A comparative phytochemical investigation and Dermal toxicity study of Polyherbal Ointment

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ABSTRACT
Phytochemical screening is a crucial process in evaluating plant extracts to identify their potential bioactive components. This study examines the methods used in initial phytochemical screening, their importance in medicine, agriculture, and nutraceuticals, and their many uses. The information is derived from an extensive analysis of existing literature. Understanding the first phytochemical screening procedure is essential for investigating the abundant source of biologically active substances found in plants, which shows potential for the creation of innovative medications, agrochemicals, and functional meals. Polyherbal ointments, made from a blend of herbal extracts or botanical compounds, are used to treat various dermatological conditions, sports injuries, and arthritis management. The synergistic interactions between constituent herbs in polyherbal ointments enhance their therapeutic outcomes, resulting in heightened anti-inflammatory, antimicrobial, antioxidant, analgesic, and wound-healing effects. However, caution should be exercised when using these ointments, especially for those with known sensitivities. In-vivo studies have shown that the ointment can enhance well-being through botanical wisdom.
KEYWORDS: Phytochemical, Toxicity, anti-inflammatory, antimicrobial, antioxidant, analgesic

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1-INTRODUCTION

Herbal medicine, also known as botanical medicine or phytotherapy, has been utilized by various cultures for millennia. Its significance spans across traditional practices, modern pharmaceuticals, and alternative healthcare systems. [1,2] This comprehensive review delves into the importance and scope of herbal medicine, exploring its historical roots, cultural diversity, scientific validation, therapeutic applications, economic implications, regulatory frameworks, and future prospects. Through an interdisciplinary lens, this paper aims to elucidate the multifaceted nature of herbal medicine, highlighting its role in global healthcare and the challenges and opportunities it presents.

Medicinal plant materials are classified based on their microscopic and macroscopic features, with crude pharmaceuticals being standardized through various approaches. The quality and standardization of herbal medicines involve several stages, including botanical identification, phytochemical screening, and standardization. Phytochemicals, biologically active compounds derived from plants, are classified as secondary metabolites due to their potential health advantages. [3,4]They are found in various parts of the plant anatomy, such as bark, leaves, stem, root, flower, fruits, seeds, and more. Phytochemicals exhibit a wide range of biological activities, including antioxidants, anti-inflammatory actions, and antibacterial properties against bacteria, fungus, and viruses. They may have a significant impact on reducing the likelihood of developing chronic illnesses like cardiovascular disease, cancer, and neurological disorders. The abundance and level of phytochemicals in plants can vary based on factors such as plant species, ambient conditions, and plant part. Advanced methods such as chromatography, spectroscopy, and bioassays are used to extract and analyze phytochemicals, which are essential for understanding their effects and creating applications in medicine, nutrition, and industry. [5,6]Alkaloids, a broad category of secondary chemical compounds, include indole alkaloids, iso-quinoline alkaloids, tropane alkaloids, and pyrrolidine alkaloids. Indole alkaloids are psychoactive substances found in hallucinogenic plants, while iso-quinoline alkaloids are derived from tyrosine or phenylalanine and have pain-relieving, calming, or antibacterial properties. Tropane alkaloids are derived from ornithine and have anticholinergic properties. Pyrrolidine alkaloids possess a pyrrolidine ring and are known for their stimulating and addictive properties. Alkaloids, a group of naturally occurring chemicals found in plants and animals, have been extensively researched for their pharmacological properties and potential medical uses. They possess strong biological activity, such as analgesic and anesthetic effects, psychoactive effects, and antibacterial effects. However, they can also present dangers due to their strong actions and possible toxicity when taken in excessive amounts. Extracting and purifying alkaloids from plants can be difficult due to inconsistency in concentration and the possibility of interactions with other chemicals present in the plants. Glycosides are organic substances derived from plants and animals that provide glycones coupled with an aglycone component. [7,8]They can be classified based on their chemical structure and properties, such as O-Glycosides, C-Glycosides, and N-Glycosides. Glycosides have various biological functions, including serving as defensive mechanisms, enticing pollinators, and storing energy. Animals can also metabolize glycosides, such as herbivores, which can transform cyanogenic glycosides into harmful cyanide molecules. Phenolics, phenols, or polyphenolics are ubiquitous chemical molecules that function as inherent pigments, imparting color to flowers and fruits of plants. They are predominantly synthesized from phenylalanine and have various biological roles, including plant defense against viruses and herbivore predators, and controlling human pathogenic diseases. Phenolics can be classified into three categories: flavonoids, flavones, flavanones, flavan-3-ols, anthocyanins, and isoflavones. [9,10]Flavonoids possess antioxidant, anti-inflammatory, and anti-cancer characteristics. Phenolic acids contribute to antioxidant activity and are involved in plant defense mechanisms. Stimulants, like resveratrol, are known for their antioxidant properties and potential health benefits, particularly in terms of cardiovascular protection. Phenolic compounds have various biological activities that contribute to their potential health benefits.

