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Formulation Evaluation and Antifungal Activity of Cream of *Swertia chirata* Plant Extract

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ABSTRACT

Introduction: Medicinal plants are of great value in the field of treatment and cure of diseases. Over the years, scientific research has expanded the knowledge of the chemical effects of various Phytoconstituents which are responsible for the different therapeutic activity**.** *Swertia chirata* is commonly known as Chirata, Charayatah, Chiarayata etc belonging to family Gentianaceae. In present study, an antifungal cream of Swertia chirata was formulated and evaluated as per the standard guidelines. **Method**: The various creams of ethanolic extract *Swertia chirata* was formulated by permutation and combinations. Out of which three creams consisiting 1% extarct having suitable spreadability and consistency by physical observations were selected for further studies. The selected herbal creams of *Swertia Chirata* were evaluated for various parameters like appearance, excrudilibility, spreadibility, pH, Viscosity, antifungal activity etc. **Result:** The present findings found to be significant for the development of alternative, inexpensive and perhaps safer strategies for the treatment of fungal diseases.**Conclusion**: From the result obtained it can be concluded that the extracts could serve as lead for development of prospective anti-fungal agent in the form of topical herbal cream.

INTRODUCTION:

Medicinal plants are of great value in the field of treatment and cure of diseases. Over the years, scientific research has expanded the knowledge of the chemical effects and various Phytoconstituents, that are responsible for the different therapeutic activity.[1] The traditional system of medicine is so organized in our culture that, even now 75% of the Indian population still depend on this indigenous system for relief with such huge sector of escalating population depending on herbal remedies, it is has been very important to investigate the traditionally claimed activity of plants which are being used from ancient time for the efficacy. Presently numerous pure compounds are isolated from plant for this purpose.[2] Natural products as a basis for new drugs have great promise and it is gratifying to note that the World Health Organization have shown an abiding interest in plant derived medicines, described in the folklore of various countries. [3].

Candidiasis is most commonly encountered opportunistic mycosis worldwide. Candida albicans is the most common species in the genus which has been implicated in Candidiasis [4] The infections range from superficial of skin to systemic diseases. *C.albicans, C. tropicalis, C. glabrata* and *C. parapsilosis* are part of the normal flora of human and can be isolated from oral cavity, vaginal and other parts of body sites from normal healthy people. Under certain circumstances, these organisms may gain access to many organ systems such as lung, spleen, kidney, liver, heart, brain, eye, skin and others. Lesions may occur in patients who have disseminated infections. [5] Current Treatment available for Candidiasis are as: Topical antifungal: Ketoconazole, Miconazole, Nystatin, for Systemic: Amphotericin B, Fluconazole, and Itraconazole and for Chronic mucocutaneous: Amphotericin B, Fluconazole, Itraconazole but with these synthetics drugs there are certain side-effects. Most Candida species multiply by blastospore formation producing either Pseudo hyphae or true hyphae. Candida species are identified and specify according to their ability to assimilate and ferment various carbohydrates as well as certain physiological tests and morphologic responses when they grow under controlled nutritional conditions. Candidiasis Treatment: a) Topical antifungal: Ketoconazole, Miconazole, Nystatin b) Systemic: Amphotericin B, Fluconazole, and Itraconazole c) Chronic mucocutaneous: Amphotericin B, Fluconazole, Itraconazole. [6,7]

Swertia chirata is commonly known as Chirata, Charayatah, Chiarayata, belonging to family Gentianaceae. It is small, erect, herbaceous plant which grows upto the height of 1.5 meters.

