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FORMULATION AND EVALUATION OF ANTIFUNGAL POLYHERBAL OINTMENT OF DIFFERENT PLANT EXTRACT

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ABSTRACT

The major goal of the current investigation is to create and assess a multi-herbal ointment with antifungal activity. Ointments made from ethanolic extracts of *Azadirachta indica, Allium sativum* and *Psidium guajava* 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 utilising the fusion process with emulsifying ointment as the basis. The formulations' physicochemical characteristics, such as loss of drying, pH, spreadability, 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.

KEYWORDS

Azadirachta indica, *Allium sativum, Psidium guajava Trichophyton rubrum*, *Trichophyton mentagrophytes* spreadability and Extrudability.

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INTRODUCTON

Ayurvedic medicine is a tried-and-true medical method that has been used in India's hospitals for many years. Being a tried-and-true system, it offers an advantage over other health management systems now in use[1]. 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 practise alternative medicine, and herbal therapy is making a striking comeback. The frightening side effects of synthetic drugs have

recently been joined by the possibility of herbal and herbal-synthetic medication interactions[2]. 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. Therefore, it is necessary to identify and formulate plant entities originating from natural sources into appropriate dosage forms for the management and treatment of diverse antifungal illnesses. The plant *Azadirachta indica*, also known as Neem, belongs to the Meliaceae family. Its leaves are highly effective against both Gram +ve and Gram -ve bacteria, such as *M. tuberculosis* and Vibrio cholera[3].

The leaves, bark, and seeds of A. indica are known to have antibacterial and antifungal properties against several pathogenic microorganisms, as well as antiviral properties against the vaccinia, chikungunya, measles, and Coxsackie B viruses. The cardiovascular system benefits from its therapeutic uses, which also include antibacterial, anticancer, anti-inflammatory, hypoglycemic, and hormone-like properties. Gram-positive and Gram-negative bacteria such E. coli, Salmonella spp., Streptococcus spp., Staphylococcus aureus, and Klebsiella are both highly resistant to the antibacterial effects of garlic.*spp., Proteus mirabilis, Shigella senteriae, Pseudomonas aeroginosa* and *Helicobacter pylori*, also it's effective even against those strains that have become resistant to antibiotics[4]. It is well known that Psidium guajava, a tropical fruit guava belonging to the Myrtaceae family, is a source of numerous herbal remedies[5]. Comarins, essential oils, flavonoids, triterpenes, and ellagitannins, among other chemical components found in guava leaves, 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. Traditional medicine has employed the plant's different parts to treat a variety of ailments, including malaria, gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothaches, coughs, sore throats, swollen gums, diabetes, hypertension, and obesity[6,7].

MATERIAL AND METHODS

Collection of plants

We purchased *Allium sativum* (garlic) leaves at the Lucknow neighbourhood market. The seeds of *Azadirachta indica* (Neem) were purchased from a regional supplier of medicinal plants in Aminabad, Lucknow. We gathered Psidium guajava (guava) leaves from the National Botanical Research Institute at Lucknow campus. Allium sativum and Psidium guajava 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.

Chemicals and reagents

Emulsifying wax, Liquid paraffin, White soft paraffin Methanol, n-hexane, Sulphuric Acid, Drangendroff's reagent, Molisch's reagent, Acetone e.t.c.

Equipments

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

Prepration of plant extract

Five grammes of the powdered, fully dried material were 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.

Prepration of culture media

38 g of Mueller Hinton Agar (MHA) medium were dissolved in 1000ml of distilled water to create the media. The media was put into sterile Petri plates

after being autoclaved at 121°C for 15 minutes. The media's pH was changed by adding 0.1M HCl or 0.1M NaOH[8].

EVALUATION

Physicochemical parameters

Preliminary evaluation of formulations at different concentrations was carried out as follows:

Colour and odour

Colour and odour was examined by visual examination.

