

Advance in recent design, development and screening of a polyherbal antifungal ointment for external use from various medicinal plant extract

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Abstract

Now day herbal therapy and herbal drugs are predominates in traditional medicine as well as in alternative medicine practiced in the developed world. Among the various indications where traditional herbal medicines are used, skin and skin related disorders. The major goal of the present investigation is to be designing, formulating and evaluating a polyherbal ointment with antifungal activity. Ointments were formulated using Methanolic extracts made from ethanolic extracts of Azadirachta indica, Psidium guajava, Ipomoea Carnea Curcumalonga and Datura metal were tested for their physical and chemical properties, including their antifungal efficacy. Different extract concentrations, such as 2 percent, 4 percent, and 6 percent w/w, were used to make ointments utilizing the fusion process with emulsifying ointment as the basis. The formulations' physicochemical characteristics, such as loss of drying, pH, spread ability, Extrudability and diffusion studies, were then examined, and the findings were good. Additionally, the formulations were stable at 4°C, 25°C and 37°C. Additionally, using the agar diffusion method

and miconazole (5 percent w/w), polyherbal formulations were tested for their anti-fungal efficacy against Trichophyton rubrum and Trichophyton mentagrophytes. Thus, it could become a media to use medicinal properties of herbs effective and easily as simple dosage form.

Material and Method: We are taken Azadirachta indica, Psidium guajava, Ipomoea Carnea Curcumalonga and Datura metal leaves, Rhizome from Herbal garden of L. N. Pharmacy college Baitalpur Deoria up. We gathered leaves were cut into small pieces measuring 1-2cm, then the plant parts were washed under running water, dried for 10 days in the shade. Neem seeds were dried for 15 days in the shade. Using a dry grinder, the shade-dried plant material was ground into a coarse powder (sieve no. 10/44). For future use, the powder was kept in an airtight container. Five-Five gram of the powdered, fully dried material was taken in a separate container. 100ml of ethanol was added to this, which was then stored for 24 hours while being periodically shaken. The filtrate was then collected. The filtrates were kept in airtight bottles at 4°C

Result: Azadirachta indica and Psidium guajava are the three plants chosen because of their antioxidant and antifungal properties

Conclusion: Ipomoea Carnea, Psidium guajava, and Azadirachta indica combined ethanolic extracts showed superior in-vitro antifungal activity.

Keywords: Azadirachta Indica, Ipomoea Carnea, Psidium Guajava, Curcumalonga And Datura Metal Spread Ability and Extrudability.

Introduction

Nature has been a source of medicinal agents for thousands of years ^[1]. Various medicinal plants have been used for years in daily life to treat disease all the world. ^[2] The use of basic herbs for medical purposes is increasing because of less side effects or no side effects and effective remedies. The golden fact about the use of herbal treatment is it is independent of any age group. Certain European and oriented countries have been exploring the use of herbs and has been in practice since the centuries. Great work has been done which eluded the common man's reach and knowledge with the techno-savvy lifestyle in 21st century human suffering are coming out with different name ^[3]. Formulations are referred to as polyherbal when they contain two or more herbs. Herbal medicine and Ayurveda both have their roots in medicinal plants and have been used for ages. A growing number of people are visiting clinics that practice alternative medicine and herbal therapy is making a striking comeback. The herbal ointments refer a viscous semisolid preparation containing any plant's seeds, berries, roots, leaves, bark or flowers for the topical use on a variety of body surfaces. The most active compound of Turmeric is curcumin which has many scientifically proven health benefits, such as the potential to improve heart health and prevent against Alzheimer's and cancer. It's a potent anti-

inflammatory and antioxidant. It may also help improve symptoms of depression and arthritis. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacological therapeutic agents in medicinal plants. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore ^[4]

Herbal medications are a great benefit to our culture. These herbal medications are regarded as a medicinal weapon to combat numerous illnesses in birds, humans, and animals without causing any negative side effects. Further research into the idea of polyherbal formulations should be done given the current situation. Therefore, in the current work, we developed a polyherbal ointment that has improved antifungal and anti-oxidant action and may be used to skin diseases. In wealthy nations, there has been a significant increase in the demand for products made from plants in recent years. According to the literature, using traditional medicines to treat illnesses of the skin has a significant positive impact. In recent years, there has been a great demand for plant derived products in developed countries. The literatures have reported that the usage of the traditional medicines brought a great benefit in skin related diseases.

