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RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY ASSOCIATED WITH *Psoralea corylifolia* Linn. (BAKUCHI) SEED AND CHEMICAL PROFILE CRUDE METHANOL SEED EXTRACT

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ABSTRACT

Objective: Present study aims to evaluate antifungal efficacy of Bakuchi (*Psoralea corylifolia*) seed extracts prepared in methanol solvents and the bakuchi oil. Bakuchi seed used in the formulations against skin related diseases and disorders in Ayurvedic system of medicine.

Method: Antifungal assay was performed by agar well diffusion method against common fungal skin pathogens Candida albicans, Aspergillus niger and Malassezia furfur.

Results: Bakuchi seeds extract in methanol was observed the most promising antifungal activity against the selected skin pathogens. The phytochemical and GC MS analysis confirmed the presence of several bioactive components including phenol derivatives as coumarin – psoralen, isopsoralen which might be accountable for its antifungal activity.

Conclusion: The study has unveiled the antifungal potential of *P. corylifolia* seed extract.

Key Words: Bakuchi seed extract; Antifungal Activity; GC/MS.

INTRODUCTION

Fungal infections of the skin, hair, and nails are common worldwide. The skin infections caused by pathogenic fungi are consider as an emerging threat to public health [1] particularly in immunocompromised population constitutes from tropical and subtropical developing countries [2]. The fungal pathogens are known as dermatophytes, produces an extracellular enzyme keratinases, capable of hydrolyzing keratin and thus able to colonize the keratin tissues and inflammation is caused by host response to metabolic by-products. They are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host [3]. Some common fungal skin infections which are caused by dermatophytes includes the nail infections (*Tinea unguium*), thrush (*Candida albicans*) and Pityriasis versicolor (*Malassezia*) caused by yeasts. In present scenario, an emergence of multiple drug resistance in human pathogenic fungi and the small number of antifungal classes available, there is continuous and urgent need to discover new antifungal compounds with diverse chemical structures and novel mechanisms of antifungal action. Based on the knowledge that plants develop their own defense mechanism against various pathogens and produce secondary metabolites, amongst them phenols, phenolic acids, flavonoids, alkaloids, terpenoids, essential oils, coumarins , quinones, peptides and proteins are known to exhibit antifungal activity [4].

Psoralea corylifolia Linn. commonly known as '*Bakuchi*' is conventionally used in ayurvedic system of medicine for the treatment of various kinds of human disorders but especially for treatment of skin disorders such as psoriasis, leucoderma and leprosy in the form of internal medication [5] as well as external applications [6]. *P. corylifolia* L. seed has been reported to contain several phytoconstituents includingcoumarins and flavone components, such as psoralen, isopsoralen, psoralidin, neobavaisoflavone, bavachin, corylin, bavachalcone [7] and possess antibacterial, anti-inflammatory[8], antifungal [9], antioxidant[10-11], antiflarial [12], estrogenic [13], antitumour [14], and immunemodulatory activity [15].

In continuation with the previous studies, *Psoralea corylifolia* seed extracts which was demonstrated a promising antibacterial activity, against both gram positive and gram negative skin pathogens [16], the present study was undertaken to evaluate antifungal activity of *Psoralea corylifolia* seed extract and seed oil against fungal pathogens such as *Candida albicans ,Aspergillus niger* and *Malassezia furfur* by agar well diffusion method along with phytochemical analysis and GC MS profile.

MATERIALS AND METHODS

Plant material

The seeds of *Psoralea corylifolia* plant and seed oil (*Bakuchi oil*) were procured from local Ayurvedic medicine supplier. The dried seeds were cleaned and disinfected with 15% H_2O_2, crushed into powder sample using an electronic blender. The powdered sample was stored in sterile screw capped bottle at room temperature prior to subject for an extraction.

Extraction of seeds of *Psoralea corylifolia*

The fine powder of the seeds (25g) with 250ml of methanol was taken in a round bottom flask. For successive extraction with methanol, seed powder was subjected for soxhlet extraction at 50^0 C for 8 h. The liquid extract so obtained was filter sterilized. Methanol extract of *Psoralea corylifolia* was stored at -20°C in air tight sterile glass bottle and used within 1 week after preparation.

Test Microorganisms

The fungal organisms used in the present study were *Aspergillus niger, Candida albicans and Malassezia. furfur.* The fungal cultures were maintained on potato dextrose agar (PDA) (*Aspergillus niger, Candida albicans*) and Pityrosporum Agar (*Malassezia furfur*) and preserved on the same medium at 4^oC. The cultures were sub-cultured periodically (5-7 days) under stationary condition on the same medium at 28 $\pm 2^oC$.

Chemicals

Methanol used for extractions was of HPLC grade and purchased from M/S Merck ltd. Mumbai, and all the microbiological media used were purchased from Hi Media Ltd., Mumbai, India

Antifungal Assay

The antifungal activity of the methanol extract of *Psoralea corylifolia* seed extract and Bakuchi oil were determined by the agar well-diffusion method. Each fungal inoculum viz *Aspergillus niger, Candida albicans* and *Malassezia furfur* consist of o.1g biomass were aseptically inoculated and evenly spread using sterile glass spreader on the surface of potatos dextrose agar and pityrosporum agar plates respectively. Wells of about 6.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells, and the edge of the petri dish. Fixed volumes (50µl) of the test extract were then inoculated into the wells. The

the diameter of zones of inhibition was recorded in mm for each test sample.

