Enamine–Amine Reduction	Ritonavir, an antiretroviral medication, is a member of the protease inhibitor family and is used to treat HIV infection and AIDS. It is produced by converting an intermediate enamine into a primary amine by treating it in the presence of methane sulfonic acid with sodium borohydride.
Esterification	Esterification is also catalyzed by sulfonic acids. An ACE inhibitor is fosinopril. A processing step in its manufacture comprises esterifying a carboxylic acid with methanol in the presence of TsOH.

3.4 Alkyl Halides

DMTMM, an alkyl halide coupling agent utilized in chemical processes like esterification, undergoes self-conversion, yielding genotoxic methyl chloride and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-morpholine, but is unstable in organic solvents[10].

3.5 Acetamide

Acetamide, a carcinogen, is vital to monitor during API synthesis; while not directly used, its derivatives like trifluoroacetamide and 2-bromoacetamide generate acetamide impurities, which may also originate from the 2- and N-derivatives, contingent on reaction conditions[10].

3.6 Genotoxicity and Carcinogenicity Due to Organic Solvents

In the manufacturing of pharmaceuticals, organic solvents are commonly employed as mediums for reactions and purification (such as extraction), separation phases (such as mobile phases for chromatography), as well as for cleaning equipment [14]. Solvents are categorized into four classes in accordance with the Q3C guideline, as shown in Table 3.

Table 3. Common Organic Solvents

Class	Examples	Description
Class1	Benzene, Carbon tetrachloride, 1,2-Dichloroethane	These are carcinogens, unless absolutely necessary, these solvents should be avoided.
Class 2	2-Methoxyethanol, Chloroform, Pyridine	They produce irreversible damage, including teratogenicity, or are nongenotoxic animal carcinogens.
Class 3	Ethanol, Acetone	Incorporate solvents like acetone and ethanol, which have acceptable daily doses of 50 mg or up to 5000 ppm (0.5%) when it is anticipated that 10 g is provided each day.
Class 4	Methyl isopropyl ketone	There are no carcinogenic studies on this group.

4. CHIRAL IMPURITIES

Naturally, biosynthesized compounds with high enantioselectivity may result in one enantiomer of a synthetic chiral molecule being clinically effective while the other is considered an impurity. Examples include levalbuterol, esomeprazole, and levofloxacin. Pharmaceutical chiral impurities are individually significant; for instance, the levo isomer might exhibit greater biological activity than the dextro isomer [15,16].

4.1 Sources Chiral impurities

Chiral impurities stem from chiral starting materials like mandelic acid and thalidomide, chiral catalysts and reagents such as chiral oxazoline ligands, intermediates and by-products like 4-hydroxy-2-methyl-2H-1, 2-benzothiazine-3-carboxylic acid, resolution processes including chiral chromatography, degradation products like 3'-hydroxywarfarin, racemization, and non-equimolar enantiomer mixtures exemplified by citalopram and albuterol, emphasizing the need for stringent control during drug synthesis and purification.

The above examples are just a few examples of sources of chiral impurities, and it's important to note that each drug substance may have different potential sources of chiral impurities. It is important to note that the sources of chiral impurities can vary depending on the specific drug substance and its synthesis process. Therefore, it is crucial to carefully assess the possibilities sources of chiral impurities and to establish effective control strategies to minimize their presence in the finished pharmaceutical product.

5. REGULATORY CONSIDERATIONS FOR GENOTOXIC AND CHIRAL IMPURITIES

Regulatory agencies such as the USFDA, ICH and EMEA have established guidelines and limits for genotoxic and chiral contaminants found in pharmaceutical goods. Compliance with these regulations is critical to ensure the effectiveness and safety of pharmaceuticals and requires effective impurity profiling techniques. Various regulatory bodies all over the world set limits on the amount of contaminants that drug substances and medication products can contain.

5.1 International Council on Harmonization (ICH)

The International Council on Harmonization(ICH) provides general guidelines for impurities, which can be organic or inorganic [17-19]. The threshold for Genotoxic impurities as per ICH guidelines is given in Table 4. Figure 2 gives the identification pathway and qualification of impurities and outlines factors to take into account for the qualification of contaminants. The decision tree highlights factors for characterizing contaminants when threshold levels are exceeded, prioritizing safety information transmission over lowering impurity levels in some cases. Specific tests, including assays for genotoxicity, are conducted as per guidelines to qualify contaminants. Techniques from ICH Q3C aid in estimating permissible daily exposure limits for genotoxic impurities. For chiral impurities, ICH recommends treating enantiomers as impurities, necessitating investigation into their effects on efficacy and safety, as well as potential interconversion, using a decision tree as depicted in Figure 3.

5.2 - Other Guidelines

Table 4. The threshold for identifying, reporting and quantifying the impurities as per ICH guidelines.

Maximum Daily Dose, g	Reporting Threshold, %	Identification Threshold	Qualification Threshold
≤2	0.05	0.1% or 1.0 mg/d (whichever is lower)	0.15 or 1.0 mg/d (whichever is lower)
>2	0.03	0.0005	0.0005

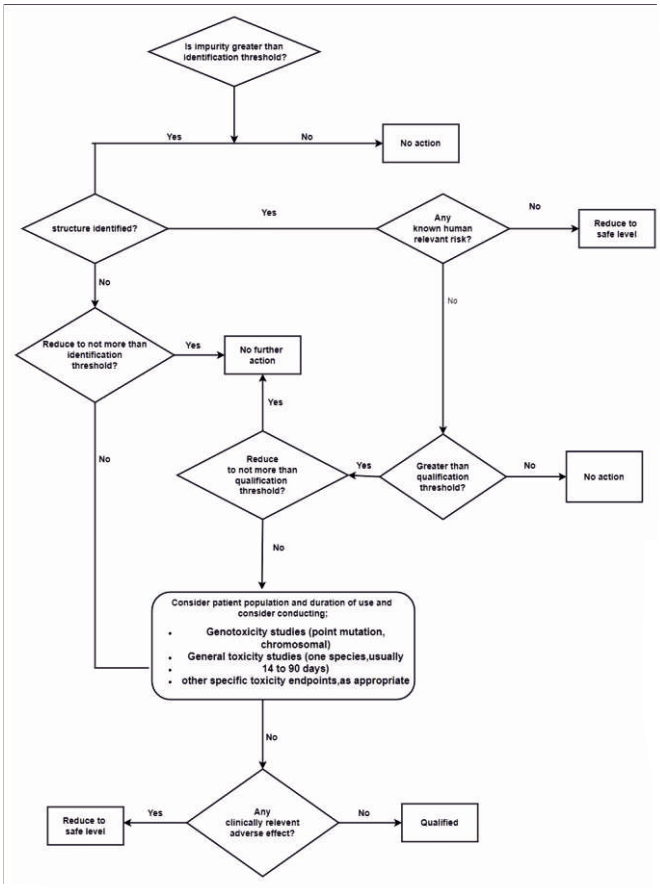

Figure 2: ICH Decision tree for Genotoxic impurity

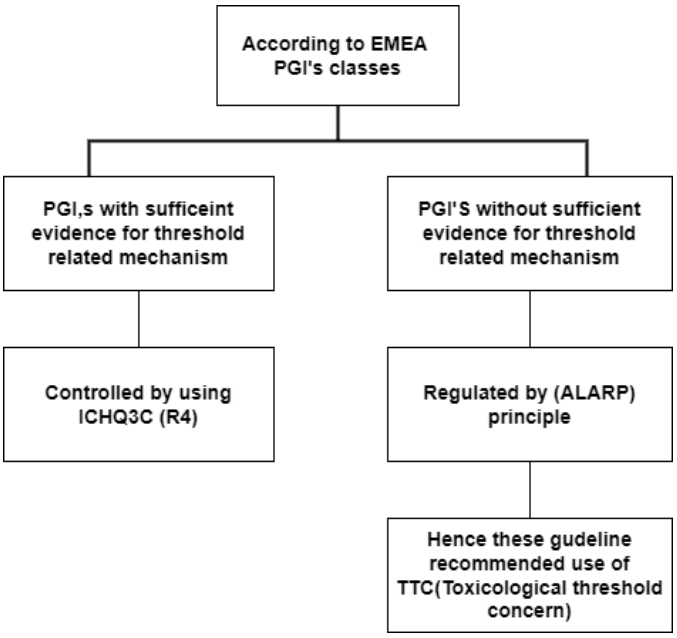

Figure 3: According to EMA guidelines, the limit of PGIs is classified into two categories

European Medicines Agency (EMA) is a regulatory body which enforces detailed guidelines to address the genotoxic impurities[20,21]. According to the According to EMEA guidelines, any component of the drug that may be genotoxic (PGIs) should be discovered, either by screening for "structural alarms" or by using genotoxicity data that is already available. According to EMA guidelines, the limit of PGIs is classified into two categories as shown in Figure 4. EMA first introduced the idea of threshold toxicological concern (TTC) is limit genotoxic contaminants and also introduced a question and answer on guidelines on the 2010 publication of the limit of genotoxic impurities helped to clarify and harmonize genotoxicity[20,4]. According to EMEA The acceptance criteria for TTC of 1.5g/day intake of PGI's is linked to a hazard that is acceptable. Specific recommendations for genotoxic impurities are not included in ICH guidance documents. However, The EMA Committee on Human Medicinal Products (CHMP) has made published a recommendation on genotoxic impurity thresholds.

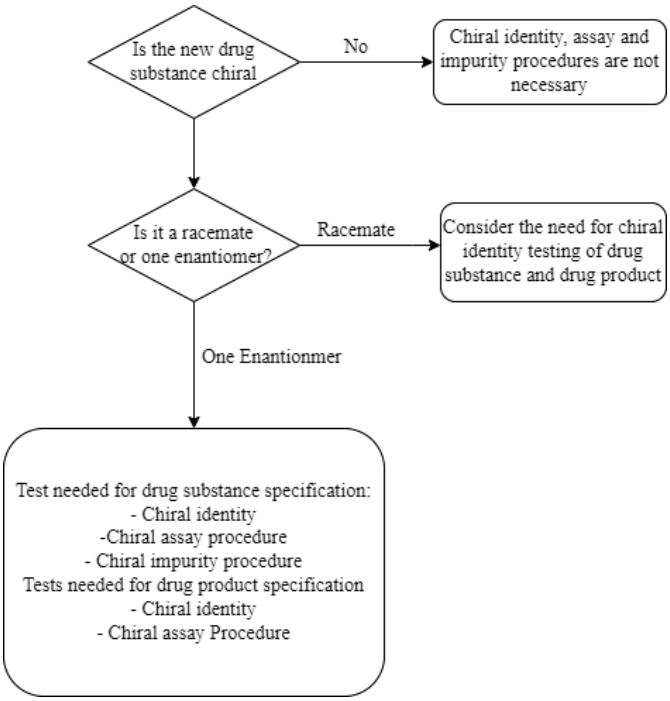

Figure 4: To determine the identity, assay, and chiral impurity procedures in a novel drug product, including a chiral component, use a decision tree

The USFDA issued a draft of a guidance document in March 2018 entitled M7(R1) guideline on "Assessment and Control of DNA Reactive Impurities (PGIs) in Medications to Minimize Potential Carcinogenic Risk [23,15] Figure 5 shows Genotoxic Functional groups. This guidance document also adopted as recently been replaced by the ICH M7 (R1)[24]. The USFDA guidance documents highlight Particular safety advice certification of pollutants with suspected or known genotoxicity [3,25].

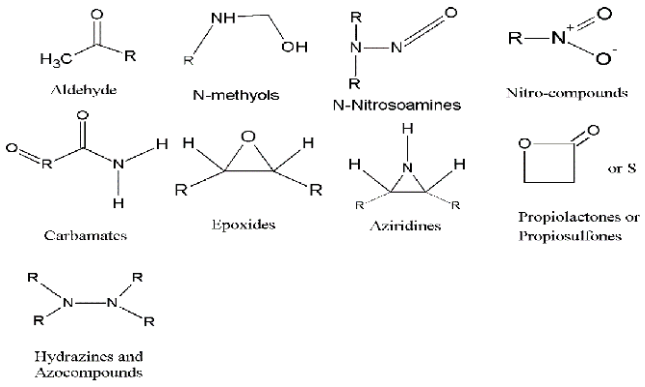

Figure 5. Genotoxic Functional Groups

R = alkyl, aryl or H
X = F, Cl, Br or I

With respect to genotoxic impurities. The USFDA approach includes:

(I) limiting the growth of genotoxic and cancer-causing contaminants,

(ii) decreasing their level (the daily maximum objective is 1.5 g),

(iii) characterizing the risk of genotoxicity and carcinogenesis, and Therapeutics Goods of Administration that is (TGA) gives recommendations on contaminants in new drug substances and drug product version 1 published in 2013.This guidance gives the limit of genotoxic impurities however the information provided is very brief. Moreover, the guidance document does not provide any information on chiral impurities [26].

In case of chiral impurities, the USFDA released a policy statement on the manufacturing of novel stereoisomeric pharmaceuticals in the year 1992. As most pharmaceuticals generate an acceptable limit for control, permissible intake of a mutagenic impurity of TTC with a dose of 1.5 g per day is generally accepted with minimal risk and can be used as a default[27]. The guidance document recommends that a decision tree should be used to address chiral impurities in chiral novel drug ingredients and novel therapeutic products[28]. Control of quality of chiral impurities is equally important like any other impurity generation in and out of the synthesis's basic ingredients. When there are multiple chiral centers, such as three or more, or when evaluation in a new drug substance at an acceptable sensitivity is technically unfeasible, restrictions can be set on suitable starting materials or intermediates. A chiral assay can be required if chiral impurity analysis is carried out using a technique that is equivalent to an assay procedure.

6. THRESHOLD OF TOXICOLOGICAL CONCERN (TTC APPROACH)

The Threshold of Toxicological Concern (TTC) method estimates impurity risk based on toxicity, with concentration limits derived from TTC and patient dosage. For chiral impurities, TTC usage is cautioned due to potential differing pharmacological and toxicological properties, necessitating specific scientific evaluation based on impurity properties [29,30].Figure 6 depicts Flow Chart for Assessment of Acceptability of Genotoxic Impurities.

$$\text{Concentration limit (ppm)} = \frac{\text{TTC [µg/ day]}}{\text{Dose [g/ day]}} \quad \text{....Equation (1)}$$

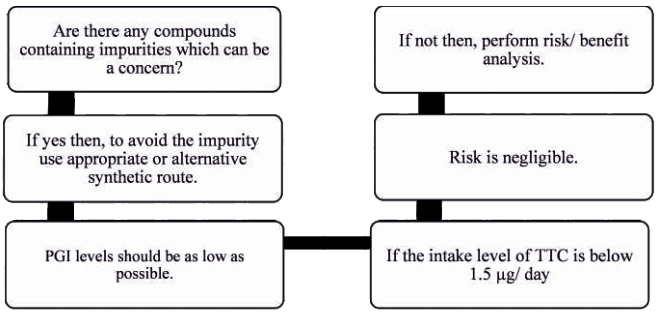

Figure 6. Flow Chart for Assessment of Acceptability of Genotoxic Impurities

7. ANALYTICAL TECHNIQUES USED FOR IDENTIFICATION, ISOLATION AND CHARACTERIZATION

Detecting and quantifying trace levels of genotoxic and chiral impurities in drugs poses a significant analytical challenge due to their potential health risks, requiring highly sensitive methods[31]. In case of genotoxic and chiral impurities, very low level, i.e. 0.01% - 0.03% is determined and hence it is major challenge. At times, there is interference due to Active Pharmaceutical Ingredients[32]. Some reactions result in changes in the functional group which leads to formation of genotoxic impurity and hence an undesirable product as shown in Figure 5. Sometimes impurities are low molecular weight so volatile in nature and hence their analysis is difficult. The major analytical techniques utilized for identification, isolation and characterization of genotoxic and chiral impurity are summarized below (Figure 7 provides an overview of the advanced analytical techniques used for identifying, isolating, and characterizing impurities):

· HPLC: Widely utilized for detecting genotoxic and chiral impurities due to its cost-effectiveness and ease of operation [33,34].

