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Formulation and Evaluation by Phytochemical Analysis of Herbal Ointment



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ABSTRACT

Objective: The main objective of the work was to analyze the phytochemical constituents from Herbal Ointment prepared by using the extract of Neem leaves, Tulsi leaves, Lemon leaves, Ashoka leaves, and Turmeric bark. **Methods:** Herbal Ointment extract was prepared by leaves of Neem leaves, Tulsi leaves, Lemon leaves, Ashoka leaves, and Turmeric bark by solvent extraction. **Results:** Herbal Ointment extract was analyzed by phytochemicals test like Tests for carbohydrates, Tests for alkaloids, Tests for steroids and sterols Tests for glycosides, Test for proteins and amino acids, Tests for Saponins and Test for flavonoids. **Conclusion:** Methanol extracts of leaf showed presences of carbohydrates, alkaloids glycosides proteins and amino acids Saponins flavonoids.

INTRODUCTION:

Herbal medication is also known as Botanical treatment or Phyto-medicine. Herbal medication refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. An herbal drug treatment gives healthy life¹. It was generally used to furnish first-line and common health provider². Since ancient time in India, herbal medicines have been the basis of treatment and cure for various diseases. Physiological conditions in traditional methods were practiced such as Ayurveda, Unani, and Siddha³. Herbal medicines having various therapeutic uses like healing wounds, treating inflammations due to infection, skin lesions, leprosy, diarrhea, scabies, venereal diseases, snake bite and ulcers etc. Many infectious agents such as virus, fungi, and parasites may harm the plants. The basic herbs have the answer with no side effects and effective medication. When two or more herbs are used in the formulation they are known as poly herbal formulations⁴. Numerous studies have been conducted with the extracts of Neem leaves (*Azadirachta indica* Family-Meliaceae) and extract of Tulsi leaves with the combination of many other herbal drugs like lemon Ashoka and Turmeric. Along with other dosage forms herbal drugs are also available in the form of the herbal ointment which is semisolid preparation used for washing of hands and for several purposes⁵⁻⁶.

The requirement of an herbal ointment cream⁷⁻⁹:

1. The ointment is a topical medication applied on the body surfaces.
2. An ointment is defined as homogeneous, viscous, semisolid preparation with a high viscosity.
3. An ointment is used for external application.
4. An ointment is used topically for several purposes like antiseptic, emollient, antipyretic, astringent.
5. An ointment is used to protect the skin against moisture, air, sunrays and other external factors.
6. An ointment is usually very moisturizing and good for dry skin. An ointment is used topically on a variety of body surfaces. These include the skin and the mucous membrane of the eye, chest, vulva, anus, and nose.

7. They have a low risk of sensitization due to having few ingredients beyond the base oil or fat, low irritation risk. There is typically little variability between brands of a drug. They are often disliked by the patient due to greasiness.

Plant Profile¹⁰⁻¹²:

1. Neem leaves:



Azadirachta indica (*A. indica*) belongs to the botanic family Meliaceae. It is commonly known as Neem. It is used in traditional medicine as a source of many therapeutic agents. Neem leaves are known to contain antimicrobial & antifungal activities against different pathogenic microorganisms like *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Neem is the multipurpose tree with multiple health benefits. Different parts of the tree were shown to exhibit antimicrobial effects against a wide variety of microorganisms. Furthermore, Neem leaves may be used for the treatment of various diseases including eczema, ringworm, acne, inflammation, chronic wound infection, hyperglycemia, diabetic foot & gas gangrene.

2. Tulsi leaves (Basil leaves):



Basil (*Ocimum basilicum* L) is a member of the Lamiaceae family. It is an annual herb which

grows in several regions around the world. It is commonly known as Tulsi. It has the characteristic odor. Basil is one of the medicinal plants. It gives antibacterial & antioxidant properties. Leaf extract of basil shows antibacterial activity against some human pathogenic bacteria (*Staphylococcus aureus* & *Escherichia coli*).

3. Lemon leaves:



Citrus Limon belongs to the family Rutaceae. Lemon leaves show antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus Subtilis*, *Pseudomonas aeruginosa* & *Salmonella species*. Leaves extracts are active against both Gram-positive and Gram-negative bacteria. Lemon leaves have great potential as antimicrobial compounds against microorganisms.

4. Turmeric powder:

It has powerful anti-inflammatory effects and is a very strong antioxidant. It has powerful anti-inflammatory effects and is a very strong antioxidant.