Polyherbal formulations refer to formulations that include two or more herbs. The drug formulation in Ayurveda is guided by two principles: the use of a single medication and the use of many drugs. [11] The last one is referred to as a polyherbal composition. Poly herbalism is a distinctive concept in Ayurveda,

Polyherbal formulations, also known as herb-herb combinations, are a popular approach in Ayurveda, a traditional medicine system that uses various plant species to treat various illnesses. The concept of polyherbalism, introduced in Ayurvedic literature, aims to enhance medicinal effectiveness by combining multiple herbs in a precise ratio. This approach results in improved therapeutic impact and reduced toxicity. However, individual plant components may not have significant pharmacological effects. Traditional medicine relies on whole plants or combinations of plants, rather than individual chemicals. Polyherbalism offers advantages that cannot be obtained from individual herbal compositions through synergism, which involves the absorption, distribution, metabolism, and elimination of other herbs.[12]

The market for herbal medicines has reached an annual value of $1.5 billion, with a growing availability. Traditional herbal medicine relies on the use of plants or plant material to cure injuries or diseases. Polyherbal treatment, also known as herb-herb combinations, has been used in Chinese medicine for millennia but lacks empirical data supporting their therapeutic advantages. The combination of drugs often yields a favorable outcome in the treatment of illnesses compared to using a single medicine. The Ayurvedic style of medicine primarily uses polyherbal chemicals to treat various illnesses. However, there is a lack of recorded information about their safety and efficacy, impeded by the absence of assessment.[13]

Dermal toxicity refers to the adverse effects that occur when a chemical substance comes into contact with the skin. These effects can range from mild irritation and allergic reactions to more severe outcomes such as chemical burns, systemic toxicity, or even cancer. The severity and nature of dermal toxicity depend on various factors, including the chemical's properties, concentration, duration of exposure, and the condition of the skin. Some substances can penetrate the skin barrier and enter the bloodstream, leading to systemic effects that impact internal organs.

The objective of present study will be the development and evaluation of poly-herbal ointment of *Datura stramonium, Nerium indicum, Curcuma longa, Acorus calamus, Emblica officinalis*.

It could become a medium to use the medicinal properties of extracts effectively and easily as a simple dosage form.Research literatures have revealed that plants taken under study are medicinally important. These plants materials having many research reports on analgesic, anti-inflammatory, anti-oxidant, anti-microbial properties and few of them posses even anti arthritic activity.

2-MATERIAL AND METHODS

2.1 Plant Material.

Datura stramonium seeds, Nerium indicum leaves, Curcuma longa rhizome, Acorus calamus rhizome, and Emblica officinalis fruits were collected from Veer Bahadur Singh Purvanchal University, Jaunpur, Uttarpradesh Campus.

2.2 Identification and authentication of plant materials

Datura stramonium, Nerium indicum, Curcuma longa, Acorus calamus and Emblica officinalis were identified and authenticated by Arti Garg, Scientist Head of office, Botanical survey of India and voucher specimens were deposited at that Institute. The certificates for the authentication were obtained.

2.3 Extraction of plant materials

The study preserved phytoconstituents from Datura stramonium seeds, Nerium indicum leaves, Curcuma longa rhizome, Acorus calamus rhizome, and Emblica officinalis fruits by desiccating them at 40°C. The coarse powder was extracted using ethanol, macerated with distilled water, and refined for concentrated extracts, which were preserved in desiccators.[14,15]

2.4. Preliminary Phytochemical Screening

This talk discusses preliminary phytochemical screening assays, crucial for early assessment of plant extracts, detecting bioactive chemicals, and their relevance in research on plant therapeutic characteristics and potential pharmaceuticals or herbal treatments.[16,17,18,19]

2.4.1. Alkaloid Testing

Alkaloids, nitrogen-containing chemicals found in plants, have significant pharmacological effects. They can be detected in plant extracts using methods like Dragendorff's test, Mayer's reagent, and Wagner's reagent. A positive result indicates the presence of alkaloids, which have various pharmacological properties, including analgesic, antibacterial, and anti-inflammatory actions, indicating their potential medicinal value.