Hence the plant entities derived from the natural source need to be identified and formulated in to suitable dosage form for the management and treatment of various antimicrobial diseases.

Azadirachta indica (Family-Meliaceae) known as Neem is well known for its medicinal properties. Its leaves possess broad spectrum of activity against Gram +ve. and Gram -ve bacteria including M. tuberculosis, Vibrio cholera. The leaves, bark, and seeds of A. indica are known to have antibacterial and antifungal properties against several pathogenic microorganisms^[5]

The **Psidium guajava**, a tropical fruit guava belonging to the (Family- Myrtaceae) is a source of numerous herbal remedies. Comarins, essential oils, flavonoids, triterpenes, and ellagitannins, among other chemical components found in guava leaves, and are known for their antifungal activities. Guava leaves, roots, and fruits have been used to cure and prevent diarrhoea as well as to fight off common food-borne diarrhea- causing bacteria like Pseudomonas species and Staphylococcus species as well as Shigella species, Salmonella species, Bacillus species, E. coli and Clostridium species. "Ointment is a semisolid preparation which is used topically for various medicinal purposes or as cosmetic"

Curcumma longa consist of dried as well as fresh rhizomes of plant known as Curcuma longa belonging to (Family- Zingibaraceae). It is used as antiseptic, expectorant, as coloring agent and condiment^[6]

Ipomoea Carnea is medicinal plants belonging to (Family-Convolvulaceae) have number of activity like Heels wound, antibacterial, antifungal, and anti-inflammatory and treat to skin problems.

Datura metal is also a medicinal plant which is belonging to (Family-solanaceae) have various medicinal used like anti fungal, antibacterial and anti-inflammatory action.

Hence, an effort has been made to establish the scientific validity to investigate the possible antimicrobial and antioxidant activity of the formulated ointments made from the Methanolic extracts of the above four herbs.

From this investigation and the results this polyherbal ointment posse's significant antimicrobial as well as antioxidant activity can be used for the treatment of burns, wounds, rashes etc.

Objectives

- 1-To check different evaluation test for antibacterial and antifungal ointment
- 2-To prepare polyherbal ointment of turmeric extract, Neem extract, Ipomoea carnea and Datura metal so as to deliver the herbal drugs locally for treatment of inflammation, wound bacterial and fungal skin cream.
- 3- To evaluate the anti -fungal, anti-bacterial and anti-inflammatory activity of the drug.

Material and Method

Collection of plants

We are collected Azadirachta indica, Psidium guajava, Ipomoea Carnea, Curcuma longa and Datura metal leaves and Rhizome from Herbal garden of L.N. Pharmacy college Baitalpur deoria u.p. The gathered leaves were cut into small pieces by measuring 1-2cm, then the plant parts were washed under running water, dried for 10 days in the shade. Neem leaves were dried for 15 days in the shade. Using a dry grinder, the shade-dried plant material was ground into a coarse powder (sieve no. 10/44). For future use, the powder was kept in an air tight container. Five gram of the powdered, fully dried material were taken in a separate container. 100ml of ethanol filtrate was then collected. The filtrates were kept in air tight bottles at 4°C.

Equipment

Incubator, Digital balance, Soxhlet apparatus, Bunsen burner, pH meter, Glass wares,

Chemicals and reagents

Emulsifying wax, Cholesterol, Glycerin, Liquid paraffin, Stearyl alcohol, White soft paraffin Methanol, n-hexane,

Sulphuric acid, Dragendroff's reagent, Moloch's reagent, Acetone etc.

Process of plant extract

We taken five gram of fully dried of the powdered material in a separate container. Add 100ml of ethanol to this container, which was then stored for 24 hours. But occasionally shake in this period. The filtrate was then collected. The filtrates were kept in airtight bottles at 4°C.

Phytochemical analysis

These Methanolic extract obtained after extraction procedure was subjected to various phytochemical screening parameter as per the standard procedure to reveals the presence of various active phyto-constituents.

