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To Develop and Evaluate Of Poly Herbal Formulation for Antifungal Activity

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ABSTRACT
The present work is to formulate and evaluate the ointment of Neem and Turmeric extract for anti-Fungal activity. The ethanol extract was prepared by Maceration extraction method. The ointment base was prepared and seven formulations of ointments were done by incorporating the extract in the base by levigation method. The antifungal activity of ointment was carried out by well diffusion method. Formulation (F4) and Formulation (F6) shows the best result against Aspergillus Niger and Aspergillus Varis. All the formulations were evaluated for their physicochemical parameters like colour, odour, pH, spreadability, extrudability, consistency, diffusion study, solubility, washability. Also the formulation was evaluated for its stability at various temperature conditions which shows no change in the irritancy, spreadability and diffusion study.

Keywords : Herbal Ointment, Well Diffusion method, Azadiracht Indica, Curcuma longa**,** Antifungal activity

I. INTRODUCTION

Plants are the oldest source of pharmacologically active compounds and have provided human kind with many medicinally useful compounds from centuries. Today more than two thirds of the world's population relays on plant derived drugs. The origin of many effective drugs is found in the traditional medicinal practices and in view of this it is very important to undertake studies pertaining to screening of the medicinal plants for their proclaimed biological activity. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new, antimicrobial compounds 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.[(1)] Curcuma longa (Family-Zingiberaceae) is a rhizomatous plant known as Turmeric. It is used for the treatment of wounds, cuts, burns, galactose induced cataract formation, ulcer etc. It is also used in protection against vascular dementia due to antioxidant activity. Both curcumin and the oil fraction suppress growth of several microbes like Streptococcus, Staphylococcus, Lactobacillus. A.flavus, P.digitatum, A.parasiticus, etc. This ointment can be used in the treatment of sun burns, rashes, burns, wounds and other skin infections. It can also be used in the treatment of superficial mycosis.[(2)]

II. MATERIALS AND METHOD

Collection of Plant material
Leaves of neem were collected from the local area of Bhopal and dried rhizomes of turmeric were purchased from the local market of Bhopal (M.P.).

Preparation of Neem extract:
Leaves of the plant were collected and washed thoroughly with distilled water and shade dried for 20 days. Dried leaves were ground into powder form. 100gm powder was imbibed with 500ml of ethanol and kept for maceration procees for about 3-4 days. . After maceration the extract is filtered and the filtrate was collected and used for making ointment.

Preparation of Turmeric extract :
Rhizomes of the turmeric were collected and washed thoroughly with distilled water and shade dried for 20 days. Dried leaves were ground into powder form. 100gm powder was imbibed with 500ml of ethanol and kept for maceration procees for about 3-4 days. After maceration the extract is

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filtered and the filtrate was collected and used for making ointment.[3]

FORMULATION OF OINTMENT:

Table 1: Formulation of ointment base

S.no.	Name of ingredient	Quantity taken
1.	Wool fat	0.5gm
2.	Cetosterayl Alcohol	0.5gm
3.	Hard paraffin	0.5gm
4.	Soft yellow paraffin	8.5 gm

Procedure for preparation of ointment base:

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

Table 2 Formulation of poly herbal ointment

S.no	Ingredients	Formulation Code						
		F1	F2	F3	F4	F5	F6	F7
1.	Prepared extract of Neem	5	5	5	5	5	5	5
2.	Prepared extract of Turmeric	5	5	5	5	5	5	5
3.	Ointment Base	15	15	15	15	5	15	15
4.	Total	25	25	25	25	25	25	25

Herbal ointment was prepared by mixing accurately weighed Neem and Turmeric extract to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container.

EVALUATION OF POLYHERBAL FORMULATION[4]

The polyherbal composition was tested by the following physicochemical parameters :

Color and odour

Physical parameters like colour and odour were examined by visual examination.

Loss on drying

Drying loss is determine by placing the ointment in a container of Petridish in a water bath and then suspended until a permanent weight is obtained.

pH

pH of the formulation was recorded using a digital pH meter. The bulk of the sample weight was dissolved in water and kept for two hours and the pH value was measured.

