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Evaluation of Antibacterial and Anti Fungal Activity of Hexane and Methanol Extracts of *Psoralea Corylifolea* Seed.

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ABSTRACT:

Objective: To evaluate antibacterial and antifungal activity of hexane and methanol extracts of Psoralea Corylifolea Seed.
Method: Psoralea Corylifolia seeds were extracted by using different solvents hexane and methanol and the test extracts were assayed for antibacterial and antifungal activity. The antibacterial activity was tested against Staphylococcus aureus and antifungal activity was tested against Candida albicans and Aspergillus niger by agar well diffusion method and measuring the zone of Inhibition.
Results: The hexane and methanol extracts of *Psoralea corylifolia* seed was very effective against *Staphylococcus aureus, Candida albicans* and *Aspergillus niger*. The results showed unique characters of the plant in inhibiting bacterial and fungal growth.
Conclusion: In the present study antibacterial and anti fungal activity of hexane and methanol extracts of *psoralea corylifolea* seed was confirmed.

1. INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine (Okeke et al., 2005). One of the more alarming recent trends in Infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections. Numerous classes of antimicrobial agents have now become less effective as a result of the effective pressure of antimicrobial usage (Soulsby et al 2005).Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanism of action (Ahmad et al., 2007; Barbour et al 2004). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Fungal related diseases may not be as common as other microbial infections but, when present, they are difficult to treat especially in immunosuppressed persons (Abubakar et al., 2010).

Plants and plant based materials are less toxic and have acceptable side effects. It is therefore essential to bring the use of these remedies into an existing frame work of rational scientific use of medicine based on the strong traditional knowledge. Medicinal plants are an important therapeutic aid for various ailments (Nickavar B *et al*., 2008). Scientific experiments on the antimicrobial properties of plants were documented in the late 19th century. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today there is widespread interest in drugs derived from

plants (Szollosi R *et al*., 2001). This interest primarily steps from the belief that green medicine is safe and dependable, compared with synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or micro organisms. The short comings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants (Nair *et al*., 2006)

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 as well as external applications (Mishra et al., 2009). P. corylifolia L. seed has been reported to contain several phytoconstituents including coumarins and flavone components, such as psoralen, isopsoralen, psoralidin, neobavaisoflavone, bavachin, corylin, bavachalcone (Wang et al., 2011) and possess antibacterial, anti-inflammatory (Gidwanier et al., 2010), antifungal (Prasad et al., 2004), antioxidant (Tang et al.,2004; Haraguchi et al., 2000), antiflarial (Qamaruddin et al., 2003), estrogenic (Zhang et al., 2005), antitumour (Latha et al., 2000), and immunemodulatory activity (Latha et al., 1999).

2. MATERIAL AND METHODS

2.1 Collection of seeds

The seeds were collected and certified from Aptus Therapeutics Lab, Hyderabad central university. The seeds were dried under dark in shade conditions, without exposing to sunlight. After drying the seeds were powdered using mixer. This seed powder was passed through a sieve no. 40 to get fine powder. The powder was stored in a cool and dry place until its use.

2.2 Method of extraction

About 100 g of the shade dried powder of *Psoralea corylifolia* seeds was filled separately in thimble and subjected to soxhlet extraction by using 500ml 2 different solvents hexane(non-polar) and methanol (polar) until about completion of 20-25 cycles were completed with each solvent. After extraction the solvent is removed, typically by means of a rotary evaporator. After complete solvent evaporation, each of these solvent extracts were weighed and preserved at 4^0C in airtight bottles until further use and were used as the test extracts for anti-bacterial assay, antifungal assay.

2.3 Antimicrobial Activity

2.3.1 Preparation of microbial plate cultures

Human pathogenic bacteria *Staphylococcus aureus* was collected from Microbiology department, SIT, JNTUH. All the test bacterial species were maintained on nutrient agar media.

2.3.2 Preparation of Inoculum

The gram positive bacteria *Staphylococcus aureus* pre-cultured in nutrient broth overnight in a rotary shaker at 37^0C.

2.4 Antibacterial assay

Antibacterial assay of hexane and methanol extracts of *Psoralea corylifolia* seeds was determined by Agar well-diffusion method (K. Das, 2010).