Formulation of Ointment

The required quantity of the chemicals was weighed and the polyherbal ointment was formulated by levigation method using emulsifying ointment base. Take the required apparatus wash it and dry it. Weight all the ingredients accurately on electronic balance. Initially ointment base was prepared by melting cholesterol, stearyl alcohol, white petroleum and white wax which was placed in evaporating dish on water bath. Stir gently to aid melting and mixing homogeneously followed by cooling of ointment base. Herbal ointment was prepared by mixing accurately weighed turmeric extract, Azadirachta indica extract, honey and eucalyptus oil to the ointment base also adds glycerin as a levigation agent. By levigation method to prepare smooth paste with 2/3 times its Weight of base gradually incorporating more bases until to form homogeneous ointment and transferred in to a suitable container.

Formula of herbal ointment

Sn.	Composition	Quantity
1	Azadirachta indica Extract	1.5 gm
2	Curcumma longa Extract	1.5 gm
3	Ipomoea Carnea	1.0 gm
4	Datura metal	0.5 gm
5	Psidium guajava	0.5
6	Stearyl alcohol	0.3 gm
7	White soft paraffin	8.6 gm
8	Cholesterol	0.3 gm
9	Glycerin	0.5 ml

Preparation of culture media for evaluation of anti fungal activity

Nutrient agar medium

For the preparation of nutrient agar 3gm of nutrient agar was taken and dissolved in 100 ml distilled water. The mixture was boiled for 10-15 min. After that the mouth of conical flask was covered by using cotton plug the assembly was kept in autoclave for 25 to 30 min for sterilization. Then the agar media was ready to use.

Nutrient broth medium

1.3 gm of nutrient broth was taken and dissolved in 100ml distilled water. The mixture was boiled for 10 to 15 min then the mouth of conical flask was covered by using cotton plug.

After that the conical flask was kept in to autoclave for 10 to 15 min for sterilization. The media was ready to use.^[7]

Evaluation parameter of poly herbal ointment

The formulation was evaluated for its different pharmaceutical properties

Physical Appearance

Colour- Dark yellow

Transparency- Non-transparent

Consistency- Smooth and no grittiness is observed

PH

Using a digital pH meter, the pH of several formulations was determined. 100cc of distilled water were used to dissolve one gram of ointment, which was then left to sit for two hours. It was done to measure the pH of each composition

Spreadability

The Spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as Spreadability. It is calculated by following formula

Where $S = \text{Spreadability}$

$$S = M \times L / T$$

$M =$ Weight tied to upper slide

$L =$ length of glass slide

$T =$ Time taken to separate the slide.

Extrudability

The formulation was filled in collapsible container. The extrudability was determined in terms of weight of ointment required to extend 0.5 cm^[8]

Stability studies

The stability studies were carried out for the prepared formulations at different temperature conditions (4°C, 25°C and 37°C) for 3 months^[9].

Wash ability

Formulation was applied on the skin and then ease extends of washing with water was checked.

Solubility

Soluble in boiling water, miscible with alcohol, ether and chloroform^[10]

Evaluation of antimicrobials activity

The required amount of agar was prepared and inoculated into it by microorganisms (Trichophyton rubrum and

Trichophyton mentagrophytes). Then Nutrient agar solution was poured into the Petri plates and allowed to stand to solidify for a few minutes. After solidification, a borer was used to generate the appropriate size of bores. The prepared ointments (of various concentrations) were then filled in the specified agar well diffusion experiment, the antifungal activity of ethanol extracts of *P. guajava*, *A. indica* and *Azadirachta indica*, *Ipomoea Carnea*, *Datura metal*, *Curcuma longa* was evaluated. Each well's zone of fungal growth inhibition is measured, and the susceptibility is calculated. In sterile Petri plates, agar (3.8 gm/100ml of distilled water) was produced, autoclaved at 121°C for 15 minutes at 15 pounds, and then poured up to a uniform thickness of around 5-6 mm. The agar was then allowed to set at room temperature before being utilized. Using a sterile borer, 6 mm-diameter wells were punched out of the plates. The plant extract solution in the combinations was then added to the wells. Petri plates were incubated at 37°C for 24 hours after being placed in the refrigerator for 30 minutes to allow extracts to diffuse. The zone of inhibition was assessed at the conclusion of the incubation period 10-12 hour.