· LC-MS: Commonly employed for characterizing genotoxic impurities [35,36].

· GC and GC-MS: Analyze volatile compounds, with headscape injection preferred to reduce contamination, capable of detecting halide, sulfonate, and epoxide impurities.

· ICP-OES: Specifically used for determining metallic impurities causing DNA alteration [37,35].

· Nuclear Magnetic Resonance spectroscopy: Provides stereochemical and structural information, valuable for detecting trace genotoxic and chiral impurities.

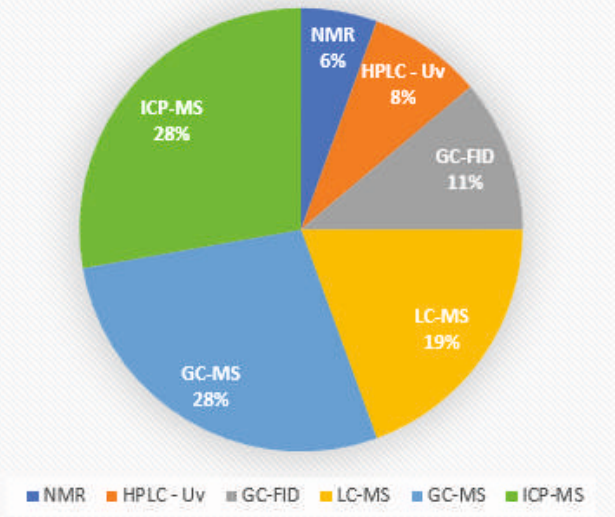

Figure 7. Advanced analytical tools for the identification, characterization of genotoxic and chiral impurities

Tables 5 and 6 are examples of Techniques used for Genotoxic and Chiral Impurities identification, isolation and characterization.

Table 5. Chiral impurities

Sr. No	Drug	Chemical Name of Chiral Impurities	Analytical Method	Reference
1	vilanterol trifenatate	(R)-tert-butyl (2-(2,2-dimethyl-4H-benzo[1,3]dioxin-6-yl)-2-hydroxy-ethyl)carbamate	HPLC	-34
2	Brivaracetam	(R,S)-brivaracetam, (R,R)- brivaracetam and (S,S)-brivaracetam	RP HPLC	-35
3	Amlodipine	(R)-amlodipine	nano liquid chromatography and capillary electrochromatography	-36
4	Besifloxacin Hydrochloride	7-[(3S)-3-aminoazepan-1-yl]-8-chloro-1-cyclopropyl-6-fluoro-4-oxoquinoline-3-carboxylic acid; hydrochloride	HPLC	-37
5	Etomidate	(S)-etomidate, (R)-metomidate, and (R)-etomidate acid	Capillary electrophoresis	-38
6	Pregabalin	R enantiomer S enantiomer	GC-MS	-39
7	Zopiclone	(+)-(S)-ZO enantiomer (eszopiclone) RP29307	capillary electrophoretic enantioselective method with UV detection	-40
8	RS-glycopyrrolate	SR-enantiomer RR-diastereomer, SS-diastereomer	capillary electrophoresis using sulfated-[1]-cyclodextrin as chiral selectors	-41

Table 6. Genotoxic Impurities

Sr no.	Drug substance/ drug product	Chemical name of Genotoxic impurity	Analytical method	% Threshold	Reference
1	Lumefantrine	Desbenzylketo derivative	HPLC/DAD/UV/ ESI/MS	0.002	42
2	MLN9708	2,5 Dicholorobenzyl chloride(DC)	UPLC	8.9 Mg/day M/Z 188.9515	43
3	Losartan	NDMA N-Nitrosodimethylamine	SFC	-	44

4	HIV tablets	EMS Ethyl Methane sulphonate	Micronucleus test lacz gene test	0.4 mg/kg day <0.5 mg/kg day	45
5	Losartan	Nitrosamine impurities	HPLC-UV	NDMA-0.693	46
6	Meropenem	318 BP M9 S5	LC-MS/MS	-	47
7	Imatinib mesylate	Methyl-3-N[4-(3-Pyridinyl)-2-pyrimidinyl]-1,3- benzenediamine (PNMP) 4-(4-Methylpiperazinomethyl) benzoic acid dihydrochloride (MPBA)	LC-MS/MS	-	48
8	Dalfampridine	A) Pyridine-4-carboxamide B) 3-Aminopyridine C) 1,2-bis (4-pyridyl hydrazine) D)1,3-Di(pyridin-4-yl) urea E) 4-Aminopyridine-N-oxide	RP-HPLC HILIC MODEL	-	49
9	Rabeprazole	A) CPAR(Chloro-propoxy analogue of rabeprazole B) FBCI (Free base chloro intermediate)	LC-MS/MS	-	50
10	sacubitril/valsartan	sacubitril Ena Ester	LC-MS/MS		51
11	Ibrutinib	Hydrazine hydrate	RP-Liquid chromatography		52

8. CHALLENGES IN PROFILING OF GENOTOXIC AND CHIRAL IMPURITIES

Impurity profiling of genotoxic and chiral impurities has several challenges; some can be easily overcome, while others are insurmountable. Nevertheless, some of the major challenges must be taken into account and overcome as much as possible Figure 8. summarizes the major challenges involved in profiling of genotoxic and chiral impurities [57,8].

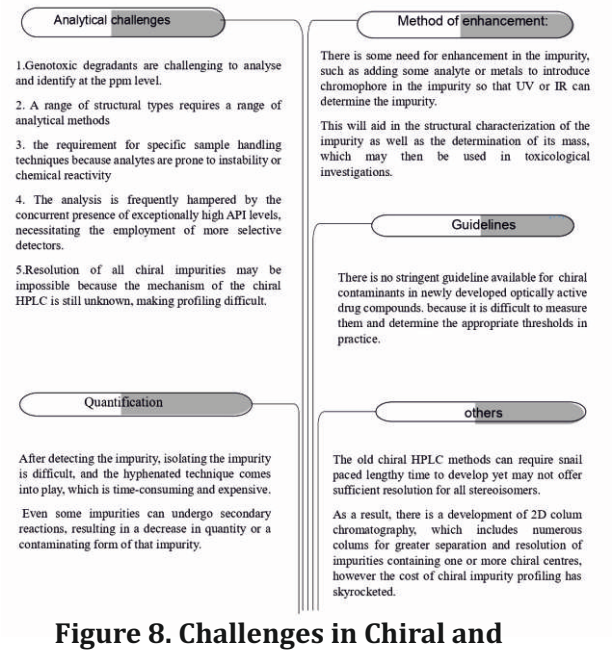

Figure 8. Challenges in Chiral and Genotoxic impurity profiling

1. CONCLUSION

Chiral and genotoxic impurity profiling is crucial for ensuring the safety and quality of drug products, as well as for complying with regulatory requirements and optimizing manufacturing processes. These impurities can exert significant biological effects, making it essential to control and monitor them to protect patient safety. Health authorities must prioritize the identification and management of contaminants present in drug substances and products, as even trace amounts can alter the biological and therapeutic efficacy of medications.

This review discusses various sources of chiral and genotoxic impurities, highlighting current challenges and the regulatory landscape surrounding them. It underscores the importance of these impurities in the

pharmaceutical industry, emphasizing that their presence can jeopardize the integrity of drug formulations.

Methods and techniques for impurity profiling are diverse and include chromatographic and spectroscopic methods, which are integral for accurate analysis. Impurity profiling has broad applications across drug development, quality control, and regulatory compliance, making it a vital component of the pharmaceutical lifecycle.

Future research should focus on developing new analytical techniques for identifying and measuring chiral and genotoxic impurities. Innovations such as chiral chromatography and enantioselective mass spectrometry hold promise for refinement and improvement, enabling more precise and efficient impurity profiling. This advancement will not only enhance safety standards but also facilitate compliance with increasingly stringent regulatory requirements, ultimately benefiting patient health and well-being.

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AUTHORS' CONTRIBUTIONS

SK and AK collected the information and drafted the manuscript. AA and OA edited the manuscript.

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CONFLICT OF INTEREST

Regarding this work, the authors disclose no competing interests, financial or otherwise.

ETHICS APPROVAL

None to declare

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Formulation and Evaluation of a Film-Forming Gel for Enhanced Burn Wound Healing

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ABSTRACT

Burn injuries present significant challenges in wound care, often leading to complications such as infections, pus accumulation, and delayed healing. To address these issues, this study focused on developing and evaluating a curcumin-loaded film-forming gel designed to create a durable protective barrier over burn wounds, adhere securely to the skin, and gradually release therapeutic agents. The gel was formulated using varying concentrations of HPMC grades such as K100LV and K100M and Eudragit RL 100, with aloe vera mucilage and PEG 400 added to enhance bioadhesion and penetration. Thin Layer Chromatography confirmed curcumin's purity, while anti-inflammatory studies revealed its dose-dependent inhibition in protein denaturation assay, demonstrating anti-inflammatory potential. Ten batches of curcumin-loaded film-forming gels were prepared, optimized, and assessed for physical characteristics, pH, spreadability, drying time, film-forming capability, and folding endurance. Batch J, containing 1.25% HPMC K100M and 1.5% Eudragit RL100, was identified as the optimal formulation, exhibiting a rapid drying time of 2 minutes and 30 seconds, good spreadability, excellent film-forming properties, and depicted comfortable movement of the applied area. The film-forming gel produced a durable and flexible film, providing a protective barrier over the wound and promoting the healing process. The gradual release of curcumin, coupled with its anti-inflammatory effects, is expected to accelerate wound healing, reduce the risk of infection, and improve patient outcomes. This innovative film-forming gel represents a promising solution for burn wound care, effectively combining the therapeutic benefits of curcumin with advanced formulation techniques.

Keywords: Burns, Anti-inflammatory, Curcumin, Eudragit RL100.

INTRODUCTION

Burn injuries are among the most severe types of trauma that the skin can endure, leading to complex challenges in wound care. The nature of burn wounds is characterized by extensive tissue damage and a disrupted skin barrier that makes them particularly susceptible to complications such as infections, pus accumulation, and delayed healing1. Effective treatment is essential to minimize these risks and promote faster recovery. Understanding the pathophysiology of burn wounds is crucial for developing effective treatment strategies. Burn injuries are classified based on the depth and extent of skin and underlying tissue damage, which influence the healing process and treatment approach.

Burn wounds are classified into four categories based on depth of the burn. The first-degree burns (superficial) affects only the epidermis, these burns cause redness, pain, and minor swelling without blistering. Typically, they heal within 3-6 days without scarring. The second-degree burns (partial-thickness) affect the epidermis and the upper dermis, causing redness, pain, swelling, and blisters. Healing occurs within 7-21 days, often with minimal scarring. The deep partial-thickness burns penetrate deeper into the dermis, appearing red or white with significant pain and swelling. Healing takes over 21 days, frequently resulting in scarring. The third-degree burns (full-

thickness) extends through the entire dermis and affecting underlying tissues, these burns exhibit a white, charred, or leathery appearance and are typically numb due to nerve damage. They do not heal on their own and require surgical intervention and the fourth-degree burns extend beyond the skin into underlying fat, muscle, and bone, characterized by black or charred skin and severe functional impairment[2,3].

The burn injuries are characterized by three distinct zones including zone of coagulation, it is the central area of maximum damage where tissue is irreversibly damaged, zone of Stasis surrounding the zone of coagulation, characterized by decreased perfusion and the potential for further damage and zone of hyperemia, the outermost area with increased blood flow due to the inflammatory response, generally viable with proper care[4].

The pathophysiological processes of burn wounds include initial injury and cellular damage, inflammatory responses, immune responses, and healing and repair. Immediate heat exposure damages skin cells and proteins, leading to necrosis. The inflammatory response involves vasodilation and increased permeability, resulting in edema and the release of inflammatory mediators. The immune response recruits neutrophils and macrophages to clear necrotic tissue and combat infection. Healing involves fibroblast proliferation, collagen production, angiogenesis, re-epithelialization, and collagen remodeling[5].

Curcumin, a bioactive compound found in turmeric (Curcuma longa), has garnered significant attention for its potential in burn wound healing due to its anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties. Curcumin reduces inflammation by inhibiting the production of pro-inflammatory cytokines and enzymes like COX-2 and TNF-α, which are often elevated in burn injuries. This helps in reducing swelling, pain, and the overall inflammatory response at the wound site [6]. The burns generate reactive oxygen species (ROS), which can cause further tissue damage. Curcumin's antioxidant properties help neutralize these ROS, thereby minimizing oxidative stress and promoting faster healing[7]. Curcumin exhibits antimicrobial activity against a range of pathogens, including bacteria, viruses, and fungi. This is particularly beneficial in preventing infections in burn wounds, which can complicate and delay the healing process [8]. Curcumin enhances collagen production, which is crucial for wound healing. Collagen provides structural integrity to the skin and helps in the formation of new tissue at the wound site [9]. Curcumin promotes angiogenesis, the formation of new blood vessels, which is essential for supplying oxygen and nutrients to the healing tissue. By modulating the expression of growth factors and matrix metalloproteinases, curcumin may help reduce excessive scar formation, leading to better cosmetic outcomes in burn wound healing [10].

Aloe vera mucilage, derived from the inner gel of the Aloe vera plant, is widely recognized for its soothing and healing properties, especially in treating burn wounds. Aloe vera mucilage reduces pain and promotes faster healing of burn wounds. Aloe vera contains compounds like bradykinase, which help reduce inflammation. The mucilage also inhibits the production of pro-inflammatory cytokines, thereby decreasing swelling and redness associated with burns [11]. Aloe vera mucilage exhibits antimicrobial effects against various bacteria, fungi, and viruses, which helps prevent infections in burn wounds. The presence of compounds like anthraquinones and saponins contributes to this antimicrobial activity [12]. Aloe vera stimulates fibroblast activity, which is crucial for collagen synthesis and wound contraction. The mucilage also accelerates epithelialization, helping in the rapid closure of burn wounds[13,14]. Regular application of Aloe vera mucilage can help reduce scar formation by promoting balanced collagen synthesis and reducing excessive tissue buildup [15]. Aloe vera also contains enzymes like amylase and lipase, which may assist in breaking down the mucilage matrix and facilitating the penetration of active compounds through the skin [16].