Loss on drying

Loss on drying was determined by placing ointment in petridish on water bath and dried for 105^{O}C.

pH

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

Spreadability

Spreadability is a phrase used to describe the size of the region that the ointments easily cover after being applied to the skin or affected area. 9 Multimer has created a unique equipment to investigate the spreadability of formulations. The spreadability was measured in terms of the number of seconds it took for two slides to separate from ointment that was placed in between the slides when a specific load was applied. The capacity to convey information is improved when two slides are separated more quickly. The formula was used to determine spread ability. S = (M.L/T)

Where, S = Spreadability, M = Weight tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides

Extrudability

A simple method was adopted for this study. The formulations were filled in the collapsible tubes after the ointments were set in the container. The extrudability of the different ointment formulations was determined in terms of weight in grams required to extrude a 0.5 cm of ribbon of ointment in 10 second.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any Concentration. It was poured into petridish. A hole bored at the centre and ointment was placed in it. The time taken for the ointment to get diffused was noted.

Stability studies

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

Evaluation of antifungal acitvity Test microorganism

Trichophyton rubrum and *Trichophyton mentagrophytes*

Standard used

5% w/w Miconazole ointment.

Sample preparation

About 10mg of ointments (2%, 4%, 6% w/w) were weighed and dissolved in DMF (dimethyl formamide) and used for activity studies.

Methodology

Using the specified agar well diffusion experiment, the antifungal activity of ethanol extracts of *P. guajava, A. indica* and *Allium sativum* was evaluated. Each well's zone of fungal growth inhibition is measured, and the susceptibility is calculated. Hinton, Medium-Muller In sterile petri plates, agar (3.8 gm/100ml of distilled water) was produced, autoclaved at 1210C 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 utilised. Using a sterile borer, 6 mm-diameter wells were punched out of MHA 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].

RESULTS AND DISCUSSION

Azadirachta indica, Allium sativum, and *Psidium guajava* are the three plants chosen because of their antioxidant and antifungal properties. This led to an attempt to create a polyherbal ointment, which was then evaluated for its physical properties, in vitro antioxidant activity, and antifungal activity in comparison to a commercial formulation (5 percent w/w miconazole). Using ethanol as a solvent, extraction and phytochemical screening were

carried out. The presence of different phytoconstituents like carbohydrates, glycosides, flavanoids, and tannins was confirmed by phytochemical analysis. In the current work, emulsifying ointment was used as the substrate to create polyherbal ointments using the fusion method. The formulations were then assessed for their physical characteristics and their antifungal activity was contrasted with that of commercial 5 percent w/w miconazole ointment. These physico-chemical characteristics were acceptable. The formulations appeared to be stable based on the stability analyses that were conducted. 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. Antifungal research therefore demonstrates that the produced ointments have less action against Trichophyton rubrum and Trichophyton mentagrophytes than the usual ointment containing 5 percent miconazole. Antioxidant activity implied that the ascorbic acid-based topicals displayed similar activity to that of normal ascorbic acid, indicating that the presence of flavanoids and tannins is the cause of this action. So, the study's findings include that ethanolic plant extracts of *Azadirachta indica, Allium sativum* and Psidium guajava can be utilised to create an effective antifungal ointment with antibacterial and antioxidant activity that can also be used for wound healing and a variety of skin illnesses.

Table No.1: Formulation of ointment

S.No	Ingredients (ethanolic extract)	F1(2%)	F2(4%)	F3(6%)
1	*Azadirachta indica (seeds)*	2gm	4gm	6gm
2	*Allium sativum(leaves)*	2gm	4gm	6gm
3	*Psidium guajava(leaves)*	2gm	4gm	6gm
4	*Ointment base*	qs to 100gm	qs to 100gm	qs to 100gm

Table No.2: Phytochemical screening of the ethanolic extracts of *Allium sativum, Psidium guajava, Azadirachta indica*

S.No	Constituents	Name of the test	Ethanolic extract of *Allium sativum*	Ethanolic extract of Psidium	Ethanolic extract of Azadirachta indica
1	Carbohydrates Reducing sugars	Molisch's test	+	+	+
		Fehling's test	+	+	+
		Benedict's test	+	+	-
		Barfoed's test	+	+	-
2	Alkaloids	Dragendorff's test	+	-	-
		Wagner's test	+	-	-
		Mayer's test	-	-	-
3	Glycosides	Legal's test	-	-	+
		Keller Killiani test	-	-	+
		Borntrager's test	-	-	+
		Modified Borntrager's test	-	-	+
4	Flavanoids	Shinoda test	+	+	+
		Lead acetate test	+	+	+
		Sodiumhydroxide	+	+	-
5	Tannins	Goldbeater'sskin test	-	+	-
		Gelatin test	-	+	-
		Catechin test	-	+	-