Result

Azadirachta indica, *Ipomoea carnea*, *Datura metal*, *Curcuma longa*, and *Psidium guajava* are the five plants chosen because of their antioxidant and antifungal properties. Using specific species of microorganism, such as *Trichophyton rubrum* and *Trichophyton mentagrophytes*, the antifungal activity of prepared ointments was compared with 5 percent w/w miconazole ointment. It was found that formulations like F2 and F3 showed greater activity against *Trichophyton rubrum* and *Trichophyton mentagrophytes* than F1 formulation and less activity against 5 percent miconazole

Formulation of ointment

Sn.	Ethanol extract of herbal Ingredients	F1(2) percentage	F2(4) percentage	F3(6) percentage
1	Azadirachta indica (leaves)	2gm	4gm	6gm
2	Psidium guajava(leaves)	2gm	4gm	6gm
3	Ipomoea carnea(leaves)	2gm	4gm	6gm
4	Datura metal(leaves)	2gm	4gm	6gm
5	Curcuma longa (Rhizomes)	2gm	4gm	6gm
6	Ointment base	q.s to 100gm	q.s to 100gm	q.s to 100gm

Physical evaluation of the developed Formulations

Sn.	Physical Appearance	F1 (2 %)	F2 (4 %)	F3 (6 %)
1	Colour	Dark green	Dark green	Dark green
2	Odour	Characteristic	Characteristic	Characteristic
4	pH	7.01	6.87	6.86
5	Spreadability(seconds)	10	11	14
6	Extrudability	174gm	178gm	181gm
7	Storage (4°C, 24°C, 37°C)	Stable	Stable	Stable

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of single & combination extract

Sn.	Plant	Part used	T. rubrum Ethanol extractmg/ml		T. mentagrophytes Ethanol extractmg/ml	
			MIC	MFC	MIC	MFC
1	Ipomoea carnea	Leaves	0.25	0.25	0.28	0.28
2	Azadirachta indica	Leaves	0.55	0.55	0.55	0.55
3	Psidium guajava	Leaves	0.46	0.46	0.43	0.43
4	Datura metal	Leaves	0.48	0.48	0.47	0.45
5	Curcuma longa	Rhizomes	0.49	0.49	0.49	0.47
6	Ipomoea carnea + Azadirachta indica	Leaves	0.22	0.22	0.24	0.24
7	Ipomoea carnea+Psidium guajava	Leaves	0.17	0.17	0.19	0.19
8	Azadirachta indica+ Psidium guajava	Leaves	0.38	0.38	0.42	0.42
9	Datura metal+ Curcuma longa	Leaves+ Rhizomes	0.39	0.39	0.42	0.42
10	Ipomoea carnea+Curcuma longa	Leaves+ Rhizomes	0.39	0.43	0.43	0.43

11	Ipomoeacarnea + Azadirachta indica+ Psidium guajava +Datura metal+ Curcuma longa	Leaves+ Leaves+ Leaves+ Leaves+ Rhizomes	0.16	0.16	0.19	0.19
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Antifungal activity of (formulated ointment)

Sn.	Ointments	Zone of inhibition in cm Ointments	
		Trichophyton rubrum	Trichophyton mentagrophytes
1	F1 (2%)	2.2	1.9
2	F2 (4%)	2.4	2.2
3	F3 (6%)	2.5	2.3
4	Standard	2.6	2.5

Conclusion

Ipomoea carnea, Curcuma longa, Datura metal Psidium guajava, and Azadirachta indica combined ethanolic extracts showed superior in-vitro antifungal activity (MIC and MFC) compared to the individual extracts, according to the findings of the current study. The most effective antifungal activity was shown by the combination group that contained all three extracts. This reinforces the fundamental idea behind the synergism of herbal extracts. When evaluated on a guinea pig model, the manufactured ointments F1 (2 percent), F2 (4 percent), and F3 (6 percent) displayed appreciable in-vivo antifungal activity in contrast to Miconazole (Standard) ointment.

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