The aim of this research is to prepare and evaluate a Curcumin based film-forming gel that will provide a robust protective barrier over burn wounds, adheres securely to the skin, releases therapeutic agents gradually, and integrates bioactive compounds to expedite the healing process. The present study involves incorporating antimicrobial and anti-inflammatory substances to expedite healing, create a protective barrier that shields the wound from contaminants, reduces infection risk, and maintains a moist environment conducive to healing, secure adhesion to skin using bioadhesive gelling agents, design the gel to gradually release therapeutic active ingredients for consistent wound healing.

MATERIALS

Curcumin was procured from Yucca enterprise, Mumbai, India. Grades of HPMC, Eudragit RL 100 were generously provided by Colorcon Asia Pvt. Ltd., Mumbai, India, and Evonik, Mumbai, India, respectively. All other solvents and reagents used were of analytical grade and purchased from S.D.Fine, Mumbai, India.

2. EXPERIMENTAL

2.1. Thin Layer Chromatography (TLC) of Curcumin

TLC is a widely utilized analytical technique in the study of plant extracts. This method is highly valued for its simplicity, cost-effectiveness, and efficiency in separating and identifying compounds within complex mixtures. Plant extracts, which often contain a diverse array of bioactive constituents such as alkaloids, flavonoids, terpenes, and phenolic acids, can be effectively analyzed using TLC. TLC helps in identifying individual compounds within plant extracts by comparing their Rf values and visual characteristics to those of known standards [17]. The technique can assess the purity of plant extracts by revealing the presence of any impurities or secondary metabolites. In the herbal medicine industry, TLC is employed for quality control to ensure the consistency and authenticity of plant-based products [18].

1 mg of curcumin was dissolved in 10 ml of 95% ethanol to prepare the curcumin solution. The mobile system consisting of chloroform, ethanol, and glacial acetic acid in a 9.4:0.5:0.1 ratio [19] was prepared and poured into the developing chamber, which was then sealed with a glass Petri plate to allow the chamber to become saturated with solvent vapor. The precoated silica gel 60 F254 plate was cut to the desired size. Simultaneously, the spotting was marked about 1 cm from the bottom of the plate and solvent front with a pencil. Using a capillary tube, the curcumin solution was spotted onto the marked baseline of the TLC plate. The spotted TLC plate was placed into the saturated developing chamber and allowed the solvent to ascend the plate by capillary action. Once the solvent front reached an appropriate height (usually about 1 cm from the top), the plate was carefully removed from the chamber, air-dried, and the spots were visualized under a UV light chamber at 254 nm, 366 nm, and white light. (Figure 1). The distance traveled by the curcumin spot relative to the solvent front (Rf value) was calculated.

2.2.In Silico Analysis of Anti-inflammatory Properties of Curcumin

Inflammation is a complex biological response to harmful stimuli such as injury, infection, or tissue destruction. It is characterized by heat, redness, pain, swelling, and altered bodily functions, a protective mechanism aimed at removing harmful agents and initiating tissue repair. To evaluate the anti-inflammatory properties of compounds, the protein denaturation assay is a commonly used in vitro method [20].

Curcumin solution was prepared in varying concentrations (50, 100, 150 µg/ml) dissolved in a dimethyl sulfoxide (DMSO). The reaction mixture included 0.2 ml of egg albumin (as the protein source), 2.8 ml of PBS pH 6.4 and curcumin extract. A control was maintained, where double-distilled water replaced the curcumin solution to provide a baseline for comparison. The reaction mixtures were incubated at 37 ± 2°C for 15 minutes to allow interaction between the curcumin solution and the egg albumin. Following incubation, the mixtures were heated at 70°C for 5 minutes to induce protein denaturation. After cooling the mixtures, the absorbance of each sample was measured at 660 nm using a UV-visible spectrophotometer (Figure 2). The absorbance readings indicated the extent of protein denaturation, with lower absorbance values suggesting higher anti-inflammatory activity due to inhibition of protein denaturation [21].

The percentage inhibition of protein denaturation was calculated using the formula:

$$\text{Percentage Inhibition} = \frac{(\text{Absorbance Control} - \text{Absorbance Sample}) \times 100}{\text{Absorbance Control}}$$

2.3. Preparation and optimization of Curcumin loaded film forming gels

The preparation of curcumin-loaded gel formulations involved the use of varying concentrations of HPMC grades and Eudragit RL 100 to identify the best batch. A total of ten batches were formulated, with the first four using a low viscosity grade of HPMC and the remaining six using a high viscosity grade to optimize the gel's rheology. The required amount of HPMC and Aloe vera mucilage was weighed, dissolved in water and transferred them into a labeled beaker (Beaker 1).The beaker was placed on a magnetic stirrer and stirred vigorously at a speed between 450 and 600 rpm until HPMC was completely dissolved. PEG 400 was gradually added to the mixture while maintaining continuous stirring for at least 10 minutes to ensure complete dissolution and homogeneity. In another beaker (Beaker 2) Eudragit RL 100 was weighed and curcumin previously dissolved in 95% ethanol was added. The mixture was stirred at a speed between 600 and 650 rpm for 15 minutes to ensure complete dissolution of the polymer. To ensure complete dissolution of polymers in both the beakers, both solutions are mixed under a magnetic stirrer until a translucent yellow gel forms, indicating a homogenous mixture. The final gel formulation is then carefully filled into aluminum tubes for further testing and evaluation.

2.4. Evaluation of Curcumin loaded film forming gels

Ten batches from A to J of curcumin loaded film forming gels were prepared and evaluated for following parameters:

1. Physical Evaluation and Homogeneity: The physical evaluation of the gel formulations involved a detailed assessment of their appearance, homogeneity,

color, and odor. Ideal gels were characterized by a uniform color and texture, and a neutral odor. The gels were thoroughly checked for consistency and the absence of particulate matter. A smooth, uniform texture was considered essential for effective application, ensuring that the gel could be evenly spread on the skin without causing any discomfort or irregularities.

2. pH Value: The pH of each gel formulation was measured using a digital pH meter. For gels intended for oral-topical use, maintaining a pH value between 5.5 and 7.4 was crucial. This pH range is compatible with the skin's natural acidity, which helps prevent irritation and ensures the gel is safe and comfortable for application [22].

3. Spreadability: The spreadability of the gel was evaluated using a simple but effective method. A 1g sample of the gel was placed between two glass plates, and a specific weight was added to the top plate to assess how well the gel spreads under pressure. The diameter of the spread gel was measured to determine its spreadability. This parameter is important because it indicates how easily the gel can be applied over a surface, which is critical for user comfort and effectiveness [23].

4. Film Forming Ability: The gel's ability to form a removable film was another key aspect of the evaluation. To test this, a sample of the gel was applied to a petri dish and allowed to dry. Observing the formation of a protective film upon drying helped ensure that the gel could create a lasting barrier for the active ingredients. This property is important for maintaining the gel's therapeutic effects over an extended period [24].

5. Drying Time: The drying time of the gel was measured to determine how quickly it could form a film after application. A shorter drying time is generally preferred as it allows the user to resume normal activities without a lengthy waiting period. The drying time also impacts the overall user experience, as a quick-drying gel is more convenient and practical for everyday use [25].

6. Film Folding Endurance: Film folding endurance refers to the gel's ability to withstand repeated folding without breaking. This test involved applying a layer of the gel to a surface, allowing it to dry, and then repeatedly folding the formed film. The number of folds the film could endure before breaking was recorded. High film folding endurance indicates good flexibility and strength, which are desirable qualities for a gel intended to provide a protective barrier on the skin [26].

3. RESULTS AND DISCUSSION

3.1. Thin Layer Chromatography of Curcumin

TLC is an indispensable tool in the analysis of plant extracts, offering a straightforward yet powerful means to separate, identify, and analyze the myriad of chemical constituents present in plants. TLC is a widely used laboratory technique for separating and analyzing compounds within a mixture. It is commonly employed for compound identification, purity assessment, and reaction monitoring.

The TLC analysis of curcumin demonstrated effective separation and identification of the compound. The observations under UV light at 254 nm and 366 nm, as well as under white light, confirmed the presence one spot signifying the purity of curcumin in the sample. The Rf value of 0.745 aligns with reported literature range of 0.6-0.79, validating the accuracy and reliability of the TLC method used in this experiment [27]. This consistency in result indicates that the experimental conditions were appropriate, and the TLC method effectively separated and identified curcumin.

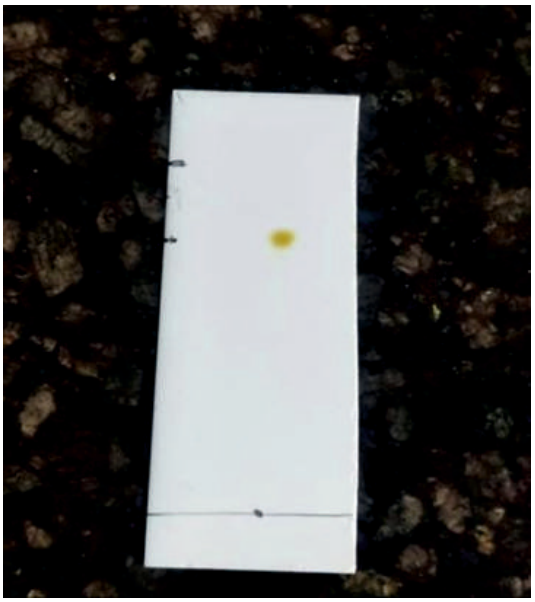

Figure 1: TLC plate image of Curcumin under white light

3.2. In Silico Analysis of Anti-inflammatory Properties of Curcumin

The study demonstrated a dose-dependent reduction in protein denaturation with increasing concentrations of curcumin. 50 µg/ml curcumin exhibited a moderate reduction in protein denaturation compared to the control, indicating initial anti-inflammatory activity, 100 µg/ml curcumin showed a significant decrease in protein denaturation, with absorbance readings markedly lower than the control, demonstrating enhanced protein stabilization and 150 µg/ml curcumin achieved the highest inhibition of protein

denaturation, reflected in the lowest absorbance values, underscoring its potent anti-inflammatory effect. The calculated percentage inhibition values confirmed the effectiveness of curcumin at all tested concentrations, with higher concentrations yielding greater inhibition. This suggests that curcumin's anti-inflammatory properties are both potent and dose-dependent.

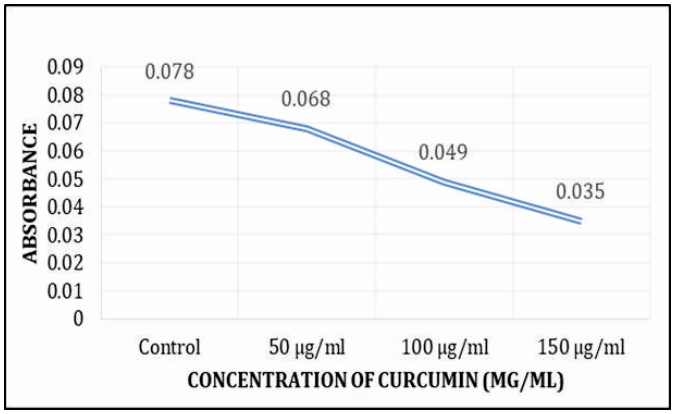

Figure 2: Anti inflammatory potential of curcumin depicted in graph plot of Concentration of curcumin Vs Absorbance

3.3. Preparation and optimization of curcumin film forming gels

Curcumin, a bioactive compound derived from turmeric, has gained attention due to its anti-inflammatory, antioxidant, and anticancer properties [28]. However, its poor water solubility and low bioavailability pose challenges in topical drug delivery. To address these issues, curcumin can be incorporated into a film-forming gel, enhancing its stability and facilitating controlled release upon application.

10 batches of curcumin film-forming gel were formulated using HPMC K100LV and K100M as bioadhesive gelling agents [29] with Eudragit RL 100 serving as the film-forming agent [30]. PEG 400 was incorporated as a plasticizer, and 95% ethanol was used as a solvent for both Eudragit RL 100 and curcumin. Aloe vera mucilage was included as a penetration enhancer in the formulation.

3.4 Evaluation of Curcumin loaded film forming gels

Initially, four batches (A-D) were prepared using varying concentrations of HPMC K100LV and Eudragit RL 100 as gelling and film-forming agents. The curcumin content was fixed at 1%, PEG 400 at 3%, and aloe vera mucilage at 1%. The ethanol and water ratios were adjusted to achieve quick drying and non-sticky films. The formulations were then evaluated for various parameters, including appearance, pH, drying time, and spreadability. All the batches were uniformly dispersed and had a translucent yellow color, indicating good dispersion of curcumin in the formulations. The pH values of the formulations ranged between 4.62 and 6.02, which is within the acceptable range for topical applications, ensuring skin compatibility. It was observed that the drying time increased with the amount of water added to the formulation. This suggests that water content plays a crucial role in the drying properties of the film. The spreadability of the formulations was relatively low, indicating that the consistency of the gel was not ideal. This was a concern as it could affect patient compliance and the effectiveness of the formulation.

Table 4: Composition and Observations of prepared batches with HPMC K100 LV

Ingredient	Batch A	Batch B	Batch C	Batch D
Curcumin (Active)	0.01	0.01	0.01	0.01
HPMC K100 LV	0.012	0.015	0.012	0.018
PEG 400	0.03	0.03	0.03	0.03
Aloevera Muilage	0.01	0.01	0.01	0.01
Eudragit RL 100	0.008	0.01	0.008	0.01
Ethanol (95% v/v)	0.3	0.4	0.65	0.65
Distilled Water	0.642	0.5356	0.3	0.3
Observation				
pH	4.62	5.3	6.02	5.67
Drying Time	15 min	13 min	8 min	8 min
Spreadability	4.2 cm	4.1cm	4.5 cm	3.9 cm

To address the issues with consistency and improve patient compliance, the formulation was further optimized by using HPMC K100M, a different grade of HPMC that offers better gelling properties. Six additional batches (E-J) were prepared, following the same criteria of achieving a gel-like consistency and quick-drying film. The new formulations (Batches E-J) exhibited improved gel-like consistency, with spreadability values ranging between 1.7 and 2.8 cm. This indicated a better balance between the gel structure and its ability to spread evenly on the skin. Among the six batches, Batch J emerged as the ideal formulation. It demonstrated the lowest drying time of 2 minutes and 20 seconds, striking a balance between quick drying and adequate spreadability. This formulation formed a durable, flexible film that adhered well to the skin, which is crucial for providing protection and supporting the healing process.

