IN VITRO ANTIFUNGAL ACTIVITY OF FORMULATED FLORAL EXTRACTS AGAINST *MALASSEZIA FURFUR*.

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Abstract

In the presence study to evaluate the antifungal activity of formulated poly herbal flower extract against *Malassezia furfur*. The Dandruff causing organism was isolated from patient's scalp and identified with standard protocols. The antifungal activity of the formulated methonolic flower extracts against *Malassezia furfur* was investigated using agar well diffusion technique. The zone of inhibition of formulated methanolic flower extracts varied from 16 to 23 mm. Among all tested formulation, formulation 1 showed a higher rate of inhibition. Formulation 1 exhibited higher rate of growth inhibition against *Malassezia furfur*, so it can be used for treatment of Dandruff disease.

Key words: Flowers, Antifungal activity, *Malassezia furfur*, Agar well diffusion

1. INTRODUCTION

Hairs are the integral part of human beauty. Publics are using many herbs for cleaning, beautifying, and managing hair since ancient days. As the time has passed, synthetic agents have taken a large share, but today people are getting aware of their harmful effects on hairs, skin and eyes. Hence the human community was attracted towards the herbal products, which are less expensive and have negligible side effects. The primary function of a shampoo is the cleansing

or detergent action, but the removal of dandruff also one of the important characteristics of a good shampoo [1].

Dandruff is a common scalp disease caused by fungal genus *Malassezia* sps such as *Malassezia furfur*, *M. globosa*, and *M. restricta*. It is a common problem for all age groups in both male and female. Affected scalp was characterized by the excessive shedding of the skin cells from the scalp. *Malassezia furfur* is a lipophilic, unipolar, dimorphic, gram positive double walled, saprophytic budding oval to round yeast. Colonization by *M. furfur* begins after birth, peak presence of yeast occurs in adolescence and early stage. Malassezia yeast requires free fatty acid for survival found in layer corneum and in pilar follicle. The yeast converts the sebum lipid into fatty acids and triglycerides, which accelerate hyper proliferation of keratinoytes [2].

Prolonged use of current antifungal such as Itraconazole, Fluconazole, and Terbinafine to treat infections caused by *Malassezia sp.* has its drawbacks by causing side effects such as burning, stinging, or redness when applied to the skin [3]. Plants have a limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total [4]. The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide [5]. Medicinal plants are widely used as effective antimicrobial and antifungal agents due to presence of secondary metabolites like alkaloids, glycosides, triterpenoids, terpenoids, flavonoids, polyphenols, reducing sugars, saponins, steroids and tannins etc.,

Traditonally flowers are very commonly used as decorative, ornamental and ritual practice all over the world. The richness and variety of colours in flowers are due to pigmented compounds like Carotenoids, flavonoids, xanthophylls, anthocyanin, betacyanin and lycopene

etc., Floral extracts are used to treat many bacterial and fungal diseases. The present work was framed to prepare poly herbal flower extracts from *Senna auriculata* L. (Fabaceae), *Ixora coccinia* (Rubiaceae), *Sesbania sesban* (L.) *Merr.* (Fabaceae), *Polianthes tuberosa* (Agavaceae), *Tabernaemontana divaricata* (Apocynaceae) and *Bauhinia purpurea* L. (Fabaceae). to determine the anti-dandruff activity on *Malassezia furfur.*

2. Materials and Methods

2.1. Collection of plant Materials and Preparation extracts

The flowers of the selected species was collected, washed and cleaned to remove the dust particles and subsequently they were dried under shade. The dried plant materials were powdered using pulverizer. The methanolic crude extract was prepared using soxhlet extraction method.