Spreadability

The spread is temporarily expressed in seconds taken by two slides from the ointment placed between the slides under the load direction. Spreadability is calculated by using the following formula :

S= (M.L / T)

When S = spread, M = Weight is tied to the top slide, L = Length of glass slides, T = time taken to split slides. [5]

Extrudability

The structure was completed in standard aluminum cross – sectional tubes that are closed at the end. The weight of each tube was determined

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and recorded. The tubes are then inserted between two glass slides held at a standard weight of 0.5 kg above the glass plate. After that, the cap is made to remove and measure the ointment extracted from the tube. Calculated the percentage of ointment extracted.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium. A hole board at the center of medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted. (after 60 minutes).[6]

Solubility

Soluble in boiling water, miscible with alcohol, ether, chloroform.

Washability

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

Non irritancy

Test Herbal ointment prepared was applied to the skin of human being and observed for the effect.

Stability study

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2oC, 25oC and 37oC. The herbal ointment was found to be physically stable at different temperature i.e. 2oC, 25oC, 37oC within four weeks.[7]

TEST MICROORGANISM:

Fungi: A.varis, A.niger, P.notatum. Standards used. Anti-fungal: Miconazole and Flucinolone ointment.

Anti-fungal activity:

Determination of zone of inhibition:

- Anti-microbial activity was checked by agar gel diffusion method.
- The cultures were grown in nutrient broth and incubated at 37ºc, for 24 hrs.
- After incubation periods was over, 0.1 ml of culture was seeded in 25 ml molten nutrient agar butts, mixed and poured into sterile petri plates and allowed to solidify.
- The well was bored with 6 mm borer in seeded agar. 0.1 g of each ointment sample was added in each well.
- Plates were kept at 10ºc as a period of pre diffusion for 30 minutes.
- After it normalized to room temperature; the plates were incubated at 37ºc for 24 hrs.
- After incubation period was over, the zone of inhibition was measured with help of Hi-antibiotic zone scale.[8],[9],[10]

III. RESULT AND DISCUSSION

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using simple maceration process to obtain a good yield of extract and there was no any harm to the chemical constituents and their activity.

The levigation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occured which was stable during the storage.

Physicochemical evaluation of formulated ointment

Table : 3 Physicochemical evaluation

S.no	Physicochemical parameter	Observation Formulation Code						
		F1	F2	F3	F4	F5	F6	F7
1	Colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
2	Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic

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3	Consistency	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
4	pH	6.2	6.5	6.3	6.5	6.8	6.5	6
5	Spreadability (seconds)	7	6	8	7	7	6	7
6	Extrudability	0.4gm	0.4gm	0.3gm	0.4gm	0.5gm	0.4gm	0.5gm
7	Diffusion study	0.7cm	0.6cm	0.7cm	0.5cm	0.6cm	0.5cm	0.7cm
8	Loss on Drying	30%	28%	30%	27%	28%	30%	30%
9	Solubility	Soluble in boiling water, miscible with alcohol, ether	Soluble in boiling water, miscible with alcohol, ether	Soluble in boiling water, miscible with alcohol, ether	Soluble in boiling water, miscible with alcohol, ether	Soluble in boiling water, miscible with alcohol, ether	Soluble in boiling water, miscible with alcohol, ether	Soluble in boiling water, miscible with alcohol, ether
10	Washability	Good	Good	Good	Good	Good	Good	Good

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11	Non irrtancy	Non irritant	Non irritant	Non irritant	Non irritant	Non irritant	Non irritant	Non irritant
12	Stablity study (2^oC, 25^oC, 37^oC)	Stable	Stable	Stable	Stable	Stable	Stable	Stable

Antifungal Activity

The Anti-fungal efficacy of the formulations of Poly herbal ointment was tested onAspergillus niger and Aspergillus varis by agar plate technique. The results of zone of inhibition showed that the ointment prepared from ethanol extract of the combined plant materials shown significant antifungal Activity.