Table 5: Composition and Observations of prepared batches with HPMC K100 M

Ingredient	Batch E	Batch F	Batch G	Batch H	Batch I	Batch J
Curcumin (Active)	0.01	0.01	0.01	0.01	0.01	0.01
HPMC K100 M	0.004	0.008	0.012	0.02	0.004	0.0125
PEG 400	0.03	0.03	0.03	0.03	0.03	0.03
Aloevera Mucilage	0.01	0.01	0.01	0.01	0.01	0.01
Eudragit RL 100	0.008	0.008	0.008	0.01	0.0125	0.015
Ethanol (95% v/v)	0.64	0.63	0.63	0.65	0.635	0.6225
Distilled water	0.3	0.3	0.3	0.3	0.3	0.3
Observations						
Drying Time	3 min 15 sec	4min 30 sec	4 min	3 min	5 min 30 sec	2 min 30 sec
pH	5.4	5.14	6.8	5.28	5.34	5.87
Spreadability	2.7 cm	2.7 cm	2.9 cm	2.5 cm	2.8 cm	2 cm

The study successfully optimized a topical formulation

incorporating curcumin, PEG 400, and aloe vera mucilage. The final formulation (Batch J) met the desired criteria, offering a gel-like consistency, quick drying time, and adequate spreadability, making it suitable for topical application. The formulation's ability to form a durable, flexible film that adheres well to the skin suggests its potential effectiveness in providing protection and aiding in the healing process

4. CONCLUSION

The film-forming gel for wound healing is successfully developed using Curcumin as an active ingredient and Eudragit RL100 as a film-forming agent. The film-forming gel will provide a durable and effective barrier against external contaminants, significantly reducing the risk of infection. Formulation Batch J containing 1.25% HPMC K100 M, 1.5% Eudragit RL100, 1% Curcumin, 1% Aloe Vera, 62.25% Ethanol (95% v/v), 3% PEG 400, and 30% water was selected as an optimum formula due to acceptable physical properties and drying time. The bioadhesive properties and soothing effects of the gel will enhance patient comfort, encouraging compliance with the treatment regimen. The anti-inflammatory activity of curcumin was performed which signified that it reduces inflammation on the skin and thereby healing the burn wound. With the gradual release of therapeutic actives, the gel is expected to accelerate the healing process, reducing recovery times and improving patient outcomes. The development of this innovative gel formulation will represent a significant advancement in burn wound management, contributing to the broader field of wound care research and technology. Burn wound management requires innovative solutions to overcome the challenges of infection and delayed healing. This project seeks to develop a specialized film-forming gel that not only protects the wound but also promotes faster and more effective healing. By leveraging advanced materials and bioactive compounds, the formulation aims to improve patient care outcomes and set new standards in burn wound treatment. The successful development and implementation of this gel could revolutionize burn wound management, offering a potent tool for healthcare providers and significantly enhancing the quality of life for patients suffering from burn injuries.

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LEAD IDENTIFICATION FOR TREATMENT OF PCOS-A COMPREHENSIVE REVIEW ON CHROMONE ANALOGUES

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ABSTRACT

Polycystic ovarian syndrome (PCOS) affects about one in ten women globally, making it one of the most prevalent endocrine disorders. Its symptoms, such as insulin resistance, hirsutism, hyperandrogenism, and weight gain, vary widely among patients. Despite the variety of treatments available, including lifestyle changes, medications like insulin sensitizers, and surgeries, there is no definitive cure for PCOS. Treatments like ovarian drilling and cosmetic interventions may alleviate specific symptoms but fail to address the root causes.

Additionally, psychological, and occupational stress, coupled with infertility, worsens the condition for many women. This complexity has prompted the exploration of new therapeutic options. Recent attention has turned to coumarins, natural compounds with potential benefits in managing PCOS due to their anti-inflammatory, anti-androgenic, and insulin-sensitizing properties. These properties suggest that coumarins, when combined with existing treatments, could offer more holistic management of PCOS.

Future therapeutic strategies may also involve polyherbal formulations, which could lower costs, reduce side effects, and improve overall treatment outcomes by targeting multiple facets of PCOS. The inclusion of coumarins as a natural, widely available resource highlights the potential for developing novel treatments that integrate traditional herbal medicine with modern medical approaches

KEYWORDS: PCOS, Hyperandrogenaemia, Insulin, Herbal, Nutraceuticals, Coumarin

INTRODUCTION

PCOS is also termed as Stein-Leventhal Syndrome. The actual prevalence of PCOS is unclear, although it varies greatly over the world from 2.2 to 26%. As per Rotterdam's criteria, it has been studied that South India and Maharashtra has prevalence reported as 9.13 and 22.5 %. PCOS has a prelude with noticeable anomalies that existing as the metabolic syndrome like obesity, dyslipidaemia associated with decrease in high density lipoprotein cholesterol and hypertriglyceridemia, high blood pressure, atherosclerosis, gestational diabetes, increased risk of development of type II diabetes, respiratory tract infection with cough and cardiovascular diseases for prolong time. [1] As per the researchers, PCOS largely affects the females of age group of 18-44 years. [4] There are mild to severe variations for each symptom. Hyperandrogenism, a biochemical hallmark for PCOS, is the excessive level of androgens marked in female mainly responsible for PCOS include cutaneous indicators such as male pattern baldness, alopecia and hirsutism which results from disturbances in GnRH pulses that give rise to increased level of LH/FSH ratio. [5][6]

The enhanced testosterone effect is a result of both the overproduction of testosterone and the decreased sex hormone binding globulin (SHBG) levels seen in PCOS, both of which are brought on by excessive insulin. Unusually irregular amount of testosterone hormone in the ovaries block ovulation which leads to infertility. According to the literature, eating high-calorie foods like milk, fruits, and starchy foods during menstruation causes vayu to build up in the uterine cavity. This causes the organ's blood flow to be limited, which in turn causes obesity, abdominal pain, and ultimately conception failure. [2] [3] Aromatase enzyme converts androgen to estrogen so this may be responsible for the syndrome to occur as low aromatase activity has also been demonstrated in women with PCOS. To maintain optimal insulin levels, a low glycemic index diet (GI) is advised, consisting of lentils, fruits, raw pineapple, carrots, cherries, cereals, and whole grain wheat. A fried, spicy, oily, processed, and junk food are to be avoided. [28] Table 1 enlists the common symptoms associated with PCOS.

EPIDEMIOLOGY OF PCOS:

Ethnic prediction and hereditary are one of the factors. The pituitary gland secretes irregularly high amount of Luteinizing hormone in the blood stream interrupting the normal menstruation cycle of a woman ultimately creating imbalance between progesterone, estrogen, FSH and LH leads to PCOS causing anovulation. [9] Cyst or fluid filled sacs is nothing but an undeveloped follicle which remains in undissolved state as illustrated in Figure 1. Hyperandrogenaemia cannot be diagnosed by measuring the blood testosterone levels. [10],[14],[15]

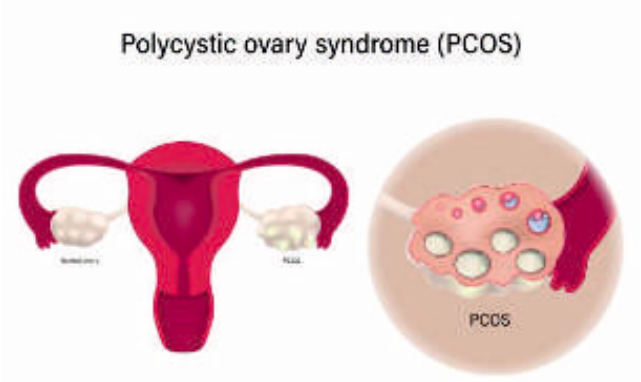

Figure 1: Schematic explanation of polycystic ovarian syndrome [11]

Table 1: Common symptoms of PCOS [14]

Period Problems	Irregular Periods, Period Cramps, Spotting in between Periods, Heavy Bleeding, Scanty Menstrual Flow
Poor Fat Profile	High Cholesterol, High Triglycerides, Fatty Liver
Poor Mental Health	Depression, High stress, Anxiety, Mood swings, Irritability
Hair Health	Hair loss or Alopecia, Hirsutism: Excessive hair on the face, arms, neck
Sleep Problems	Inability to sleep, Waking up in the middle of the sleep, Sleep Apnoea
Skin Problems	Cystic Acne, Dark spots on the skin, Skin Tags, Canker sores, Bleeding gums
Cysts and Lumps	Ovarian cysts, Benign cysts in breast or other body parts, Follicular cysts
Digestive Health	Bloating and Constipation, Uncontrolled hunger, Sugar cravings, Food cravings
Difficulty to get Pregnant	Frequent miscarriage, Inability to conceive, Infertility, Low sexual drive
Weight Problems	Digestive Health

Excess of androgen leads to acne which are very difficult to treat, hirsutism and androgenic alopecia/ baldness. [7] Figure 2 depicts the signs and symptoms associated with PCOS. Long term risk associated with PCOS are Diabetes, Hypertension, Heart disease, Dyslipidemia, Endometrial cancer, Breast cancer and recurrent pregnancy loss. [8] Reduced Melatonin concentration in the follicles and mild sleep disturbances have been found among PCOS women.

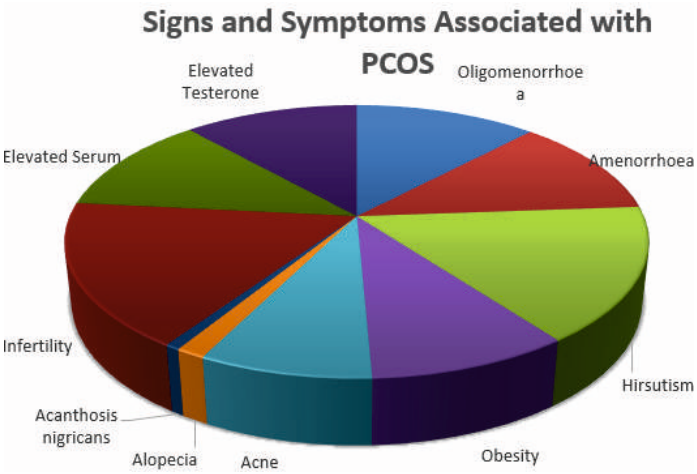

Figure 2: Signs and Symptoms associated with PCOS

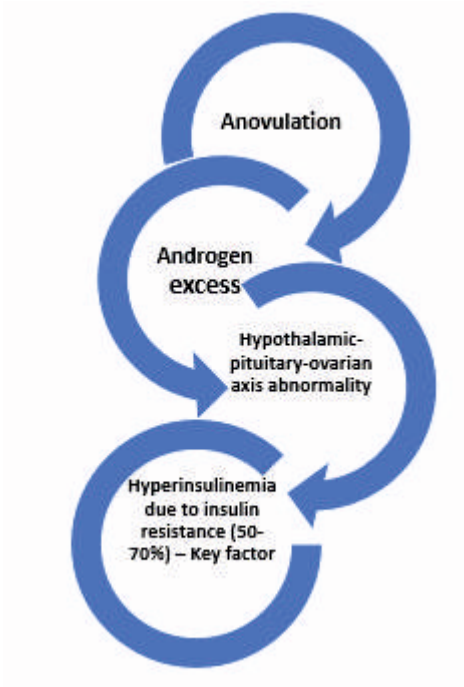

Figure 3: Factors responsible for PCOS

Allopathic: The Allopathic remedies available for PCOS are enlisted in Table 2. Surgical methods like Laparoscopic ovarian drilling by Laser, Cyst Aspiration and Oophorectomy also available as one of the treatment methods. [6]

Table 2: Treatment Available for PCOS [38]:

MOA	Drugs
A specific gonadotropin-releasing-hormone agonist	Nafarelin
Insulin sensitizing and ovulation improving agent	Troglitazone
Non-steroidal selective estrogen receptor modulator, ovulation improving agent	Tamoxifen
Partially selective estrogen receptor modulator	Clomiphen citrate
Inhibits hepatic gluconeogenesis, decreases serum lipids and androgen	Metformin
Blocks androgen receptor and prevent testosterone from binding and exerting hirsutism	Spironolactone
Blocks Estrogen receptor by inhibiting negative estrogen feedback	Estrogen
Inhibits GnRH and decreases the LH secretion	Progestins
Hirsutism treatment	Eflornithine

The GnRH agonist like Nafarelin is typically used in severe cases where other treatments fail. Its prolonged use reduces ovarian hormone production, addressing both menstrual irregularities and hyperandrogenism, although it is often a second-line treatment due to side effects like menopausal symptoms. An insulin sensitizer (Troglitazone) that addresses the underlying insulin resistance in PCOS. However, glitazone derivatives have been withdrawn in many countries due to concerns over liver toxicity. Alternatives like metformin are now more commonly used. Tamoxifen and Clomiphene Citrate induce ovulation in women with PCOS, especially those seeking fertility treatments. Clomiphene is more commonly used than tamoxifen, but tamoxifen can be an alternative for clomiphene-resistant cases. For managing hirsutism and acne in women with PCOS Spironolactone is used to provides symptomatic relief However its anti-androgenic effects are cause for concern. Another alternative is Eflornithine used topically and / or systemically. Though it reduces hair growth but does not affect the underlying hormonal

imbalance Often used in hormonal therapies progestin and estrogen regulates the menstrual cycle, preventing endometrial hyperplasia in PCOS patients. Such combination of progestins and estrogen is prescribed for long-term management.