2.4.2. Flavonoid Test

Flavonoids, polyphenolic chemicals found in plants, have antioxidant, anti-inflammatory, and anticancer effects. They are detected through the Shinoda test or UV light exposure. The test produces a pink, crimson, or violet hue in plant extracts, indicating the presence of flavonoids. A positive result signifies the plant extract's medicinal worth, as flavonoids exhibit a wide range of biological actions.

2.4.3. Tannin Test:

Tannins, polyphenolic chemicals found in plants, are known for their astringent properties. They are detected in plant extracts through the Ferric chloride test, which produces tannin-iron complexes with a blue-black or greenish-black hue. A positive test indicates the presence of tannins, which have historically been used for antibacterial and wound-healing properties, indicating their potential medicinal importance.

2.4.4. Saponins Test:

Saponins, compounds in plants that create froth or foam when agitated with water, are identified in plant extracts using the Froth test. This method, which involves forcefully agitating the extract with water, indicates the presence of saponins. Saponins have historically been used as expectorants, diuretics, and hemolytics, indicating their potential medicinal importance.[193]

2.4.5 Glycoside Test:

Glycosides are chemical substances with a sugar molecule bonded to an aglycone. They include cardiac glycosides, flavonoid glycosides, and cyanogenic glycosides. The Keller-Kiliani test is a widely used procedure for identifying glycosides in plant extracts. The test involves hydrolysis using hydrochloric acid and ferric chloride solution, resulting in complex ions with a green or blue tint. Glycosides have various pharmacological activities.

2.4.6 Phenolic compound test

The phenolic compound test is a method used to identify phenolic compounds in plant extracts. These compounds, including phenolic acids, flavonoids, and tannins, can undergo oxidation or reduction processes, creating pigmented substances. A positive result indicates the presence of phenolic compounds, which have antioxidant, anti-inflammatory, and antibacterial properties, indicating their potential medicinal importance.

2.5 Formulation of Ointment

The substance of the ointment was manufactured via fusion. [20]The components of the base were consolidated in the basin and subjected to melting at a temperature of 70oC. The ingredients were chilled while being agitated continuously at a temperature of 70oC for specific time intervals following melting. The process of ointment formulation involved the trituration of the base material with the active ingredients using a mortar and pestle. The components utilized in the formulation of ointment base and polyherbal ointment are as follows: Datura stramonium seeds, Nerium indicum leaves, Curcuma longa rhizome, Acorus calamus rhizome, and Emblica officinalis fruits. The excipients included in the ethanolic extract are as follows: stearic acid (15 g), white wax (2 g), yellow vaseline (8 g), propylene glycol (8 g), methyl paraben (0.2 g), and propyl paraben (0.1 g). Each extract contains 1 g.

2.6 Pharmacological Screening

2.6.1 Animals:

Wistar albino male rats (200-300g each) were housed in standard animal housing conditions and were utilized to assess the anti-inflammatory activity of the ointment formulation that was prepared. Every animal experiment was conducted at Deshpande Laboratories Pvt. Ltd, Bhopal ,India. An Institutional Animal Ethics Committee-Certified Drug Testing Laboratory with ISO 9001:2008 Accreditation and Register No. CPCSEA Consensus: 1410/c/11/CPCSEA