2.4.1 AGAR- WELL DIFFUSION METHOD (Cup plate method)

Before performing the microbial assay, glass ware, media was sterilized, antibiotic solution (standard) and plant samples were kept ready.

The first step of assay involves the preparation of inoculated plates. For this 2% of microbial suspension is added to the quantity of medium per plate i.e., 0.5 ml of suspension per 25 ml of nutrient agar medium. Allow the petriplates for solidification for about 10 min. By using sterile glass (Pyrex) bores, cups were made by maintaining approximate distance between cups (cup diameter: 6 X 8 mm2). Cups were labeled properly to enable the introduction of the test sample, standard and control precisely.

The concerned samples were introduced into appropriate wells with the help of micropipette; all the cups were filled with equal volumes of methanol and hexane extracts of different concentration i.e., 25µg/ml, 50 µg/ml, and 100 µg/ml. To minimize the effect of variants the petriplates were allowed to store at room temperature for 1 - 4 hrs, and then the plates were allowed to incubate for a time period of 18 - 24 hrs. The zone of inhibition was examined and measured (The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well) (Munir Anwer *et al., 2011*). Streptomycin (10µg/ml) and cefotaxime (10 µg/ml) were used as a standard.

2.5 Antifungal Activity

2.5.1 AGAR WELL DIFFUSION METHOD

In vitro antifungal activity of the extracts were studied against the fungal strains, *Candida albicans* and *Aspergillus niger*, by Agar Well Diffusion Method.

PROCEDURE:

The Potato Dextrose Agar (PDA) medium was suspended in distilled water (39 g in 1000 ml) and heated to boiling until it dissolved completely, the medium and Petri dishes were autoclaved at pressure of 15 lb/inc^2 for 20 min. Agar well bioassay was employed for testing antifungal

activity**.** The medium was poured into sterile Petri dishes under aseptic conditions in a laminar air flow chamber. When the medium in the plates gets solidified, 0.5 ml of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the extracts in methanol and hexane and different concentrations were made. After inoculation, wells were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each well different concentrations of test solutions were added. Controls were maintained. The treated and the controls were kept at 27^0 C for 48 h. Inhibition zones were measured, the diameter calculated in millimeter and the corresponding results were tabulated (Arumugam, et al.,2012).

3. STATISTICAL ANALYSIS

The antifungal and antibacterial activity was determined by measuring the diameter of zone of inhibition. Test were carriedout in triplicate and represented using MS Windows based graph pad prism (version 6) software. Results were expressed as graphically / mean ± SEM. The results were subjected to Student –Newman-Keuls Multiple comparision test. The p value is < 0.0001, considered extremely significant.

4. RESULTS

The anti-bacterial activity and anti-fungal activity of *Psoralea corylifolia* seed were studied. The hexane and methanol extracts of *Psoralea corylifolia* seed was very effective against *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. The results showed unique characters of the plant in inhibiting bacterial and fungal growth. The hexane and methanol extracts of seeds of *Psoralea corylifolia* presented a better inhibitory effect on the test organisms. The results of anti bacterial activity and anti fungal activity by Agar diffusion method of both the plant extracts against selected micro organisms as shown in Table 1& 2 and graphically represented in graph 1 & 2.

Table 1. Antibacterial activity of *Psoralea Corylifolea* seed crude extracts

S.No.	Concentration (mg/ml)	Zone of inhibition in mm	
		Methanolic extract	**Hexane extract**
1	25	22.66±0.66	18.33±0.88
2	50	29±1.55	23.33±0.33
3	100	33.66±0.33	30.66±0.88

Zone of inhibition for Standard drug Streptomycin (10µg/ml) - 38mm
Zone of inhibition for Standard drug Cefotaxime (10µg/ml) - 42mm
From the above results the methanolic extract was found to be having more anti bacterial activity than hexane extract.