Herbal: Natural alternatives available for treatment of PCOS are adequate intake of Calcium, Vitamin D, and Magnesium (low levels of Mg associated with Diabetes). It also includes increased intake of Chromium & Turmeric (essential for insulin and blood glucose level), omega-3 supplements (decreases Androgen level in women with PCOS), rich fibre diet, anti-inflammatory foods like olive oil, tree nuts, tomatoes, leafy green vegetables, fatty fish like Mackerel and Tuna, totally cut off the coffee as it causes changes in estrogen level.[39],[40],[41] Auraptene is a natural bioactive monoterpene coumarin isolated from Citrus aurantium and Aegle marmelos (family Rutaceae) has clinically proven acting via anti-inflammatory effect as the inflammatory mediators like TNF α increases in PCOS patients and eventually induces apoptosis in antral follicles granulosa cells. [29], [30] Vitamin B12 and Vitamin B9 are mostly prescribed as the level of homocysteine has been found increased in the blood eventually causing damage to the lining of arteries, increases formation of blood clots in blood vessels and high chances of blockage of blood vessel.[12] Vitamin B12 and Vitamin B9 (folate) helps to lower the inflammation by breaking down amino acid homocysteine level in the blood. Vitamin C and Vitamin E are also prescribed to protect the blood from clotting.[13] Poppy seeds one of the home remedies contains Magnesium, Calcium, Vitamin D, Zinc provides nutrition and acts as anti-inflammatory.[16] Mohammed Azeemuddin et al reported in the study that a multiherbal preparation "DXB-2030" formulation consisting of Trigonella foenum-graecum, Aloe vera, Sphaeranthus indicus, Nardostachys jatamansi, and Symplocos racemosa extracts was found to be successful in managing PCOS and can be suggested as a line of treatment.[67] Eating healthy, balanced diet rich with green vegetables, getting good sleep, and keeping oneself away from stress is recommended as a best effective medicine by Gynaecologist. The hepatic synthesis of the sex hormone binding globulin is decreased by hyperinsulinemia, which increases the levels of circulating androgens. Insulin resistance affects both adipose tissue and skeletal muscle. The hepatic synthesis of the sex hormone binding globulin is decreased by hyperinsulinemia, which increases the levels of circulating androgens. Insulin resistance affects both adipose tissue and skeletal muscle. [17]

As per Ayurvedic literature, similar kind of features are observed in Pushpadhni jataharini (disease like polycystic ovarian syndrome with hyperandrogenism and irregularity in menstrual cycle) and Nashtartva (improper growth of follicles and frequent anovulatory cycle). It encompasses the inequality ratios of dosha, dhatu and upadhatu. [18]

NUTRACEUTICALS AVAILABLE FOR TREATING PCOS:

Allopathic treatment, despite of causing severe side effects, is used majorly in the society for women suffering from PCOS. To overcome these problems, many Research Scientists from the industry are working on nutraceuticals and recently some nutraceuticals are available in the market as a symptomatic treatment. A Nutraceutical powder formulation launched by Unived industry containing D-chiro Inositol (DCI) and Myoinositol with the composition of 1:40. Myoinositol is a natural source obtained from carob seeds responsible for reducing Insulin resistance. D-chiro Inositol can be synthesized from Myoinositol. Vegan capsules/powder as a nutraceutical contains D-chiro Inositol: Myoinositol in the range of 1:40, key supportive nutrients like alpha lipoic acid for obesity, chromium picolinate to reduce glucose level, Vitamin D3, folates for cellular metabolism, natural calcium from algae to increase the bone density. [31] Higher DCI concentration is used to compensate epimerase inactivity. Epimerase is the enzyme responsible for properly release of insulin

after signalling. For the fertility treatment, regularization of menstrual cycle and healthy ovarian functioning D-chiro Inositol (Caronositol): Myoinositol capsules in the ratio of 1:3.6 can be used as its clinically tested, proven, and has shown 65.52% fertility success rate in the clinical trials. A nutraceutical named Lipifuse contains L-Carnitine Tartrate, Alpha Cyclodextrin, Phaseolus vulgaris extract, Coffee bean extract tablets under its brand name. [32]

COUMARIN

Plant origin of Coumarin [23][21]: Scientist Vogel (1820) separated coumarin first from tonka beans (Dipteryx odorata) using extraction technique. Then subsequently it was identified in many plants belonging to different families such as Apiaceae, Caprifoliaceae, Clusiaceae, Guttiferae, Nyctaginaceae, Oleaceae, Rutaceae and Umbelliferae.

Table 3: List of Plants Containing Coumarin

Botanical Name	Plant	Family
Coumarouna odorata	tonka bean	*Leguminosae*
Anthoxanthum odoratum	Vanilla grass	*Poaceae*
Melilotus alba and Melilotus officinalis	sweet clover	*Fabaceae*
Glycyrrhiza glabra	liquorice	*Fabaceae*
Cinnamomum cassia	cassia cinnamon	*Lauraceae*
Asperula odorata	sweet woodruff	*Rubiaceae*
Myroxylon pereirae	balsam of Peru	*Fabaceae*
Justicia pectoralis	chamba	*Acanthaceae*
Prunus serrulata	cherry blossom	*Rosaceae*

Coumarin was found to be present in majorly in certain herbs particularly Thuja occidentalis, Tephrosia purpurea, Camellia sinensis, Labisia pumila, Nardostachys jatamansi, Tribulus terristris, Symplocos racemosa, Aloe barbadensis, Commiphora mukul, Mentha piperita, Corylus avellana, Commiphora wightii, Cimicifuga racemosa, Cinnamomum cassia, Curcuma longa, Glycyrrhiza spp., Matricaria chamomilla, Paeonia lactiflora, Silybum marianum, and Vitex agnus-castus [9], [19], [20] Coumarin is from plant origin and oils are rich source of coumarins like cinnamon bark oil (0.1-1.6% or 7000 ppm (mg/kg)), cassia leaf oil (0.03-0.08% or 17000-87300 ppm), peppermint oil (20 ppm), cinnamon leaf oil (40600 ppm), also found in lavender oil, sweet clover oil, woodruff, whereas Australian cassia recorded 15.3% of coumarin. [22] Approximately 1200 coumarin has been recognized from different plant origins till date. According to the studies conducted in Pakistan on fresh mature seeds of Ferula foetida indicates mixture of coumarins approximately 7.5-7.8%. It has a very good skin penetration ability. [23]

To establish the relationship between these herbal components and anti-PCOS action, a literature survey led to very less yet convincing data pertaining to utility of coumarin. A preliminary study on rat models' liquorice had been found to alleviate the symptoms of PCOS hinting connection with regulation of imbalanced hormonal levels. [24]

Utility of Cinnamon powder had been proved to be effective in PCOS through Clinical trials. [25] Among other related compounds, Auraptene had been found to be effective in improvement of oocyte maturation and fertilization rate in PCOS Mouse Model. [26]

Synthesis of Coumarin:

Coumarin is widely distributed in plant kingdom, so extraction techniques can be used to obtain coumarins; but for commercial use it has been mostly synthesized in the laboratory. Various methods can be used to synthesize coumarins like from (Phenol) Pechmann reaction- green synthesis, (Salicylaldehyde) Knovengel condensation (Salicylaldehyde) Perkin reaction, (O-hydroxyaryl ketones) Kostanecki acylation, (Phenol) Pschorr synthesis, Reformatsky, Wittig reaction. By using different means several numbers of coumarins can be synthesized. [27] Coumarin possesses immunomodulatory, antitumour, antifungal, antiviral, antibacterial, antitubercular, anticoagulant, anti-inflammatory, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, antioedematous, anti-HIV and neuroprotective activity. [23]

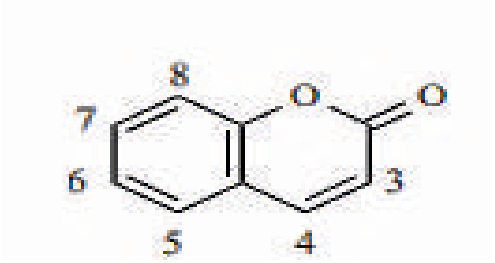

Figure 4: Structure of Coumarin

Challenges and Opportunities while treating PCOS-

Although coumarins may be manufactured in a wet lab using a number of synthetic processes, there are certain challenges in using coumarins as ligands to treat PCOS.

a) The research does not yet show evidence of target-specific treatment. The illness is managed by keeping an eye on the symptoms and the ovarian structure.

b) No biological testing is mentioned in the literature for synthetic chromone counterparts. Chromone extraction and activity have been documented in the literature. The development of appropriate animal models has emerged as an important area of research to better understand the genesis and association of metabolic factors to the outcome of chronic disease in PCOS

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c) Computer simulations: Chromones are not investigated in computer simulations for managing PCOS. No information was discovered in the older records.

d) No reports have been discovered about the use of synthetic analogues to treat PCOS.

Certain herbs can be used to screen for PCOS are classified as follows in Table 4

In PCOS, uncharacteristically high amount of Luteinizing hormone was released by the pituitary gland in blood stream which eventually upsets the normal menstruation cycle of a patient. Consequently, it affects the maturity of the follicle and subsequently ovulation which leads to anovulation. The undissolved immature follicle remains as fluid filled sacs or cysts. These cysts bring about a hormonal imbalance due to an increased amount of testosterone. The outcome is production of acne in facial and body hair. Table 2 illustrates the list of various herbs to be used with their appropriate body parts containing coumarin for the management of PCOS.

Literature survey of various related studies of coumarins acting as anti-PCOS agent reveals some possibilities of mechanism of actions. Coumarins could act as insulin sensitizers, like metformin or thiazolidinediones (e.g., troglitazone). Several studies have shown that coumarins improve insulin sensitivity by modulating glucose uptake, enhancing insulin signaling pathways, and reducing insulin resistance, which is central to the pathology of PCOS. Studies on certain coumarin derivatives, such as esculetin and umbelliferone, have demonstrated their ability to lower blood glucose levels and improve insulin sensitivity in diabetic animal models. [33]

PCOS is associated with chronic low-grade inflammation and oxidative stress, both of which contribute to insulin resistance and hyperandrogenism. Coumarins have well-documented anti-inflammatory and antioxidant effects, which could mitigate these underlying issues in PCOS. Coumarins like scopoletin and 7-hydroxycoumarin may inhibit the nuclear factor-kappa B (NF-κB) pathway, reducing the production of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β, which are elevated in PCOS. Furthermore, they could upregulate antioxidant enzymes such as superoxide dismutase (SOD) and catalase, neutralizing reactive oxygen species (ROS). [34] Coumarins could potentially act as anti-androgenic agents, modulating the synthesis and action of androgens. This could be particularly relevant for women with PCOS, as hyperandrogenism leads to symptoms like hirsutism, acne, and anovulation. Although direct evidence of coumarins' effect on

androgen synthesis in PCOS is limited, compounds such as coumarin derivatives from Angelica sinensis have been shown to reduce testosterone levels in animal models, providing a basis for further exploration. In this connection there is a report of coumarin analogue with new aromatase inhibitors will be worth mentioning. [35]

There is a possibility that coumarins may help regulate ovarian function by restoring hormonal balance, particularly the levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In fact, Coumarin based selective estrogen receptor modulators (SERMs) and coumarin-estrogen conjugates have also been described as potential anti-breast cancer agents, advocating this prospect. [36]

Coumarins may have a role in modulating lipid metabolism and providing cardiovascular protection. Coumarins such as esculin may inhibit lipid peroxidation, improve cholesterol metabolism, and reduce serum triglyceride and LDL cholesterol levels, which are often elevated in women with PCOS. [37]

The coumarins have been known to have a vast number of pharmacological effects and reach to systemic circulation. Hence, possibility that it is acting on many systematic targets including the hormonal sites, CNS sites and offshore sites cannot be negated. In future more would be explored about the targets in PCOS and hence design of coumarin analogues or testing of known analogues on them will be possible through all computational, in-vitro and in-vivo models.

Among many challenges to treatment of PCOS by herbal components is one misconception of complete safety of herbal products, especially in Indian subcontinent. With the complex nature of disease it can be speculated that it will involve long-term treatments, hence long-term safety of herbal treatments for PCOS, particularly when combined with conventional medications, would be a cause of concern. Herbal compounds can interact with conventional medications used to treat PCOS, such as metformin, clomiphene, or spironolactone. These interactions may either enhance or inhibit the effects of these drugs, leading to adverse reactions. For example, Angelica sinensis (Dong Quai) has coumarin derivatives, which have mild anticoagulant effects. When combined with medications like spironolactone, it may increase the risk of bleeding. The coumarins are known to have inducer abilities to cytochrome P450 system, potentially reducing effectiveness of co-administered drugs. Another hiccup with Herbal remedies is lack of standardization, which may lead to inconsistent dosages. Third cause of concern will be side effects like liver toxicity or exacerbate hypertension if used long-term or in high doses. For example, berberine, while helpful for insulin resistance, may lead to gastrointestinal upset and affect liver enzyme levels when used chronically. Hence it implies that long-term clinical trials to assess their safety in treating chronic conditions like PCOS will be required which is unavailable as of now. Also, Regulatory Oversight over herbal remedies can result in contamination, poor-quality ingredients, or variations in active compounds, leading to unpredictable outcomes when used with PCOS medications. If consultation, standardization, and ADR monitoring is observed a safer usage can be ensured.

CONCLUSION:

PCOS, being a multifactorial disorder, number of symptoms at a time in the patients. Patients with PCOS experience undergoing repeated fertility treatments like IVF, IUI with poor pregnancy outcomes. Not all PCOS symptoms can be managed with birth control pills. Many women have discovered natural remedies for PCOS and other restorative concerns, which have improved their long-term health. Despite being a condition, evidence suggests that reducing inflammatory reactions and apoptosis rates in PCOS ovaries can improve the oocytes' capacity for IVF and in vitro maturation. Despite being a condition, evidence suggests that reducing inflammatory reactions and

apoptosis rates in PCOS ovaries can improve the oocytes' capacity for IVF and in vitro maturation. This study focuses on several herbs that can be used to treat PCOS depending on the symptoms. Coumarin is widely utilized in the global market and is of natural origin. Table 1 lists the number of herbs utilized for the symptomatic and centrally acting therapy of PCOS. Future research can be planned by executing biological activities on animal models and determining the mode of action for centrally and peripherally acting coumarins. Given the opportunities and challenges, there should be plenty of potential for pharmaceutical discovery and development.

NA1:D12ame of the Herb	Botanical name	Parts used	Pharmacological activities
Centrally acting			
Ashwagandha	*Withania somnifera*	Leaves	Stress levels are minimised by balancing Cortisol levels [42]
Maca Plant	*Lepidium meyenii*	Roots	Hormonal balance and in Depression [43]
Liquorice	*Glycyrrhiza glabra*	Roots	Antiandrogenic and reduces blood glucose level [44], [76]
Cinnamon	*Cassia cinnamon*	Bark	Reduce Insulin resistance, help to regulate menstruation [45], [77]
Fenugreek	Trigonella foenum	Seeds	Reduce Insulin resistance, Lowers the Cholesterol, reduce the size of ovarian cysts, Regularize the menstrual periods [46], [78]
Shatavari	*Asparagus racemosus*	Leaves, Powder	Regulates Menstrual cycle [56]
Holy Basil	*Ocimum sanctum*	Leaves, Seeds	Antiandrogenic [47], [79]
Black Cohosh	*Actaea racemose*	Roots	Induce ovulation [48]
Peppermint	*Mentha piperita*	Leaves	Antiandrogenic [49], [80]
Maitake Mushroom	*Grifola frondose*	Extract	Induce ovulation and in management of Diabetes [51]
Varuna	*Crataeva nurvala*	Powder	Helps to reduce cyst size, and normalize the menstrual cycle [52]
Flax seeds	*Linum usitatissium*	Seeds	Suppression of Testosterone levels and Hirsutism [53]
Sunflower	*Helianthus annuus*	Seeds	Promote progesterone development [54]
Turmeric	*Curcuma longa*	Powder	Antiandrogenic [53]
Shatapushpa	*Anethum graveolens*	Seeds	Reduce insulin resistance [54]
Chamomile	*Chamomilla recutita*	Flower	Reduces Luteinizing hormone and Improves Ovarian Morphology [55]
Stinging Nettle	*Urtica dioica*	Roots	Reduces level of free Testosterone by increasing production of sex hormone binding globulin [57]
Fennel	*Foeniculum vulgare*	Extract	Reduces Testosterone level, increases the serum levels of FSH and decreased the LH hormones [58]
Pumpkin	*Cucurbita pepo*	Seeds	Manage Cholesterol Level, Anti-androgen, and Treat Hirsutism and prevent hair loss [65]
Peripherally acting			
Guduchi	*Tinospora cordifolia*	Herb	Hypoglycemic [56]
Bael/ Bilva	*Aegle marmelos*	Fruits	Helps in producing more amount of Insulin, provide nourishment to hair, Improves lactation [59]
Sesame	*Sesamum indicum*	Seeds	Regulate blood glucose level [61]
Amla	*Phyllanthus emblica*	Extract	Hormonal balance and Reduces Cholesterol [60]
Red Clover	*Trifolium pratense*	Flower	Purify blood and treat Acne associated with PCOS [62]
Green Tea	*Camellia sinensis*	Extract	Controls Insulin Level [63], [81]
Punarnava	*Boerhaavia diffusa*	Powder	Used to reduce obesity and regulates blood sugar level [64]
Chia	*Salvia hispanica*	Seeds	To inhibit or normalize insulin resistance [66]

Table No.4: List of herbs used in PCOS treatment with their role

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DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Comparative analysis of granulation equipment and their influence on granule properties

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ABSTRACT:

Plain granules were manufactured without drug by using the granulation equipments like high-shear mixture granulator and fluid bed granulator. Various parameters such as size, shape, density and a variety of different flowability measurements were used to compare the granules. This comparison depicted that granule formation was dissimilar for both the equipments. The high-shear mixer granulation requires short process time and demonstrated better overall flow properties as compared to the fluid bed granulator. The granules formed after using high-shear granulator were denser as compared to the granules formed from fluid bed granulator which were less dense. Even though the make-up of the granules was the similar for high-shear mixture granulator and fluid bed granulator, the properties of the granules were dissimilar, resulting in differences in flow characteristics. The granules formed by high-shear mixture granulator are large & spherical in shape with low porosity, high bulk/tapped density, wide range of particle size distribution, whereas the granules formed by fluid bed granulator with Standard distribution plate are small in size, irregular in shape, have lower value of bulk/tapped density, narrow particle size distribution and high porosity. It was observed that both Carr's index and Hausner's ratio were satisfactory for high-shear mixture granulator, whereas they were only acceptable for Fluid bed granulator.