Table No.3: MIC and MFC of single and combination of extracts

S.No	Plant	Part used	*T. rubrum* Ethanol extract mg/ml		*T. mentagrophytes* Ethanol extract mg/ml	
			MIC	MFC	MIC	MFC
1	*Allium sativum*	Leaves	0.25	0.25	***0.28***	0.28
2	*Azadirachta indica*	Seeds	0.55	0.55	0.55	0.55
3	*Psidium guajava*	Leaves	0.46	0.46	0.43	0.43
4	*Allium sativum*+ *Azadirachta indica*	Leaves+Seeds respectively	0.22	0.22	0.24	0.24
5	*Allium sativum*+*Psidium guajava*	Leaves+Leaves respectively	0.17	0.17	0.19	0.19
6	*Azadirachta indica*+ *Psidium guajava*	Seeds+Leaves repectively	0.38	0.38	0.42	0.42
7	*Allium sativum*+ *Azadirachta indica*+ *Psidium guajava*	Leaves+Seeds+ Leaves respectively	0.10	0.10	0.15	0.15

Table No.4: Physicochemical evaluation of formulated Formulations

S.No	Physicochemicalparameters	F1(2%)	F2(4%)	F3(6%)
1	Colour	Dark green	Dark green	Dark green
2	Odour	Characterstic	Characterstic	Characterstic
3	Loss on drying	38%	40%	41%
4	pH	7.01	6.87	6.86
5	Spreadibility(seconds)	10	11	14
6	Extrudability	174gm	178gm	181gm
7	Diffusion (after60 min)	0.7cm	0.8cm	.10cm
8	Storage (4^oC, 24^oC, 37^oC)	Stable	Stable	Stable

Table No.5: Antifungal activity of formulated ointment

S.No	Ointments	Zone of inhibition in cm Ointments	
		Trichophyton rubrum	***Trichophyton mentagrophytes***
1	F1(2%)	2.2	1.9
2	F2(4%)	2.4	2.3
3	F3(6%)	2.5	2.2
4	Standard	2.6	2.5

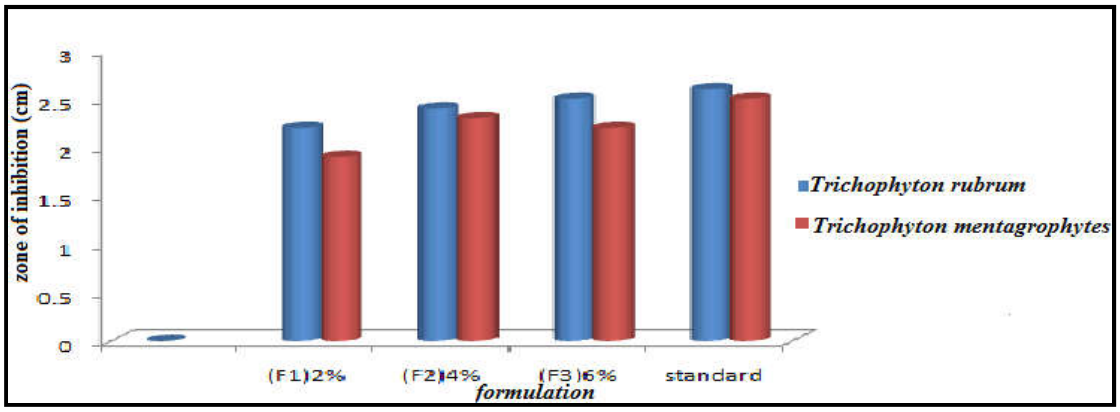

Figure No.1: Antifungal activity of formulated ointments

CONCLUSION

Allium sativum, *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.

The prevalence of dermatophytosis has significantly increased during the last ten years. Serious health issues can result from rising prevalence of fungus infections and rising resistance to conventional antifungal medications. The study's creation of a herbal ointment provides a workable answer to this issue.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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