5. Ashoka leaves:



The Ashoka tree belongs to the family of legumes and is a part of the subfamily caesalpiniaceous. The Ashoka tree is a rainforest tree. The Ashoka tree has many health benefits and has long been used in traditional Indian medicine as a key ingredient in various

therapies and cures. It is used in menstrual disorders, excessive bleeding, and pain, dysentery, piles, pain, complexion, also use in diabetes.

MATERIALS AND METHODS:

Collection of plant material

Leaves of Neem were collected from the local area and rhizomes of turmeric were purchased from the local market.

Preparation of all extract:

Leaves of the plant Neem, Tulsi, Ashoka, and Lemon were collected and washed thoroughly with distilled water and shade dried for 5 days. Dried leaves were ground into powder form. 20gm of powder was imbibed with 80 ml of 90% ethanol for 3hr and transferred to the closed container then keep it for 2days finally filter it. The extract was stored in the airtight container in a cool and dark place.

Preparation of turmeric extract:

Dried rhizomes of turmeric were ground and the powder obtained was followed for extraction same as that for Neem and other leaves extract. The extract with crimson red color was obtained and stored at the cool and dark place in airtight container.

Components of herbal ointment formulation:

1. Extract of Neem
2. Extract of Turmeric
3. Extract of Tulsi
4. Extract of Lemon
5. Extract of Ashoka
6. Ointment Base
7. Wool Fat

8. Cetostearyl alcohol

9. Hard Paraffin

10. Yellow Soft Paraffin

11. Antioxidant

Formulation of herbal ointment:

Table 1: Formulation of herbal ointment

Sr. No	Name of ingredient	F1	F2	F3
1.	Neem extract	0.18 ml	0.20 ml	0.22 ml
2.	Tulsi extract	0.18 ml	0.20 ml	0.22 ml
3.	Lemon extract	0.18 ml	0.20 ml	0.22 ml
4.	Ashoka extract	0.18 ml	0.20 ml	0.22 ml
5.	Turmeric extract	0.18 ml	0.20 ml	0.22 ml

Table 2: Formulation of ointment base

Sr. No.	Name of Ingredient	Quantity		
1	Wool fat	0.75 gm	0.75 gm	0.75 gm
2	Cetostearyl alcohol	0.75 gm	0.75 gm	0.75 gm
3	Hard paraffin	0.75 gm	0.75 gm	0.75 gm
4	Yellow soft paraffin	12.75 gm	12.75 gm	12.75 gm

Preparation of Herbal Ointment:

A) Initially, ointment base was prepared by weighing accurately grated hard paraffin which was placed in evaporating dish on the water bath. After melting of hard paraffin remaining ingredient were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.

B) The herbal ointment was prepared by mixing accurately weighed Neem, Tulsi, Ashoka, turmeric extract to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of the base, gradually incorporating more base until to form homogeneous ointment finally transferred in a suitable container.

Qualitative Phytochemical Analysis and Evaluation of Herbal Ointment⁵:

The Herbal ointment preparation was subjected to the phytochemical analysis by using various chemical tests.

Tests for carbohydrates:

➤ Molish test: Take 1 ml of Herbal ointment extract in the test tube and added with 1 mL of α -naphthol solution and few drops of concentrated sulphuric acid, it gives purple or reddish violet color gives the positive result.

➤ Fehling's test: Take 1 ml of Herbal ointment extract in the test tube and to this added the equal quantities of Fehling's solution A and B, it gives a brick precipitate indicates the presence of carbohydrate.

Tests for alkaloids:

➤ Mayer's test: Take 1 ml of Herbal ointment extract in the test tube and add 2-3 drops of conc. Nitric Acid solvent, it gives a dull white precipitate indicate the presence of alkaloids.

➤ Wagner's test: Take 1 ml of Herbal ointment extract in the test tube and 2 to 3 drops of Nitric Acid solvent and sulphuric acid is added, it gives the presence of reddish brown precipitate indicates the presence of alkaloids.

Tests for steroids and sterols:

➤ Salkowski test: Take 1 ml of Herbal ointment extract and dissolved in the chloroform, the same amount of concentrated sulphuric acid is added. Cherry red color is observed in the chloroform layer

Tests for glycosides:

➤ Baljet test: Take 1 ml of Herbal ointment extract in the test tube and added with sodium picrate solution, yellow to orange color indicates the presence of glycosides.