Tests were performed in triplicates and observed plates were kept at 4^0C for 2 h for pre-diffusion of the extract and then incubated at room temperature for 7 days. After incubation values of zone of Inhibition were expressed as mean value of the average of the three readings [17].

Phytochemical Analysis

The test sample with promising antifungal activity was subjected for phytochemical analysis for the detection of plant secondary metabolites as tannins, saponins, steroid, alkaloids and glycosides based on the earlier studies [18].

Thin Layer Chromatography (TLC)

The most promising extract was spotted on TLC plates coated with silica gel G. The plates were developed in TLC chamber previously saturated with 30% ethyl acetate in petroleum ether as mobile phase. The solvent was allowed to evaporate and different spots developed were identified by means of UV light at λ max 254 nm and the R_f value (for each spot developed was calculated and recorded [16, 19].

GC-MS analysis of Methanol extract of *Psoralea corylifolia*

Methanol extract *of Psoralea corylifolia* seeds was subjected for gas chromatography-mass spectrometry analysis with the GC MS – QP 2010 plus system (Shimadzu). The methanol seed extract with most promising activity was subjected for the GC /MS analysis. 2µl of the sample was injected to the GC-MS with the oven temperature programmed as 80°-280°C with increment of 5°C/min with Helium as a carrier gas. The injection size was 0.1 µL, and detector temperature of 270^0C. The eluted peaks were identified using NIST library by comparing with the mass spectral data and retention indices in the literature.

RESULT AND DISCUSSION

Antifungal Assay

The antifungal potential *of Psoralea corylifolia* seed oil and seed extract were evaluated against the selected fungal pathogens. All the fungal pathogens tested were susceptible to the extract as well as oil however methanol extract of *Psoralea corylifolia* seeds was found to be comparatively more effective against all pathogens selected under present investigation. Methanol extract of *Psoralea corylifolia* seeds was reported with maximum zone of inhibition (ZOI= 16.25 mm) against *C. albicans* which is followed by *M. furfur* (ZOI = 14.25mm) and *A. niger* **(**ZOI = 12.22 mm**).** *P. corylifolia* seed oil was reported with moderate antifungal activity against *M. furfur* (ZOI=9.3) followed by *C. albicans* (ZOI=1.7mm) and the least antifungal activity was observed with *A. niger* (ZOI= 1.6mm). Both methanol extract and oil of *Psoralea corylifolia* exhibit significant antifungal activity against *M. furfur*.

Table 1: Antifungal Activities of *Psoralea corylifolia* seed Extracts &Oil

	(Diameter of Zone of Inhibition (mm)*	
Fungal Pathogen	**Methanol Extract**	**Seed Oil**
M.furfur	14.25	9.3
C. albicans	16.25	1.7
A. niger	12.22	1.6

(*Average of the triplicate results)

Preliminary Phytochemical and TLC Analysis

The preliminary phytochemical screening of methanol extracts of the *Psoralea corylifolia* seeds showed the presence of alkaloids, carbohydrates, flavonoids, glycosides and saponins. However the steroids and terpenoids were absent as represented in table 2. Presence of these secondary metabolites will be accountable for antifungal activity. The retention factors (R_f) of methanol extracts was shown in table 3. The methanol extracts produces three fraction having R_f 0.18, 0.36, 0.42 and 0.93 under 30% ethyl acetate in petroleum ether as mobile phase. The results of TLC analysis signify the presence of several chemical constituents in methanol extracts.

Table 2: Photochemical analysis of *Psoralea corylifolia* seed extract

Test for	Methanol Extract
Alkaloid	**+**
Carbohydrates	**+**
Flavonoids	**+**
Glycosides	**+**
Saponins	**+**
Steroids	**-**
Terpenoids	**-**

+ = Present; - = Absent

Table 3: TLC Studies of *Psoralea corylifolia* methanol extract

Extract	Solvent System	No .of Spots	R_f values
Methanol Extract	*Ethyl acetate: Methanol (3:7)*	*04*	*0.18, 0.36, 0.42, 0.94*

GC-MS screening of *P. Corylifolia* seed extract

The GC-MS analysis confirmed the presences of several important phytoconstituents in the bioactive methanol extract .A total of 15 compounds were identified in methanol extract of *P. corylifolia* seeds. The major components identified were, 1-(+) -Ascorbic acid 2, 6-dihexadecanoate (8.76%), Coumarin –Psoralen (7.67%), Angecin- Isopsoralen (6.34%), caryophyllene oxide (2.82%). Several other components in trace amounts were also present. In earlier findings by S. Shreenivasan et al also reported the presence of the phenyl derivative of pyranocoumarin (PDP) in bakuchi oil with a potent antifungal activity against *Fusarium* species. [20]

CONCLUSION

In the present study, the antifungal activities of *Psoralea corylifolia* seed oil and methanol seed extract were explored specifically against common fungal skin pathogens. The results clearly demonstrate that, the methanol seed extract of *P. Corylifolia* comprise of a promising antifungal activity against common fungal pathogens as compare to seed oil. The phytochemical and TLC analysis of the methanol extract followed by GC- MS screening confirmed the presence of various phytochemicals. Major identified phytochemicals in methanol extract of *P. corylifolia* seeds are phenol derivatives and coumarin – psoralen, isopsoralen which may be accountable for its antifungal activity. In Ayurvedic system of medicine, *Psoralea corylifolia* seeds are commonly used in various dermatological conditions [21- 25]. The present study supports the use of bakuchi seed in the development of ayurvedic skin formulations and also explored the additional benefits associated with its traditional applications.

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