The varying mechanisms of granule growth have significantly affected both the particle size distribution and structure of the granules, depending on the equipment used. Evaluation of granule parameters concluded that the high-shear granules demonstrate superior flow characteristics than granules produced by the fluid bed granulator.

Key Words: Powder flow, fluidized-bed granulation, particle size, high-shear mixture granulation, density

INTRODUCTION

Powder flow is key requirement for pharmaceutical manufacturing process and depends on many factors of which granulation being one of the most important and critical factor. Granulation is a procedure during which the drug and excipients are mixed together by adding binding agents to form larger particles called granules[1]. Granulation process renders the material free flowing & enhances the consistency of drug distribution throughout the product. It also improves the appearance of the tablet & prevents the segregation of powder material in mixture. The process improves compaction characteristics of the powder which helps in formulation of tablets. Granulation is a crucial process that involves various challenges, including achieving size uniformity and managing physicochemical properties such as size of the granule, bulk density/tapped density, porosity, granule hardness, moisture content of granules, and compressibility, as well as ensuring the drug's physical and chemical stability[2]. The powder has the tendency to absorb moisture from atmosphere and may adhere and form cake. Whereas after granulation, granules absorb some amount of moisture and retain their flowability because of their size, thus granulation plays a very significant role in product quality & efficacy.

There are many types of equipment used for carrying out wet granulation where High Shear Mixture Granulator (HSMG) & Fluidized bed granulator (FBG) are commonly used in many pharmaceutical companies. In FBG, all steps from granulation to drying are carried out within the same equipment. On the other hand, in HSG, after granulation, the granules typically need to be dried either by vacuum or microwave drying within the granulator itself, or more commonly, by transferring them to a FBG for drying[3]. The fluidized granulator method thus saves wage expense, transfer losses, and duration and can be fully automated once all factors affecting granulation are optimized. However, a fluidized bed granulator (FBG) is costly, and optimizing both process and product parameters is crucial, necessitating extensive developmental work during the initial stages of scale-up and production. In contrast, high-shear granulation involves less developmental work because of the fewer operational parameters and limited interactions.[4,5].
In the current article an attempt is made to compare formulation and evaluation of granules by using HSG and FBG.

MATERIALS AND METHODS:

Lactose, Microcrystalline Cellulose (MCC), Sodium Croscarmellose, maize starch and HPMC E5 were received from Ganson Pvt Ltd to make the placebo formulation. All materials were passed through standard US no. 20 mesh sieves. Equipments used were Gansons Jacketed High Shear Mixer Granulator (2.5 kg) and Gansons Fluidized Bed Granulator (10 kg) with Standard Air Distribution Plate

FORMULATION OF GRANULES:

Preparation of Binder Solution & Powder Blend

An HPMC solution was created by dispersing 50 g of HPMC powder (Grade E5) into 200 ml of distilled water and continuously stirring with a mechanical stirrer at room temperature until a homogeneous solution was achieved. Product intermediates for tablet preparation were obtained after accurately measuring the final quantities and then the batches were mechanically mixed in High Shear Mixture Granulator (Batch A) & Fluidized Bed Granulator (Batch B) for 10 minutes to ensure proper mixing. The model formulations consisted of Lactose (16%), MCC (54%), Sodium Croscarmellose (10%), Maize Starch (20%) & HPMC (12.5%).

The wet granulation formulations were prepared in HSMG & FBG where the materials that were to be granulated were first dried, mixed, and then the binding solution was added during mixing and subsequently the wet mass formed was wet sieved, dried and sieved again to control agglomerate growth.

EVALUATION OF GRANULES:

Moisture content[6,7]

The moisture content of the granules was measured by determining the loss on drying at 105°C using a Mettler Toledo HG63 halogen moisture analyzer. Twice samples of 5.0 g were evaluated.

The final loss of weight is calculated by,

Percent Moisture content = Moisture Loss (g))/ Initial Sample weight (g) ×100

Moisture Loss= Initial weight of sample (g) – Final weight of sample (g),

Initial weight of sample = Weight of sample before drying

Final weight of sample = Weight of sample after drying

Sieve Analysis[6,7]

Sieve analysis evaluates the PSD of granular materials by passing the material through a series of sieves with progressively smaller mesh sizes and measuring the quantity of material retained by each sieve as a proportion of the total mass. By using the mechanical shaker the procedure was carried out in 2 phases for 20

minutes each at high speed.

% Retained = (Weight Retained on Mesh (in grams)) / (Total weight Sieved (in grams))

Angle of repose[6,7]

The angle of repose is the angle between the horizontal base of the bench surface and the edge of a conical pile of granules. Once the cone of the sample is formed, the height (h) of the cone and the radius (r) of its base need to be measured.

The angle of repose *(θ)* is calculated by using the formula

$$\text{Angle of Repose } (\theta) = \tan^{-1}\left(\frac{h}{r}\right)$$

Bulk and Tapped Density[6,7]

Bulk and tapped densities were measured following USP methods. The bulk volume is determined by tapping the cylinder twice manually on a flat table surface.

Bulk Density of granules (ρb) =Weight of powder/Volume of powder

Tapped Density (ρt) = Weight of powder / Tapped volume of powder

Compressibility Index and Hausner's Ratio [8,9]

The bulk density and tapped density of granules are used to calculate Carr's compressibility index (CI) and Hausner's ratio (HR), which assess the flow characteristics and powder compressibility.

Compressibility Index = 100 ×(tapped density- bulk density)/(tapped density)

Hausner's Ratio = Tapped density/bulk density

Result & Discussion:

The size & shape of particles play a very significant role in compression of a tablet. If the particles are spherical they have less resistance to flow. The large size particles have wide size distribution whereas smaller particles have narrow size distribution. Smaller particles have good porosity than the larger ones[10,11]. Therefore for better compression particles should be large & spherical.

PSD (PSD) of granular material is a mathematical function that describes the relative amount, usually by mass, of particles present based on their size. Granules with a diameter ranging from 150 to 600 µm, a spherical shape, and uniform formulation components are deemed optimal. Particles with diameters under 150 µm are classified as fines, while those exceeding 600 µm in diameter are considered oversized.

Table1: Results of PSD for HSMG and Fluid bed granulator

Mesh Size	% Retained	
	HSMG	FLUID BED GRANULATOR Std. plate
10	2.63	0.45
20	5.44	2.07
44	47.7	21.4
52	26.82	16.59
85	91.52	18.62
100	36.9	17.16
200	64.98	12.15
Not Retained on 200	60.53	17.9

PSD has effect on the flowability which increases with particle size. Increasing the particle size decreases the bulk density and a wide PSD will lead to lower viscosity thus increasing flowability. In the present study the large agglomerates were observed for the high shear granulation contributing to its wide PSD and reverse was observed for FBG showing particles with narrow size distribution. The reason for different size distribution can be because of differences in granulation process including liquid spray rate of binder and processing time.

Table 2: Loss on Drying

Sr. No.	LOD
HSMG	5.85%
FLUID BED GRANULATOR Std. Plate	4.56%

When the moisture content was low in granules, the size distributions for both the equipments was found to be narrow. On the other hand, at high moisture level, the oversized agglomerates were formed by high shear granulation while the PSD for the FBG would not change significantly as even though the larger agglomerates are formed they would be breakable and irregular in shape with high porosity and consequently will break down during attrition and mixing[10,11].

Table 3: Powder Characteristics

MACHINES	POWDER CHARACTERISTIC					
	Mean Size	Size Distribution	Granules Shape	Granules Porosity	Bulk/Tapped Density	Flowability
HSMG	Large	Wide	Spherical	Low	High	Good
Fluid bed granulator std.	Small	Narrow	Irregular	High	Low	Poor

The table 3 results depicted that the granules formed by HSMG are large & spherical in shape. Besides the granules had low porosity, high bulk/tapped density & range of size distribution is wide, whereas granules formed by FBG are small in size, irregular in shape, have

lower value of bulk/tapped density, narrow PSD and high porosity in comparison to HSMG.

In the context of fluidized-bed granulations the formation of granules progresses slowly and steadily through the coalescence of nuclei followed by formation of small size of granules as the binder was sprayed continuously onto the bed. With low shear forces in FBG, the granules formed are irregular in shape and are highly porous.

Table 4. Comparative Evaluation Parameters of granules

	HSMG	Fluid bed granulator with Std. Plate
Bulk Density	0.314	0.1956
Tapped Density	0.351	0.2477
Angle of Repose	23.96	33.81
Carr's Index	10.39	21.03
Hausner's Ratio	1.116	1.266

High bulk/tapped density shows Good Flowability. The bulk density for HSMG is 0.3142, for Fluid bed granulator (Std.) is 0.1956. The tap density for HSMG is 0.3507, for Fluid bed granulator (Std.) is 0.2477 thus indicating HSMG shows good flow.

The assessment of flow properties of the granules are dependent on critical factors like size, shape, particle size distribution and density of granules. The flowability of the granules are important to manufacture the tablet with particular properties. Both compressibility and flowability are indicated by the Carr index and Hausner's ratio. A Carr index below 15 indicates low compressibility and good flow properties, while a value above 25 suggests a highly compressible powder with poor flow characteristics.

The Carr's index for HSMG was calculated to be 10.39 and for FBG (Std.) to be 21.03 similarly the Hausner's Ratio for HSMG was calculated to be 1.116; for FBG (Std.) to be 1.266 as per the table no. 4. As per the standards we can say that the Carr's Index and Hausner's Ratio for HSMG was good [12,13], for FBG (Std.) was passable. Thus result of Carr's index and Hausner's Ratio ensures excellent compressibility and flowability. The elevated Carr index for fluidized-bed granulations indicates porous granules that are likely to be compressible. However, these granules, being of lighter density and irregular shape, may experience flow challenges. The variation in the Carr index across fluidized-bed granulation trials reflects differences in particle size distribution, with larger granules—exhibiting better flow properties—being produced at lower fluidization velocities.

The static angle of repose also serves as an indicator of flowability. The angle of repose for HSMG was calculated to be 23.963 and for FBG (Std.) was 33.81 (Table 4). As per the standards we can say that the angle of repose of HSMG was excellent and for Fluid bed granulator (Std) was good. Thus both types of granulators decreased the angle of repose and thus ensures excellent flowability[14,15]. The measurement of angle of repose alone is not adequate to identify minute changes in flowability.

The size & shape play a very important role in compression of a tablet. If the particles are spherical, they have less resistance to flow. Also, the large size particles have wide range of size distribution whereas small particles have narrow range of size distribution with good porosity than the large ones[16,17,18]. Therefore, for better compression particles should be large & spherical in combination with few fines which can be used to fill the gaps between large particles [19,20].

Conclusion

The present research study concluded that wet granulation of powders can be effectively performed in HSMG and FBG. In these equipments it was essential to select appropriate operating conditions for the manufacturing of desired granules by wet granulation. In HSMG the machine parameters like impeller speed, jacket temperature & kneading time are important whereas in FBG; temperature, flow rate of fluidizing air & temperature of product is important. Due to the varying mechanisms of granule growth, the use of different equipment has significantly influenced the particle size distribution (PSD) and structure of the granules, as evident in the results. The evaluation of granule parameters led to the overall conclusion that high-shear granules demonstrate better flow properties compared to those produced by a fluidized bed granulator.

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Evaluation of in-vitro antiurolithiatic activity of ethanolic extract of Delonix regia, and its comparison with marketed formulations.

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ABSTRACT:

Urolithiasis, commonly known as kidney stones, is a global health problem. This study investigated the in vitro anti-urolithiatic activity of Delonix regia ethanolic extract (EEDR) compared to F1 and F2. established formulations for urolithiasis. Nucleation and aggregation assays were conducted to assess the effects on calcium oxalate (CaOx) crystal formation. EEDR exhibited dose-dependent inhibition of both nucleation and aggregation, showing highest inhibition at 1000 µg/ml. Polyherbal formulation F1 displayed sustained inhibition throughout the observed time points, F2 showed a significant effect primarily within the first hour. These findings suggest that Delonix regia has potential as a therapeutic intervention for urolithiasis. Further research, including in vivo studies, is warranted to explore its clinical applications.

Key words: Delonix regia, nucleation assay, aggregation assay, calcium oxalate, kidney stone.

1.INTRODUCTION

Urolithiasis, commonly known as kidney stone disease, is a global health problem characterized by the formation of calculi in the urinary tract. These stones, which can develop in the kidneys, bladder, or urethra, consist mainly of calcium oxalate, calcium phosphate, uric acid, and cystine. As of 2019, the global burden of urolithiasis remains substantial. The age-standardized incidence rate (ASIR) for urolithiasis globally was approximately 1,394 per 100,000 population. While the ASIR has shown a slight decrease over the past few decades, the absolute number of cases has increased significantly, with a 48.57% rise in new cases since 1990[1]. This increase is primarily attributed to population growth, aging, and changes in lifestyle and dietary habits. The number of deaths associated with urolithiasis also increased by 17.12% from 1990 to 2019, reflecting the growing impact of the disease on global health. In India, the incidence of urolithiasis is particularly high, with an estimated two million new cases reported each year [2]. The management of urolithiasis typically involves pharmacological treatment, dietary modifications, and in more severe cases, surgical interventions. However, these approaches often come with side effects, high costs, and the risk of recurrence, necessitating the exploration of alternative therapies [3].