The data of zone of inhibition **of poly herbal formulation (F6) is shown in below table**

Table : 4 Zone of inhibition

S.no	Microorganism	Formulation (F6)			
		Different concentration			
		25 µg/ml	50 µg/ml	75µg/ml	Standard
1.	Aspergillus niger	3.5mm	4 mm	7 mm	6.4mm
2.	Aspergillus Varis	3.2mm	3.8mm	7.2mm	6.8mm

The anti fungal assay was performed using the well diffusion method of F6 formulation with concentration 75µg/ml **Aspergillus niger**which best showed the 7 mm zones of inhibition in diameters.

The anti fungal assay was performed using the well diffusion method of F6 formulation with concentration 75µg/ml **Aspergillus Varis** which best showed the 7.2 mm zones of inhibition in diameters.

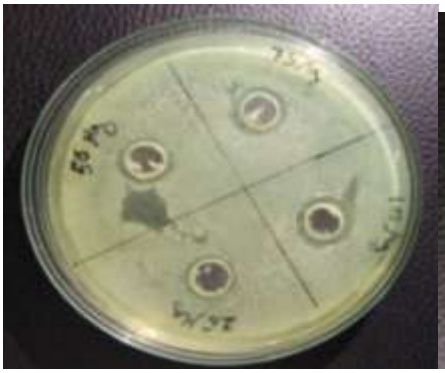

Figure: 1
Different concentration of poly herbal herbal formulation F6 with Aspergillus niger

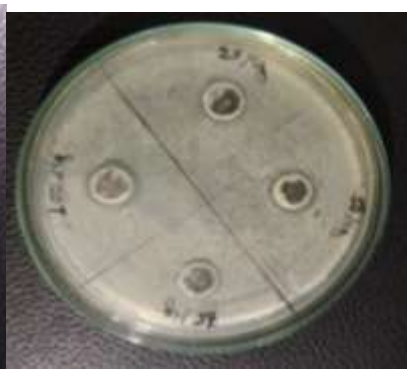

Figure: 2
Different concentration of poly herbal formulation F6 with Aspergillus varis

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In Petri Plates
25ug/ml = Poly herbal Formulation F6
50ug/ml = Poly herbal Formulation F6
75ug/ml = Poly herbal formulation F6
100ug/ml = Standard

The data of zone of inhibition of poly herbal formulation (F4) is shown in below table

Table : 5 Zone of inhibition

S.no	Microorganism	Formulation (F4)			
		Different concentration			
		25 µg/ml	50 µg/ml	75µg/ml	Standard
1.	Aspergillus niger	3.7mm	5.2 mm	7 .8mm	7.5mm
2.	Aspergillus Varis	3.5mm	4.8mm	8.3mm	8.1mm

The antifungal assay was performed using the well diffusion method of F4 formulation with concentration 75 µg/ml against Aspergillus niger which best showed the7.8 mmzones of inhibition in diameters.
The antifungal assay was performed using the well diffusion method of F4 formulation with concentration 75 µg/ml against Aspergillus varis which best showed the8.3 mmzones of inhibition.

Figure : 3
Different concentration of poly herbal formulation F4 with Aspergillus Niger

Figure : 4
Different concentration of poly herbal Formulation F4 with Aspergillus Varis

In Petri Plates
25ug/ml = Poly herbal Formulation F4
50ug/ml = Poly herbal Formulation F4
75ug/ml = Poly herbal formulation F4
100ug/ml = Standard

IV. DISCUSSION

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using simple maceration process to obtain a good yield of extract and there was no any harm to the chemical constituents and their activity.

The levigation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occured which was stable during the storage.

The physicochemical properties were studied which shows satisfactory results for spreadability, extrudability, washability, solubility, loss on drying and others. Also the formulation was placed for a stability study at different temperature conditions like $2^{o}C$, $25^{o}C$ and $37^{o}C$ within four weeks. There were no changes observed in spreading ability, diffusion study as well as irritant effect.

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