➤ Keller-Kiliani test: The Herbal ointment extract was dissolved in acetic acid containing a trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. Reddish brown color is formed which gradually becomes blue indicating the presence of glycosides.

Tests for Saponins;

➤ Foam test: Take 1 mL of Herbal ointment extract and added with the distilled water. The sample was shaking and the foam was observed.

Test for flavonoids:

➤ Shinoda test: Take 1 ml of the Herbal ointment extract in the test tube and added with the ferric chloride, followed by the addition of concentrated hydrochloric acid. A red color indicates the presence of flavonoids.

Tests for tannins:

➤ Ferric chloride test: Take 1 ml Herbal ointment extract, ferric chloride was added. Dark blue or greenish black color indicates the presence of tannins.

➤ Potassium dichromate test: In the Herbal ointment extract, potassium dichromate solution was added. A precipitate indicates the presence of tannins.

Test for proteins and amino acids:

➤ Biuret test: To the Herbal ointment extract, 1 mL of 40% sodium hydroxide and 2 drops of 1% copper sulfate solution were added. A violet color indicates the presence of proteins.

Evaluation of Herbal wash:

Organoleptic evaluation (color, odor) was done by sensory and visual inspection and compared to the marketed Herbal ointment. The evaluations were carried out on the Herbal ointment by using the following parameters;

➤ Color and odor:

Color and odor of prepared ointment were examined by visual examination.

➤ pH

The pH of ointment was determined by digital pH meter. 1 g of ointment was dissolved in 50 ml of distilled water and the pH was measured.

➤ Stability study:

The stability study was carried out for the prepared ointment at the temperature of 37°C for 15 days.

➤ Fragrance test

It was based on individual observation for its acceptability. 5 people were asked for acceptability of fragrance and their opinion was taken. And fragrance was evaluated based on the below-described criteria;

A). Fragrance was good, as good as the fragrance of reference Herbal ointment

B). Fragrance was not so good but comparable to the reference Herbal ointment

C). Fragrance of the toothpaste was poor than the reference Herbal ointment

RESULT AND DISCUSSION:

The preliminary phytochemical screening of methanolic extract of 3 Herbal ointment formulations was tested and it gives the result which was shown below. It gives the presence of carbohydrates, steroids, glycoside, proteins, amino acids and absence of flavonoids and Saponins (Table 2).

Table 2: Phytochemical analysis of leaves

Sr. No.	Sample	Solvent extraction Methanol		
		F ₁	F ₂	F ₃
	Phytochemical analysis Tests	Phytochemical analysis Tests result	Phytochemical analysis Tests	Phytochemical analysis Tests
1	Tests for carbohydrates	+	+	+
2	Tests for alkaloids	+	+	+
3	Tests for steroids and sterols	+	+	+
4	Tests for glycosides	+	+	+
5	Tests for Saponins	-	-	-
6	Test for flavonoids	-	-	-
7	Tests for tannins	+	+	+
8	Test for proteins and amino acids	+	+	+

The Quality control evaluation of formulation of methanolic extract of 3 Herbal ointments was tested and it gives the result which was shown below. Three different Herbal ointments were formulated and the physicochemical parameters such as color, odor, and pH were evaluated. All Three different Herbal ointments have different colors and characteristic odor. The pH of the three herbal ointments was in the range of 6-7.

It was observed that the phytochemical analysis of the methanolic extract of 3 Herbal ointment formulations (table 2) showed a presence of carbohydrates, reducing sugar, steroids, glycoside, proteins, amino acids and absence of flavonoids and Saponins.

Table 3: Quality control evaluation of formulation

Sr. No.	Parameters	Observations		
		F ₁	F ₂	F ₃
	Sample	F ₁	F ₂	F ₃
1	Color	Yellow	Yellow	Yellow
2	Odor	Characteristic	Characteristic	Characteristic
3	Texture	Smooth	Smooth	Smooth
4	Stability	Stable	Stable	Stable
5	pH	Acidic	Acidic	Neutral
6	Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform	Soluble in boiling water, miscible with alcohol, ether, chloroform	Soluble in boiling water, miscible with alcohol, ether, chloroform
7	Washability	Good	Good	Good
8	Nonirritancy	Non irritant	Non irritant	Non irritant

CONCLUSION:

Study concluded that it is possible to develop and evaluate preliminary phytochemical screening of methanolic extract of 3 Herbal ointments and gives good result and quality control evaluation. The formulation can also be routinely used for improving the hygiene of healthy children and adults.

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