This plant is native to temperate Himalaya at the altitude of 1200-1300 meters. It is also grown in sub temperate region at the altitude of 1500 to 2100m. *Swertia Chirata* can be easily grown in different types of sandy loamy soil rich in carbon and humus. It majorly contain chiratin, ophelic acid, Xanthones, Xanthoneglycoside and flavonoid are also found. Other minor constituents are calcium, magnesium, Iron, potassium and sodium. Traditionally his plant is claimed to have antibacterial, antifungal, antiviral, anticancer, anti-inflammatory, and antioxidant activities. [8,9,10]

Figure No. 1: ***Powder extract of Swertia Chirata***

MATERIALS AND METHODS:

Collection of Plant material and chemicals:

The leaves of plant *Swertia chirata* was collected from the local market of Indore which a was further identified on the basis of its macroscopic as well as microscopic characters. The chemicals required for the study was procured from the College.

Preparation of Extracts

Leaves of selected plant was dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of leaves powder of *Swertia chirata* was extracted using petroleum ether for the removal off fats and then dried material was extracted by using ethanol as a solvent by soxhlet apparatus for 72h. Then the ethanolic extract was dried under reduced pressure to get crude ethanolic extract. After drying the extract was weighed and percentage yield was determined. [11]

Preliminary Phytochemical Screening

Qualitative chemical tests of ethanolic extract was carried out to various phytoconstituents present in the extract. [12,13,14]

Chemical test for carbohydrate:

Molish test- To 2-3 ml of the extract add, few drops of alpha naphthol solution and was shaken. Concentrated sulphuric acid was added from sides of test tube. A Violet color ring was seen at the intersection of two liquids.

Molish reagent- Dissolve 10 gram of alpha naphthol in 100 ml 95% alcohol.

Fehling's Test: To 1ml of the extract, equal quantities of Fehling solution A and B was added and heated. There appeared ppt of red brick color which responded for the presence of reducing sugars.

Chemical test for alkaloids:

The few amount of extract was taken separately and was evaporated and filtered. To residue, dilute HCl was added and it was shaken well and filtered. With filtrate, following tests were performed:

Dragendorff's Test: In 3 ml. of filtrate, Dragendorff's reagent (potassium bismuth iodide solution) was added and thus showed the ppt of Orange brown color indicating the existence of alkaloids.

Mayer's Test: Few drops of Mayer's reagent (potassium mercuric iodide solution) were added in 3 ml of filtrate and formation of cream colored precipitate indicates presence of alkaloids.

Hager's Test: Small quantity of Hager's reagent (saturated aqueous solution of picric acid) was added in filtrate and formation of yellow colored precipitate shows presence of alkaloids.

Wagner's Test: Few drops of Wagner's reagent (iodine in potassium iodide) were added in 3 ml filtrate and formation of reddish brown colored precipitate show presence of alkaloids.

Test for saponins:

Foam Test: The drug extracts were vigorously shaken with water. Persistent foam formation indicates presence of saponins.

Liberman Burchard's Test: To drug extracts few drops of glacial acetic acid and two drops of conc. H_2SO_4 were added. Color changes from rose red, violet, blue to green reveals the presence of steroidal saponins.

Test for proteins:

To about 3 ml of the extract, add 4% sodium hydroxide and few drops of 1% copper sulphate was added. Violet of pink color appeared.

Test for amino acids:

Ninhydrin test- 3 ml of test solution was heated and 3 drops of 5% Ninhydrin solution was added in boiling water and it was boiled for 10 minutes. Appearance of Purple or bluish color shows presence of Amino acid.

Ninhydrin reagent-0.1% solution of ninhydrin in n-butanol.

Test for steroids:

Salkowski reaction- 2 ml of the extract, 2 ml of chloroform and 2 ml concentrate sulphuric acid was added. It was shaken well. The Chloroformic layer seen to be in red whereas acidic layer showed greenish yellow color fluorescence.

Test for anthraquinone glycosides:

To about 3 ml extract, dilute sulphuric acid was added. It was boiled and filtered. To cold extract equal volume of benzene or chloroform was added. After shaking organic solvent was separated well. Add ammonium, ammonical layer turned pink.