2.2. Formulation of anti-dandruff flower extract

The flower extracts of study plants were dissolved at various concentrations. Prepared poly herbal flower extract was subjected to their anti-dandruff activity against the causal organism *M. furfur.*

2.3. Isolation and culture preparation of Dandruff causing organism

Dandruff causal agent was collected by scraping of patient's scalp and stored in sterile containers and stored under refrigeration until use. The causal organism was inoculated in Sabouraud Dextrose Agar (SDA) media enriched with coconut oil. The inoculated plates were incubated at 37º C for 3-5 days. The fungal culture was stained with lactophenol cotton blue stain and examined under the high power objected microscope to identify the fungus.

2.4. Identification of *M. furfur*

M. furfur species can be identified based on their macro/microscopic and Biochemical features were as follows.

Macroscopy	Microscopy	Biochemical
Dull, smooth or slightly folded with convex elevations (averagediameter 5mm); soft/friable texture	Large, oval, cylindrical or spherical cells, broad base bud	Assimilation of glycine: This is positive in *M. furfur* only

2.5. Anti-dandruff activity

The antidandruff activity of poly herbal flower extract was studied by agar well diffusion assay [6]. About 20-25 ml of potato dextrose agar medium was added to pre-sterilized plates. After this, 0.1 ml of 12-16 hrs old culture of *M. furfur* was spread over the surface of agar plates. Petri plates were allowed to dry. About six wells in each plate of 6mm diameter were punched in agar surface with the help of sterilized cork borer. Each well filled with 100 µl of different formulated flower extract. The plates were kept in laminar air flow for 30 minutes for proper diffusion of the formulated extract and thereafter incubated at 37°C for 24 - 48 hours. After 48 hours of incubation the zone of inhibition was clearly visible and the diameter of the zone was measured. Different concentration of amoxicillin was used as positive control.

3. Results

Poly floral extract of definite formulations were prepared from the well bloomed flowers of *S. auriculata, B.purpurea, I. coccinea, T. divaricata, P. tuberosa* and *S. sesban*. The ratio of floral extracts taken to prepare the formulation was given in the Table 1.

The causal organism *M. furfur* was identified based on morphological and biochemical characters (Figure 2). The macroscopic nature of colony for *M. furfur* is dull, smooth or slightly folded with convex elevations; soft/friable texture and microscopically large, oval, cylindrical or spherical cells, broad base bud (Figure-2). Further it is confirmed through standard biochemical test by assimilation of glycine method gave positive result for the fungal species (Figure-2).

Evaluation of antidandruff activity of formulated poly herbal flower extract was executed by agar well diffusion method against *M. furfur* which is well known fungal strain responsible for dandruff in human beings. The results of antifungal activity were showed in table 2 and figure 3. All the formulation exhibited good anti-dandruff activity against the sps *M. furfur*. The maximum zone of inhibition was observed as 23 mm in F1 formulation and the minimum zone of inhibition was observed 14 mm in F2 formulation, whereas the high anti-dandruff activity was observed in antibiotic amoxicillin which is used as control (32 mm).

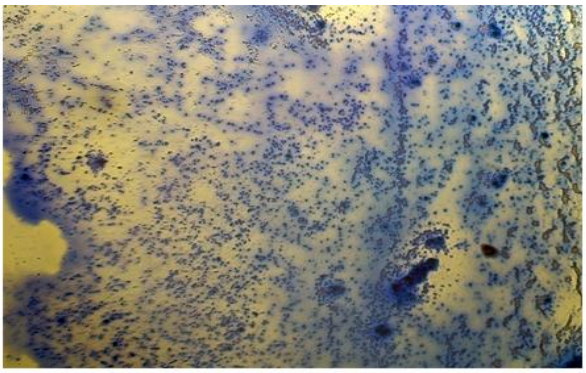
The anti-dandruff flower extract formulation, F1 showed good response against *M. furfur* than other formulation. The F1 formulation contains the methanolic flower extracts in milligram *viz. S.auriculata*-10, *I. coccinea* -60, *T. divaricata*-50, *P. tuberosa*- 40, *B. purpurea*-30 and *S. sesban*-20. However the flower extract formulation F1 was considered to be the best formulation for dandruff problem especially against the causal organisms *M. furfur*.