Herbal remedies have been used for centuries in traditional systems of medicine to prevent and treat urolithiasis. Several medicinal plants are reputed to have anti-urolithiatic properties, including Phyllanthus niruri, Tribulus terrestris, and Cratoxylum formosum. These plants are believed to exert their effects through various mechanisms, such as diuretic action, inhibition of stone-forming substances like calcium and oxalate, dissolution of preformed stones, and antioxidant activity that protects renal tissues from oxidative stress [4,5].

The bioactive compounds in these herbal extracts, such as flavonoids, saponins, alkaloids, and phenolic acids, play a significant role in their anti-urolithiatic activity. For example, flavonoids have been shown to reduce the formation of calcium oxalate crystals, while saponins may inhibit stone growth by interacting with stone-forming ions. Additionally, phenolic compounds in these plants exhibit strong antioxidant properties, which can help prevent oxidative damage to the renal epithelium, a key factor in stone formation [4-7].

Recent trends in medical research have shifted towards exploring alternative and complementary therapies, especially those derived from traditional medicine. Among these, Delonix regia, family Fabacaea commonly known as the Royal Poinciana, Flame Tree or Gulmohar, has gained attention for its potential therapeutic benefits. Traditionally used in various cultures for treating a range of ailments, Delonix regia is reported to possess anti-inflammatory, antioxidant, and diuretic properties. Delonix trees, have been used in traditional medicine for centuries. These trees have been used to treat ailments like rheumatism and stomach problems. Additionally, their leaves are believed to be effective in treating bronchitis and pneumonia in infants. This demonstrates that Delonix trees are not only beautiful but also have valuable medicinal properties. Its phytochemical profile, which includes flavonoids, tannins, and saponins, suggests a potential role in mitigating urinary stone formation [8].

In this context, evaluating the in vitro antiurolithic activity of Delonix regia is of particular interest. In vitro studies offer valuable insights into the efficacy of herbal extracts in preventing or dissolving urinary stones before clinical trials are conducted. Such studies can elucidate the mechanisms by which these extracts influence stone formation and contribute to developing effective and safer treatment alternatives.

To establish a comparative framework, this study also examines two marketed formulations F1 and F2. F1 is an Ayurvedic polyherbal formulation traditionally used to address urinary tract disorders and has shown promise in managing urolithiasis. Its composition typically includes over 15 medicinal herbs namely Bergenia ligulata, Tribulus terrestris, Boerhaavia diffusa, Berberis aristata, Crataeva nurvala, Asparagus racemosus, and Physalis alkekengi which exhibit antiurolithiatic action by inhibiting crystallisation, promote kidney stone dissolution and have anti-inflammatory, antioxidant and diuretic actions.[9]. F2, another formulation aimed at treating urinary stones, includes a unique blend of herbal and mineral ingredients which act as urine alkalizers inhibiting growth of calcium oxalate stones [10]. Comparing the antiurolithic efficacy of Delonix regia with these established formulations can provide a comprehensive understanding of its potential benefits.

This research aims to assess the antiurolithiatic activity of ethanolic extract of Delonix regia by in- vitro nucleation and aggregation assay and compare its efficacy with F1 and F2. By evaluating these interventions in a controlled laboratory setting, we seek to contribute to the growing body of evidence supporting the use of herbal remedies in urolithiasis management and potentially offer new avenues for patient care.

2. MATERIALS AND METHODS

2.1. Preparation of crude drug (D.regia leaves)

The leaves were collected from the vicinity in Chembur, cleaned, to remove any dirt or impurities and shade dried. The dried leaves were then finely ground or powdered to increase the surface area and facilitate efficient extraction of the bioactive compounds.

2.2. Extraction

Ethanol (AR grade) was used for the extraction. The powdered leaves are soaked and macerated in ethanol for 3 days with intermittent stirring and shaking. The mixture was then filtered through muslin cloth and passed through Whatman filter paper to obtain the filtrate. The filtrate was concentrated by solvent evaporation to obtain the concentrate of ethanolic extract of D.regia (EEDR) and stored under refrigeration in air tight container until further use.

2.3. F1 and F2

Marketed polyherbal formulation F1 and allopathic formulation F2 were bought from local pharmacy store. Different concentrations of the formulations with respect to the major active constituent were prepared for the study by dilution.

3. EXPERIMENTAL

3.1 Preliminary phytochemical analysis

The EEDR was subjected to qualitative analysis for phytoconstituents using well established procedures [11]. The plant extract was screened for presence of alkaloids, flavonoids, phytosterols, and terpenoids.

3.1.1 For alkaloids:

3.1.1.1 Wagner's Test: A fraction of extract was treated with 3-5 drops of Wagner's reagent and observed for the formation of reddish brown precipitate (or colouration).

3.1.1.2. Hager's Test: A fraction of extract was treated with an equal volume of Picric Acid (2 ml) and observed

for the formation of a bright yellow precipitate.

3.1.1.3. Dragendroff's Test: A fraction of extract was treated with 2 ml of Dragendorff's reagent and observed for the formation of an orange-red precipitate.

3.1.1.4. Mayer's Test: A fraction of extract was treated with 2 ml of Mayer's reagent and observed for the formation of a bright yellow precipitate.

3.1.2 For Saponins:

Foam test: To 2 ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

3.1.3. For Flavonoids:

3.1.3.1. Lead Acetate Test: A fraction of extract was treated with 2 ml of 10% lead acetate solution and observed for the formation of a white precipitate.

3.1.3.2. Shinoda Test: Few magnesium turnings and a few drops of concentrated hydrochloric acid were added to the extract and boiled for five minutes. Red coloration confirms the presence of flavonoids.

3.1.4. For Phytosterols:

Liebermann-Burchard test: 1 ml of extract was treated with drops of chloroform, acetic anhydride and conc. H2SO4 and observed for the formation of dark pink or red color.

3.1.5. For Terpenoids:

Salkowski's Test: 1 ml of chloroform was added to 2 ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish-brown precipitate produced immediately indicated the presence of terpenoid.

3.2 In vitro anti-urolithiatic activity by Nucleation and Aggregation assay.

Various stages of calcium oxalate (CaOx) crystal formation were investigated by in-vitro methods, both with and without the presence of the plant extract or drug. This was assessed through nucleation and aggregation assays.

3.2.1. Nucleation assay:

Occurrence of nuclei marks the stone formation. The inhibitory activity of the extract (200, 400, 600, 800 and 1000 micrograms/ml) and marketed formulations; F1 and F2 (20, 40, 60, 80 and 100 micrograms/ml) on nucleation of Calcium oxalate crystals was determined by spectrophotometric assay using method described by Bawari et al[11]. Solution of Calcium Chloride (5mmol/L) and Sodium Oxalate (7.5mmol/L) were prepared in buffer containing Tris (0.05 mol/L) and NaCl (0.15 mol/L) buffer at pH 6.5). 1ml of each concentration of extract was mixed with 1 ml of Calcium Chloride solution followed by addition of 1ml of sodium oxalate solution. Final mixtures were incubated for at 37°C for 30 min. The optical density (O.D) was measured at 620 nm with an UV- visible spectrophotometer.

Percentage inhibition of nucleation was calculated using the following formula:

% Nucleation inhibition = {1-(Optical density of Test/ Optical density of Control)} X 100

3.2.2. Aggregation assay:

In the assay, crystals are formed under controlled conditions, and the Drug extract being tested is added to see if it prevents the crystals from clumping together (aggregating). This inhibition of aggregation is indicative of the compound's potential to prevent the formation or growth of kidney stones.

When crystals in solution adhere to one another, larger particulate aggregates are formed. Aggregation inhibition by the extract was assessed using the method described by Bawari et al [12]. Solutions of calcium chloride (CaCl2 and Na2C2O4 (each at 50 mmol/L) were mixed, warmed at 60°C in a water bath for 1 hour, and then incubated overnight at 37°C to produce calcium oxalate (CaOx) seed crystals. After drying, a CaOx crystal solution (0.8 mg/mL) was prepared in a buffer containing 0.05 mol/L Tris-HCl and 0.15 mol/L NaCl at pH 6.5. Two milliliter of EEDR (at concentrations of 200, 400, 600, 800, and 1000 µg/mL) or marketed formulations F1 and F2 (20, 40, 60, 80 and 100 micrograms/ml) was added to 2 mL of the calcium oxalate solution, mixed, and incubated for 30 minutes at 37°C. The optical density of the mixtures was measured at 620 nm, and the percentage inhibition of aggregation was calculated as above.

4. RESULTS AND DISCUSSION

4.1. Preliminary phytochemical analysis

Qualitative phytochemical analysis of EEDR showed presence of alkaloids, saponins, flavonoids, phytosterols and terpenoids. Saponins inhibit calcium oxalate crystal growth and aggregation due to their detergent-like action, helping dissolve kidney stones [13]. Terpenoids by virtue of their anti-inflammatory and antioxidant properties that protect renal cells from oxidative damage, reducing conditions that favour stone formation [14] while flavonoids prevent calcium

oxalate crystal adhesion and promote the dissolution of small crystals by their antioxidant and anti-inflammatory actions [15]. The phytosterols in EEDR inhibit nucleation and calcium oxalate crystal growth, reducing aggregation thus promoting excretion of smaller particles. Alkaloids possess anti-inflammatory and analgesic properties thus reduce renal inflammation and inhibit calcium oxalate crystal formation, decreasing their adhesion to renal cells[16].

4.2. In vitro nucleation assay

There was a gradual increase in % nucleation inhibition observed for EEDR from 200 µg/ml to 1000 µg/ml. The highest inhibition of 67.27 % was observed at 1000 µg/ml (Table 1). The Herbal formulation F1, showed highest % nucleation inhibition of 23.696±0.009% at 100 µg/ml while F2 showed 24.487±0.001% nucleation inhibition at 100 µg/ml (Table 2). The reduction in nucleation suggests that the bioactive compounds in EEDR and F1, such as flavonoids, saponins, terpenoids, and phytosterols, may interfere with the supersaturation process of calcium oxalate crystals. These compounds likely bind to free calcium and oxalate ions, preventing their aggregation into larger crystals. Urine pH is a key factor influencing the formation of kidney stones, with varying pH levels being linked to different types of renal calculi. Alkaline pH as it reduces the crystallisation of calcium oxalate crystals, F2 demonstrated a significant % nucleation inhibition.

4.3. In vitro aggregation assay

EEDR depicted increasing % inhibition with increasing doses in aggregation assay showing highest inhibition of 60.205±0.001 at 1000 µg/ml for 60-minute incubation. The % inhibition for 61-120th min was 36.392±0.002 and 36.125±0.002% for 121-180th min which was significantly lower than that observed in the first hour of incubation (Table 3). The similar trend was observed for F2 where 100 µg/ml of formulation showed % inhibition of 85.677±0.001%, 35.517±0.004% and 38±0.018% in the 0-60th min, 61st -120th min and 121st-180th min respectively (Table 4). F1 exhibited a dose-dependent inhibitory effect on aggregation. As the concentration of F1 increased, the percentage inhibition of aggregation also rose. The inhibitory effect of F1 was observed across all time points (0-60th, 61-120th, and 121-180th minutes). The highest percentage inhibition was achieved at 100 µg/ml concentration, especially within the 61-120th minute time frame (Table 5). Kidney stones often form when particles clump together and grow larger. This process, called aggregation, is crucial in determining the size, composition, and structure of these stones. Once crystals stick together, they become difficult to separate and can form larger, more solid stones. This aggregation plays an essential role in the development of kidney stones. EEDR and F2 have demonstrated significant inhibition in the first one hour while the polyhedral formulation displayed sustained inhibition throughput all the time points this could be due to synergistic actions of phytoconstituents present in F1.

Table 1: Nucleation assay of Delonix regia ethanolic extract at different concentrations.

EEDR (µg/ml)	% Inhibition
0	0
200	1.01%
400	5.15%
600	19.60%
800	36.06%
1000	67.27%

Table 2: Nucleation Assay of F1 and F2

Concentration (µg/ml)	% Inhibition (F1)	% Inhibition (F2)
0	-	-
20	2.894±0.005	12.167±0.002
40	6.499±0.009	21.569±0.001
60	12.746±0.006	23.644±0.001
80	19.83±0.008	22.585±0.002
100	23.696±0.009	24.487±0.001

Table 3: Aggregation Assay of Delonix regia ethanolic extract at different concentrations.

EEDR	% Inhibition		
	0-60th min	61-120th min	121-180th min
Control	-	-	-
200 µg/ml	31.732±0.002	11.901±0	21.177±0.003
400 µg/ml	34.223±0.002	22.122±0.002	20.065±0.004
600µg/ml	34.79±0	24.836±0.001	22.378±0
800 µg/ml	37.782±0.001	27.172±0	23.601±0.001
1000 µg/ml	60.205±0.001	36.392±0.002	36.125±0.002

Table 4: Aggregation Assay of F2 at different concentrations

F2	% Inhibition		
	0-60th min	61-120th min	121-180th min
Control	-	-	-
20 µg/ml	11.784±0.001	1.092±0.004	7.333±0.028
40 µg/ml	21.484±0.004	4.367±0.003	18.667±0.011
60µg/ml	79.036±0.001	5.845±0.001	25.333±0.021
80 µg/ml	82.747±0.001	14.965±0.007	30.667±0.011
100 µg/ml	85.677±0.001	35.517±0.004	38±0.018

Table 5: Aggregation Assay of F1 at different concentrations

F1	% Inhibition		
	0-60th min	61-120th min	121-180th min
Control	-	-	-
20 µg/ml	12.898±0.002	18.68±0.001	41.363±0.001
40 µg/ml	14.782±0.001	54.242±0.002	42.204±0.004
60 µg/ml	30.153±0.002	56.727±0.001	49.767±0.001
80 µg/ml	44.111±0.001	61.782±0.001	55.509±0.002
100 µg/ml	53.121±0.01	66.067±0.002	90.71±0.001