Test for flavonoids:

Shinoda Test: 5 ml of (95%v/v) ethanol was added in the extract and then few drops of Conc. HCl and 0.5g magnesium turnings were added. Pink color shows the presence of flavonoids.

To small quantity of extract, lead acetate solution was added. Yellow colored precipitate formation shows the presence of flavonoids.

Addition of increasing amount of sodium hydroxide to the extract showed yellow coloration, which decolorized after addition of acid, indicates the presence of flavones.

Tests For Tannins

Take 1ml of the extract to which at ferric chloride solution which shows development of a dark blue or greenish black colour indicating the existence of tannins.

The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

Development of Antifungal Cream

Emulsifying wax, glyceryl monostearate and soft paraffin were melted together in a china dish over a water bath at a temperature of 70°C. Terbinafine hydrochloride was dissolved in propylene glycol and it was mixed with liquid paraffin. This mixture was added to the melted mixture and the temperature was maintained at 70°C. Simultaneously methylparaben and propylparaben were dissolved in freshly boiled and cooled water taken in a beaker and the temperature was maintained at 70°C. Then aqueous phase was added to the oily phase and stirred well. The fluid mixture was taken away from the water bath and stirred until cooled, avoiding aeration. The content was stirred effectively to avoid any crystallization. Three batches of 1% extract creams were subject to physical and chemical analysis (Table 1). [15]

Table No. 1: Composition of Antifungal Cream

S.No	Ingredients	C1(gm)	C2(gm)	C3(gm)
1	Ethanolic Extract	1.0	1.0	1.0
2	Emulsifying wax	5	2	4
3	Glyceryl	5	2	5
4	Soft paraffin	10	7	-
5	Liquid paraffin	-	3	10
6	Propylene glycol	1	1	1
7	Methyl Paraben	0	0	0
8	Propyl Paraben	0	0	0
9	Purified water	68	69.2	64

EVALUATION OF TOPICAL HERBAL CREAM FORMULATION

Extrudability

A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end and a clamp was applied to put off any roll back. The closure of the tube was detached and then the gel was extruded out. Then the quantity of the extruded out gel was weighed and

calculated. [16,17]

pH measurement

pH capacity of the gel was carried out using a digital pH meter by dipping the glass electrode completely into the gel system to cover the electrode. The process of measuring pH was done in triplicate.[18]

Appearance and Homogeneity

Appearance and homogeneity was checked by the visual observation.[18]

Viscosity

Viscosity is defined as the resistance to flow property of gel which was evaluated using Brookfield viscometer (S-62, model LVDV- E) at 25°C with a spindle speed of the viscometer rotated at 12 rpm. [19]

Spreadability

Standard glass slides of equal size were considered. The formulated gel was applied on one surface of slide and other slide was kept on the top of previous slide in such manner that formulation as sandwiched between the two slides. Then to it 100 g weight was kept on upper slide in such a manner that it uniformly compresses so that formulation forms a thin layer. Then the weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest trouble and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 20 g weight was tied to the upper slide cautiously. Then the moment in time taken by upper slide to cover the distance of 7.5 cm and then time taken to separate away it from the lower slide beneath the effect of the weight was noted. [20]

Spreadibility of the gel was evaluated by

$$S = m \times l/t$$

where, S= spread ability, m-weight tied to upper slides (20 g), l- length of the glass slide (7.5 cm), t- time taken in second.

Antifungal activity of herbal antifungal Ointment

Fungal Inoculum:

The inoculums was prepared by inoculating *Candida albicans* in MGYP broth and incubated at 37^0C for 24 hrs.

Procedure of media preparation:

MGYP medium:

- The respective quantity of Malt extract, Glucose, Yeast extract, Peptone, Agar were added in 200ml of distilled water.
- Heat to boiling to dissolve the medium completely.
- Then sterilized by autoclaving 15 lb at 121^0C for 15 min.

Potato Dextrose Agar medium:

40.0 g of peeled potatoes are cut into small pieces and suspended in 200.0 ml of distilled water.