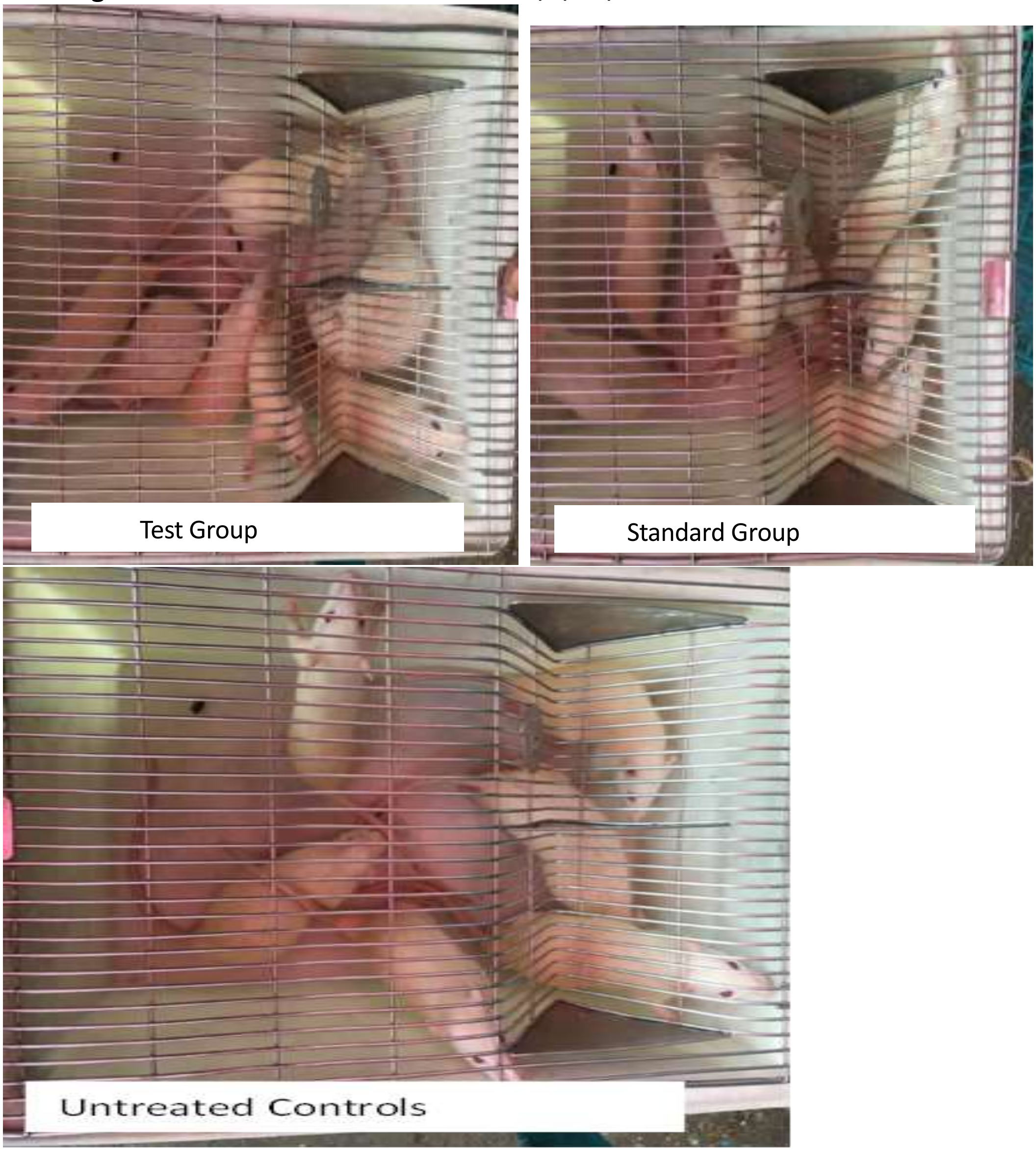

Figure. 2.1 – Images of Animal uses in different Group

2.7. Procedure for Acute toxicity study:

2.7.1 Dermal Toxicity

This method provides information on health hazard likely to arise from short-term exposure to a test chemical by dermal route. Test chemicals should not be administered at doses that are known to cause marked pain and distress due to potential corrosive or severely irritant actions.

Groups of animals, of a single sex, are exposed via the dermal route to the test chemical in a stepwise procedure using the appropriate fixed doses. The initial dose level is selected at the concentration expected to produce clear signs of toxicity without causing severe toxic effects or mortality. Further groups of animals may be tested at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing toxicity or no more than one death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose. Subsequently, observations of effects and deaths are made. Animals which die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied.

The method provides information on the hazardous properties and allows the substance to be classified for acute toxicity according to the Globally Harmonised System of classification and labelling of chemicals.

Depilated rat skin was treated with 100mg of ointment and blank formulation once daily for 7 days. Signs of redness, inflammation, itching, and other visible morphological signs were observed by two independent observers.

3- RESULT

3.1-Phytochemical screening

Phytochemical screening is crucial in the first evaluation of plant extracts to identify their potential bioactive components. This study explores the methods used in initial phytochemical screening, their importance in the fields of medicine, agriculture, and nutraceuticals, and their many uses. This study provides a thorough examination of the approaches used in screening for phytochemicals, the importance of different categories of phytochemicals, and their many uses in numerous fields. The information is derived from an extensive analysis of existing literature. Comprehending the first phytochemical screening procedure is essential for investigating the abundant source of biologically active substances found in plants, which shows potential for the creation of innovative medications, agrochemicals, and functional meals.