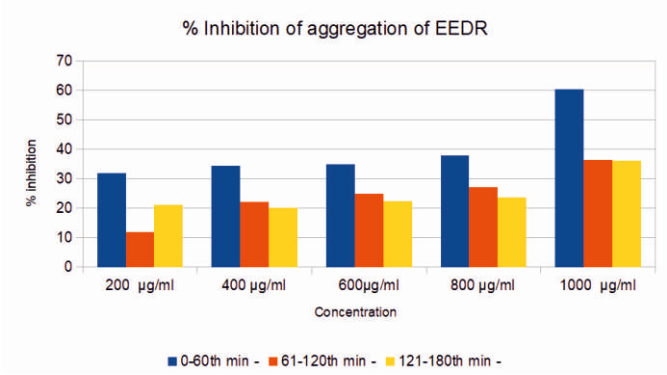

Figure 1: Percent inhibition of aggregation of EEDR

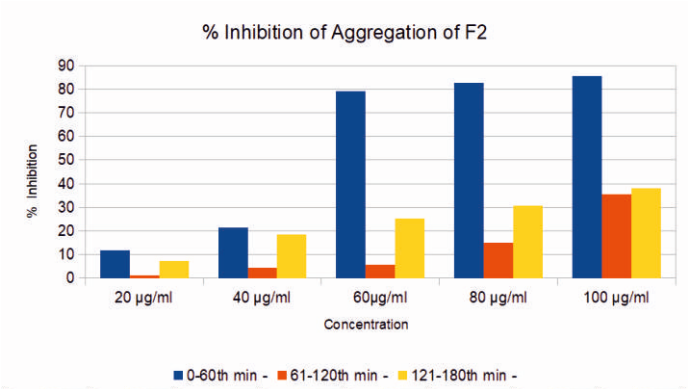

Figure 2: Percent inhibition of aggregation of F2

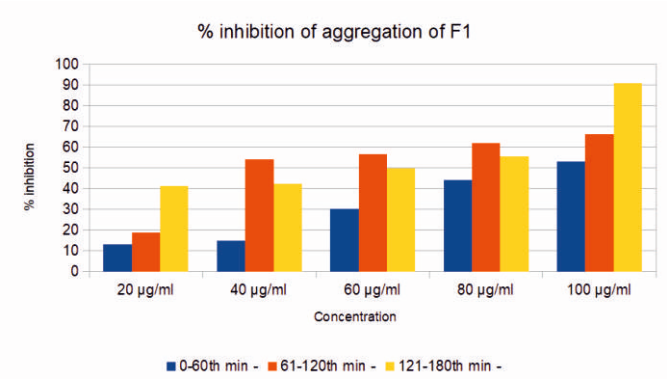

Figure 3: Percent inhibition of aggregation of F1

5. CONCLUSION

Ethanolic extract of Delonix regia exhibited inhibition of nucleation as well as aggregation comparable to F1 and F2 at all the concentrations and time points. Highest nucleation inhibition was obtained at 1000 µg/ml, in comparison to 100 µg/ml of polyherbal F1 and allopathic F2 formulations. This displays the potential of incorporating Delonix regia as a component of polyherbal formulations which can exhibit synergistic antiurolithiatic activity clinically. In conclusion, our study underscores the significant potential of Delonix regia ethanolic extract as a therapeutic intervention against urolithiasis. Through rigorous examination of critical stone formation processes, including nucleation and aggregation, our findings reveal a dose-dependent effectiveness of the extract in mitigating these pathological events in vitro. Comparative analyses against established herbal and allopathic formulations further validate its efficacy, positioning our extract as a promising alternative treatment option.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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MIGRAINE AND HERBS IN MIGRAINE

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ABSTRACT

A migraine is an episodic headache that affects more than 10% of the population. The present review will elaborate on the occurrence of migraine, along with its diagnosis, types, symptoms as well as causes. Even after significant research progress, there is no proper drug therapy that exists for preventing and treating migraine in many patients. Many herbs and herbal formulations have proved to be very effective for the treatment of migraine. Herbals are effective for prevention as well as treatment of acute migraine. Combining herbal remedies and formulations with the identification and avoidance of migraine triggers often leads to effective results. The herbal drugs discussed here include Ergot, Cannabis, Capsicum, Lavender oil, and Butterbur for the treatment and prevention of migraine.

KEYWORDS: Migraine, Ergot, Cannabis, Capsicum, Lavender oil, Butterbur

INTRODUCTION

Migraine is a new disease that has recently been added to the Global Burden of Disease study, where it is ranked as the 3rd most prevalent and 6th most disabling disease in the world, where 90% of people who suffer, cannot work or function normally during an episode[1]. Nowadays, migraine is commonly found in both genders, as per an analysis of 29.8 million Years of healthy life lost due to disability (YLDs) in 1990 increasing to almost 45.1 million YLDs in 2016[2].

In the USA 6% of men, 18% of women, and 10% of children experience migraine[3]. Previously, dilation and constriction of blood vessels in the head were the primary reason behind migraine. Therefore, early medication with a focus on blood vessels is the principal target for treatment. Migraine, on the other hand, is a severe neurovascular illness marked by typically one-sided throbbing head pain and a slew of neurological symptoms such as hypersensitivity to sound, light, and smell as well as nausea. It can also include various autonomic, cognitive, emotional, and motor problems. The pain associated with migraines is thought to result from the activation and sensitization of peripheral neurons in the trigeminal ganglion that innervates the meninges(meningeal nociceptors) and central trigeminovascular neurons in the upper cervical and medullary dorsal horn[4]. The stimulation of the trigeminovascular system (TGVS) causes

migraine, an episodic headache. This activation leads to the release of vasoactive peptides, which are believed to cause neurogenic inflammation and activate regions responsible for pain transmission and perception[5]. Two major topics in the neurobiology of migraine headaches remain unresolved: the root cause of TGVS activation and the mechanism by which pain is generated following this activation. There are two primary theories proposed to explain the fundamental cause of TVGS activation. One theory suggests that cortical spreading depression, which involves a gradual wave of cortical depolarization, plays a key role. Alternatively, another theory states that dysfunction in brainstem nuclei, which are involved in pain inhibition, serves as the main trigger for migraines. Alterations in cortical excitability appear to be linked to migraine attacks. Concerning the mechanisms of pain in migraines, two primary ideas are considered: neurogenic inflammation of the meninges and sensitization of the trigeminal nerve and its nucleus[6]. In humans and cats, the electrical stimulation ganglion enhances extracerebral blood flow and triggers the release of CGRP and substance P in the cranial region[7]. Common triggers or causes that lead to migraine are stress in the majority of conditions, then skipping meals, alcohol, improper sleeping hours, hormonal changes in women, and concussion and traumatic brain injuries. These lead to episodic headaches of migraine. While there are several rare types of migraines, the most common forms are migraines with aura and migraines without aura. Other nine rare types of migraine include silent migraine, abdominal migraine, menstrual migraine, vestibular migraine, ophthalmic migraine, hemiplegic migraine, migraine with brainstem aura, status migrainosus, and ophthalmoplegic migraine[8]. Menstrual migraine related to menstruation and pure menstrual migraine, are also different types of menstrual migraine[9]. Withdrawal of estrogen before menses also leads to menstrual migraine[10]. The precursor to the development of migraine with and without aura is observed in abdominal migraine. Symptoms of abdominal migraine were relieved in 61% of patients. However, 70 % of these patients went on to develop migraine with or without aura, compared to only 20% of matched control patients[11]. Vestibular symptomatology related to migraine spectrum disorder is described by vestibular migraine. In this type of migraine, up to 50% of patients reported occasional dizziness or vertigo[12]. Certain brain regions exhibit circadian patterns that suggest varying migraine susceptibility throughout the day, indicating that environmental triggers don't always have the same effect.

This article will focus on the role of herbs in preventing and treating migraines. However, it's important to acknowledge that natural therapies can be essential for these purposes. The present review covers the following herbal drugs - Ergot, Cannabis, Capsicum, Lavender oil, and Butterbur, focusing primarily on their properties and active compounds that aid in migraine prevention and treatment.

Global migraine drugs market size was estimated at USD 5.64 billion in 2023 and is projected to grow at a CAGR of 11.9% from 2024 to 2030. The market growth is attributed to the increasing prevalence of migraines and the global approval of new drugs for their treatment. According to the WHO article published in March 2024, approximately 40% of the global population, equating to around 3.1 billion individuals, were affected by migraine and other headache disorders in 2021.

Ergot

Ergot alkaloid from the Clavicipitaceae family contains Ergotamine, which is very effective on acute migraine. Similar efficacy is demonstrated by its derivative, dihydroergotamine[13]. Ergotamine is available in tablet and suppository form, with tablet contraindications such as severe nausea or vomiting, and suppository contraindications such as peripheral vascular disease, coronary artery disease, and uncontrolled hypertension. Dihydroergotamine derivative is

available in injection as well as intranasal form. Contraindications for these two forms are coronary artery disease, peripheral vascular disease, and uncontrolled hypertension[14]. Ergot alkaloids have complex modes of action on various receptors. Both ergotamine and dihydroergotamine have an affinity to act on 5-HT, dopamine, and noradrenaline receptors[15]. Ergotamine has only 60-70% absorption through the oral route, and administration of caffeine with ergotamine improves the rate as well as the extent of absorption. Ergotamine exhibits low oral and rectal bioavailability due to extensive first-pass metabolism. Ergotamine has an i.m. bioavailability of 47%, while its i.v. Bioavailability is less than 1%, and its rectal bioavailability ranges from 1% to 3%. Dihydroergotamine, like the triptans which are 5-HT1B and 5-HT1D receptor antagonists, was administered every 8 hours until the patient was headache-free. Dihydroergotamine often required many doses to get patients free from the headache but 40% of patients achieved freedom from headache by the 5th dose. In continuous treatment till the 12th or 13th dose, the percentage of headache-free patients increased to 67%(17). The bioavailability of dihydroergotamine when administered as a nasal spray is roughly 40%, and in intravenous or intramuscular administration is nearly 100%. The Aerosol formulation of dihydroergotamine absorbed by inhalation gives around 77% bioavailability[18].

Cannabis

Cannabis sativa contains two phytoconstituents (-)-Δ^9-trans-tetrahydrocannabinol (Δ^9-THC) and Cannabidiol (CBD); the former being the major active constituent. Cannabis sativa can be categorized into three phenotypes: Phenotype I (drug-type), characterized by high levels of Δ9-THC (>0.5%) and low levels of cannabidiol (CBD <0.5%) (Δ9-THC/CBD >>1); Phenotype II (intermediate-type), where CBD is the predominant cannabinoid, but Δ9-THC is also present in varying amounts (Δ9-THC/CBD ~1); and Phenotype III (fiber-type or hemp), which has a very low content of Δ9-THC (Δ9-THC/CBD <<1))[19]. NMDA mechanisms play a crucial role in secondary and tertiary hyperalgesia in chronic migraine. Δ9-THC reduces NMDA responses by 30-40%, providing antioxidant and neuroprotective effects. Additionally, it inhibits CGRP activity, blocks capsaicin-induced hyperalgesia, reduces 5HT reuptake, increases cerebral 5HT production, and inhibits 5HT release from platelets. These combined mechanisms have the potential to influence trigeminovascular migraine pathways[20]. It is also important to note that Cannabis sativa contains many other phytocannabinoids besides THC and CBD, with terpenes and flavonoids that may contribute to its medicinal properties. Oral cannabinoids were preferred over THC oromuscosal spray because oral administration prevents the concentration peaks that cause euphoric effects and is more effective for chronic administration due to better management because of bioavailability differences[22]. Δ9 -THC and other CB1 agonists have been shown to inhibit the amplitude, duration, and propagation velocity of cortical spreading depression in a dose-dependent manner in a rat brain model. This supports their potential to inhibit the trigeminovascular system in migraines with aura[23].

Capsicum

Capsaicin, a naturally occurring alkaloid in capsicum, has been shown to offer relief from migraine attacks for a considerable number of individuals when applied topically[24]. Capsaicin may affect the operation of the trigeminovascular system by reducing the amount of substance P in the brain, which is mainly involved in pain transmission. It also strongly inhibits platelet aggregation. A relationship discovered between migraine and platelet aggregation has been widely reported[25]. Capsaicin's unique ability to selectively desensitize nerve terminals that express the channel by targeting TRPV1 has been utilized through the use of lotions, ointments, and patches to treat various types of pain, particularly neuropathic pain[26]. The TRPV1 antagonist capsazepine, as well as CGRP blocking

therapies, prevent capsaicin-induced vasodilation. This demonstrates that capsaicin-induced vasodilation is mediated by the release of CGRP. TRPV1 activation could play a key role in CGRP release from trigeminal neurons[27]. Topical capsaicin may alleviate pain in both the absence of and during a migraine attack for many individuals who experience scalp arterial tenderness. More potent capsaicinoids could be explored in the future, perhaps providing a new treatment option for migraine attacks[28].

Lavender Oil

The Lamiaceae (Labiatae) family contains roughly 25–30 species of flowering plants in the genus Lavandula (common name: lavender). The leaves of lavender can be used in aromatherapy. Stomach upset, gas, colds, gallbladder difficulties, liver, and loss of appetite have all been treated with lavender essential oil in the past. In addition, it is beneficial for anxiety, headaches, stress, insomnia, and migraine[29]. Lavender oil extract is one of the most utilized essential oils with a sedative effect; the most potent components identified are linalyl acetate and linalool. A massage with lavender oil effectively reduces anxiety and promotes sleep quality[30]. One of the most well-known qualities of lavender oil is its analgesic effect. After therapy with lavender, there was a considerable reduction in the severity of headache from the first to the sixth attack[31]. Lavender oil works through the limbic system, specifically the amygdala and hippocampus, to relieve tension and promote relaxation. Lavender oil has been used as a stress reliever and to help students relax during exams[32].

BUTTERBUR

Butterbur derived from the rhizomes and stems of Petasites hybridus from the Asteraceae family, is used as a nutraceutical product. The efficacy of butterbur treatment in migraine prevention is good enough[33]. Butterbur is used to treat and prevent acute migraine[34]. Hepatotoxic pyrrolizidine alkaloids (PA) are found in butterbur. To ensure safety, PA toxins have been eliminated from several butterbur formulations[35]. Butterbur is used, only if it is labeled and confirmed as PA-free, according to the National Institutes of Health. Butterbur is a perennial shrub that has been utilized for medicinal purposes since ancient times. Petacin works by inhibiting peptide leukotriene production and regulating calcium channels. Inflammatory mediators including leukotrienes and other inflammatory mediators may play a role in the migraine-related inflammatory cascade[36]. The efficacy of Petadolex® has been studied in both children and adults, with encouraging results from both studies. However, the efficacy of Petadolex® in children has been mixed. Butterbur use has been linked to hepatotoxicity, however, extracts like Petadolex® are safe[37]. There have been no warnings from Health Canada that butterbur should be removed from the commercial market owing to safety concerns. The quantity of butterbur supplements accessible in the United States is unknown, and there have been no FDA warnings to remove butterbur products from the commercial market to date[38]. As per IHS guidelines for clinical migraine studies, the primary variables selected to evaluate the effectiveness of prophylactic treatment with butterbur extract were the frequency and duration of migraines, as well as the proportion of therapy responders [39]. The dependent variable was the relative reduction in headache rate, with dummy variable coding of butterbur and membership in a music therapy group as explanatory variables[40].

CONCLUSION

A variety of herbs and their phytoconstituents have been found helpful in the prevention and treatment of migraines to varied degrees. For patients experiencing relatively acute migraines, ergot (most effective), topical capsaicin, cannabis, and aromatherapy of lavender essential oil or butterbur nutraceutical can show promising results, especially for those who prefer to avoid pharmaceuticals. These all are very effective on migraine to reduce headaches, while triggers are

identified and eliminated. Ergotamine and its derivative dihydroergotamine, Δ^9 -THC, capsaicin, linalool, menthol, and petacin all these chemical constituents play an important role in reducing migraine by inhibiting CGRP activity, increasing 5HT production in cerebral and decreasing 5HT release from platelets. These are the main mechanisms behind the reduction of migraine in patients. These herbal remedies are likely used along with pharmaceutical agents to achieve a synergistic effect. Many pharmaceutical agents also have certain side effects associated with them. However Herbal drugs have minimal side effects, are safe, and economical to be used for migraine treatment.

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