- Steamed for 30 min. decant the extract or filter through muslin cloth and make the final volume to 200.0 ml.

Add 4.0 g of dextrose, 0.02 g of yeast extract and 4.0 g of agar.

The respective medium was sterilized by autoclaving at 121^0C (15lb/ln^2).for 15 min. and medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow solidification..15µl of inoculums of the bacteria and fungi was transferred to respective Petri plate. Four wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 28^0C for 48 hours. The diameter of zone of inhibition surrounding each of the wells was recorded. [21]

RESULTS AND DISCUSSION

Extractive Value Determination

Dried leaves of Swertia chirata were extracted using ethanol. The percentage yields of dried extract were determined by using the following formula.

Percentage yield = Weight of extract/ weight of drug powder taken x100

Preliminary Phytochemical Screening

The extracts showed the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds, tannins, steroids and fatty acids. In previous literature, lots of biologically active phyto compounds such as steroids, flavonoids, alkaloids, terpenoids, glycosides, tannins and phenolic compounds are reported to be responsible for significant anti-fungal activity.

Formulation Of Herbal Cream & Its Evaluation

The three herbal creams of 1 % extract was formulated and then evaluated for various physical and mechanical properties like appearance, color, pH, viscosity, Spreadibility, Extrudability, firmness, consistency, cohesiveness, hardness as per the standard procedures. The physicochemical properties of the three formulations was also compared with the marketed composition. The results for the physical and chemical parameters are shown in Table no 4.

The Antifungal activity against *Candida albican* of Herbal cream made up of *Swertia chirata* determined by cup plate method and result reveals the cream showed it activity in a dose dependent manner. Zone of inhibition were taken to determine the inhibitory concentration of extracts against fungal strains by Cup Plate method the result is shown Table no 5.

CONCLUSION

The present findings are significant for the development of alternative, inexpensive and perhaps safer strategies for the treatment of fungal diseases. The extract could serve as lead for development of prospective anti-fungal agent in the form of topical herbal cream.

Table No. 2: Extractive Value of the Extract

S.No.	Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
1	Ethanolic extracts of *Swertia Chirata*	Dark Green	Sticky	9.8 %

Table No. 3: Composition of 1% Antifungal Herbal Cream

S.No	Ingredients	Quantity (Gm)		
		C1	C2	C3
1	Ethanolic Extract of *Swertia Chirata*	1	**1**	1
2	Emulsifying wax	5	**2**	4
3	Glycerol monostearate	5	**2**	5
4	Soft Paraffin	10	**7**	-
5	Liquid paraffin		**3**	10
6	Propylene glycol	1	**1**	1
7	Methyl paraben	0	**0**	0
8	Propyl Paraben	0	**0**	0
9	Purified Water	68	69.2	64

Table No. 4: Physical properties of 1% herbal cream formulations

S.NO	Properties	C1	C2	C3	MC
1	Appearance	**4**	**6**	**9**	**9**
2	Color	White	White	White	White
3	pH	5.6	5	6	6.4
4	Viscosity (cps) at 19 rpm at 30°c	27500	26100	27400	26950
5	Spreadibility (g .cm/s)	25	26	27	28
6	Extrudability (g)	51.5	52	54	55
7	Firmness	692.32	674.52	782.70	794.57
8	Consistency (g)	2364.36	1846.12	2473.7 6	2602.23
9	Hardness (g)	21.06	22.92	25.10	27.14
10	Stickiness (g)	-12.45	-14.70	-15.28	-14.72

MC =Marketed Compositions

Table No. 5: Minimum inhibitory concentration against *Candida* albicans by Zone of Inhibition method

S.NO	FORMULATIONS	ZONE OF INHIBITION
1	C1	19.33±0.571
2	C2	20.33 ± 0.512
3	C3	22.33 ± 0.577
4	MC	23.34±0.562

*All values represented as Mean ± S.D. (n=3).

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