Table 1 Formulations and composition of anti-dandruff poly herbal flower extract

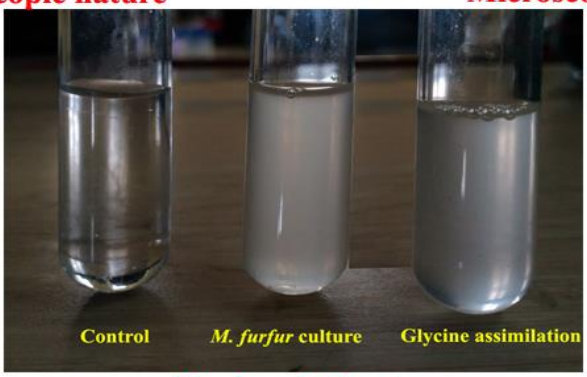
	Formulation of extracts in different concentration (mg)					
Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Senna auriculata	10	20	30	40	50	60
Ixora coccinea	60	10	20	30	40	50
Tabernaemontana divaricata	50	60	10	20	30	40
Polianthes tuberosa	40	50	60	10	20	30
Bauhinia purpurea	30	40	50	60	10	20
Sesbania sesban	20	30	40	50	60	10

Figure 2 Identification of *Malassezia furfur* by macro/microscopic and biochemical nature

Macroscopic nature



Microscopic nature



Biochemical nature

Table-2 Anti-dandruff activity of formulated poly herbal flower extract against *M. furfur*

S. No	Flower extract formulation	Zone of inhibition (mm)			
		10 µl	**25 µl**	**50 µl**	**100 µl**
1	F1	-	-	-	**23**
2	F2	-	-	-	**14**
3	F3	-	-	-	**16**
4	F4	-	-	-	**17**
5	F5	-	-	-	**18**
6	F6	-	-	-	**17**
7	**Amoxicillin**	**27**	**28**	**28**	**32**

Figure-3 Anti-dandruff activity of formulated poly herbal flower extract against *M. furfur*

4. Discussion

According to the present study, the formulation 1 showed the maximum zone of inhibition (23 mm) against *M. furfur*. A significant increase in the antifungal activity was observed in F1 formulation when compared to the other formulation. This may be due to the presence of more amounts of antifungal compounds in the methanol extracts of *Ixora coccinea*.

The earlier studies of the antifungal activity of the formulated herbal extract showed the antidandruff activity. All formulation was exhibited good anti-dandruff activity against *M. furfur*. The maximum zone of inhibition was showed in F3 formulation (30mm) and the minimum zone of inhibition was showed in F1 formulation (23 mm) Sathishkumar *et al.* (2019) [7]. Compare to the Sathishkumar *et al*. our formulation showed low antidandruff activity because of the secondary metabolite content are high in leaves and low in flowers.

The 80 % of dry and ethanol of *Calendula* flower extract showed the good antifungal activity against *M. furfur* (23mm) by the same time 50% of dry and ethanolic extract of *calendula* flower showed (19 mm) zone of inhibition against *M. furfur* [8]. In this study the formulation F1 showed the similar activity of 80% dry ethanolic extract of *Calendula* flower. The 80% of dry ethanolic extract of *Chrysanthemum* flower showed the (48 mm) zone of inhibition against *M. fufur* [8]. In our study the F1 formulation showed less antifungal activity compare to the 80 % dry and ethanolic extract of *Chrysanthemum* flower.

The 80 % of dry and chloroform extract of *Calendula* flower extract showed the good antifungal activity against *M. furfur* (49 mm) by the same time 50 % of dry and chloroform extract of *Calendula* flower showed (41 mm) zone of against *M. furfur* [8]. In the present study the formulation F1 showed the similar activity of 80 % dry chloroform extract of *Calendula*

flower. The present study confirmed that the formulation F1 was suitable to control dandruff organisms in humans. Further the studies will be extended to evaluate the shelf life period and to determine cytotoxicity of these extracts before going to complete commercial product.

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