Table 3.1: Qualitative chemical evaluations of Datura stramonium, Nerium indicum and Curcuma longa

S.N	Test	Extracts					
		Datura stramonium		Nerium indicum		Curcuma longa	
		Water	Alcohol	Water	Alcohol	Water	Alcohol
1	Alkaloid	+	+	+	+	+	+
2	Flavonoid	+	+	+	-	+	+
3	Tannins	+	+	+	+	+	-
4	Saponins	+	+	-	+	+	+
5	Glycoside	+	+	+	+	+	+

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6	Phenolic Compound	+	+	+	-	+	+

+=Presence, -=Absence

Table 3.2 Qualitative chemical evaluations of Acorus calamus, *and Emblica officinalis*

S.N.	Test	Extracts			
		Acorus calamus		Emblica officinalis	
		Water	Alcohol	Water	Alcohol
1	Alkaloid	-	+	+	+
2	Carbohydrate	+	+	+	+
3	Tannins	+	+	+	+
4	Saponins	+	+	+	+
5	Glycoside	+	+	-	+
6	Phenolic Compound	-	-	+	-

+=Presence, -=Absence

3.2 Ointment formulation

Polyherbal ointments are topical formulations made from a blend of various herbal extracts or botanical compounds, providing a holistic approach to skin care, wound healing, and discomfort relief. These ointments are crafted from a variety of herbs, including aloe vera, turmeric, neem, chamomile, calendula, and arnica, each with its unique biochemical constituents. They are used to treat various dermatological conditions, as well as sports injuries and arthritis management. The synergistic interactions between constituent herbs in polyherbal ointments enhance their therapeutic outcomes, resulting in heightened anti-inflammatory, antimicrobial, antioxidant, analgesic, and wound-healing effects. However, caution should be exercised when using these ointments, especially for those with known sensitivities. The formulation process involves extracting bioactive compounds from herbs and incorporating them into a suitable base, such as cream, gel, or ointment. These ointments are grounded in tradition but adaptable to modern needs, offering potential for enhancing well-being through botanical wisdom.

3.3 In-vivo Studies

3.3.1 Acute Toxicity Study

3.3.1.1 Dermal Toxicity

Depilated rat skin was treated with 100mg of ointment and blank formulation once daily for 7 days. Signs of redness, inflammation, itching, and other visible morphological signs were observed by two independent observers.

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Table 3.3 Acute toxicity study general appearance and behavioural observations for the treated and control groups following treatment.

Parameter	**Control Group**		**Test Group**	
	4 hr	**72 hr**	**4 hr**	**72 hr**
Body weight	Normal	No change	No change	No change
Temperature	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal
Urination	Normal	No effect	No effect	No effect
Change in skin &Fur	No effect	No effect	No effect	No effect
Eye color	No effect	No effect	No effect	No effect
Digestion	Not observed	Not observed	Not observed	Not observed
Diarrhea	Not present	Not present	Not resent	Not present
Death	None	None	None	None

4-SUMMARY AND CONCLUSION

The study involved collecting plant materials such as Datura stramonium seeds, Nerium indicum leaves, Curcuma longa rhizome, Acorus calamus rhizome, and Emblica officinalis fruits from Veer Bahadur Singh Purvanchal University. These plants were identified and authenticated by Arti Garg, the Scientist Head of office of the Botanical Survey of India. The extracts were preserved and refined for concentrated extracts. Preliminary phytochemical screening was conducted to assess the potential medicinal properties of plant extracts. Alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds were tested. Positive results indicated the presence of alkaloids, which have various pharmacological effects. Flavonoids have antioxidant, anti-inflammatory, and anticancer effects, while tannins have astringent properties and have historically been used for antibacterial and wound-healing properties. Saponins have historically been used as expectorants, diuretics, and hemolytics. Glycosides have various pharmacological activities and can undergo oxidation or reduction processes, creating pigmented substances. The ointment was manufactured via fusion, with components of the base material and active ingredients being combined. Wistar albino male rats were used to assess the anti-inflammatory activity of the ointment formulation. The acute toxicity study involved exposure to the test chemical by dermal route, with animals exposed at different doses. The results showed that the ointment had potential therapeutic properties and could be used in pharmaceuticals or herbal treatments. This study examines the importance of phytochemical screening in evaluating plant extracts for potential bioactive components. It provides a comprehensive analysis of the methods used in screening phytochemicals, their various uses in medicine, agriculture, and nutraceuticals, and their potential for innovative medications, agrochemicals, and functional meals. The study also examines the qualitative chemical evaluations of Datura stramonium, Nerium indicum, and Curcuma longa, as well as Acorus calamus and Emblica officinalis. Polyherbal ointments, made from a blend of herbal extracts or botanical compounds, are used to treat various dermatological conditions, sports injuries, and arthritis management. The synergistic interactions between constituent herbs in these ointments enhance their therapeutic outcomes, resulting in heightened anti-inflammatory, antimicrobial, antioxidant, analgesic, and wound-healing effects. However, caution should be exercised when using these ointments, especially for those with known sensitivities. In-vivo studies have also been conducted to assess the toxicity of the ointment on rat skin.

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