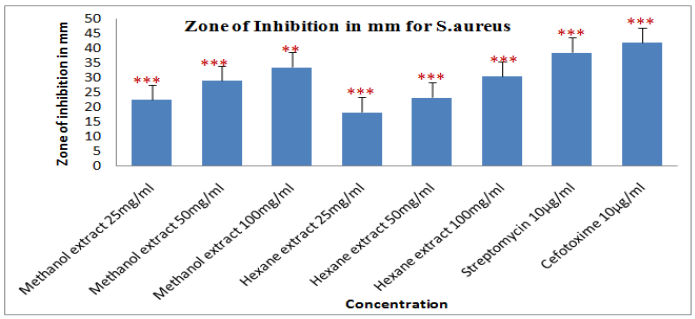

Fig 1: The antibacterial potency of Psoralea Corylifolea seed extracts

****** Indicates P < 0.01 & 8 indicates P < 0.05 & *** P < 0.001 means significant when compared with Fluconazole. Values are expressed as Mean ±S.E.M.
Test extract treated groups were compared standard and with in the groups.
(Statistically analysed by one – way ANOVA followed by Student – Newman – Keuls multiple comparision test.)

SCREENING OF *IN VITRO* ANTI FUNGAL ACTIVITY OF CRUDE EXTRACT

Table 2: Antifungal Activities of *Psoralea corylifolia* seed Extracts

S.No	Fungal pathogen	Methanolic extract		Hexane extract	
		Concentration (mg/ml)	Zone of inhibition in mm	Concentration (mg/ml)	Zone of inhibition in mm
1	*Candida albicans*	25	14.33±0.33	25	11.33±0.33
		50	17±0.57	50	13.66±0.66
		100	20.66±0.88	100	18±0.57
2	*Aspergillus niger*	25	12.33±0.33	25	8
		50	15±0.57	50	12.66±0.66
		100	18.33±0.66	100	15±0.58

The antifungal potential *of Psoralea corylifolia* seed extracts were evaluated against the selected fungal pathogens. All the fungal pathogens tested were susceptible to the extracts however methanol extract of *Psoralea corylifolia* seeds was found to be comparatively more effective against all pathogens selected under present investigation.

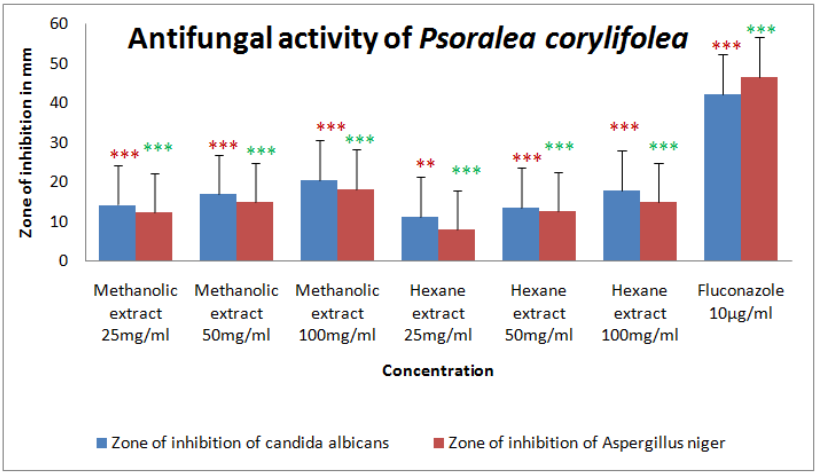

Fig 2: The antifungal potency of *Psoralea Corylifolea* seed extracts

** Indicates P < 0.01 & 8 indicates P < 0.05 & *** P < 0.001 means significant when compared with Fluconazole. Values are expressed as Mean ±S.E.M.
Test extract treated groups were compared standard and with in the groups.
(Statistically analysed by one – way ANOVA followed by Student – Newman – Keuls multiple comparision test.)

5. DISCUSSION:

The search for newer sources of antibiotics is a global challenge preoccupying research institution, pharmaceutical companies and academic since many infectious agents are becoming resistance to synthetic drugs (Latha et al., 2006). Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs. The local use of material plants as primary health remedies due to their pharmacological properties is quits common in Asia, Latin America and Africa (Bibitha et al., 2002) .Plants are the best sources of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported with biological properties like analgesic, anti inflammatory antioxidant, hypoglycemic, antibacterial and antifungal agents (Sindhu et al., 2009).

Psoralea corylifolea belongs to family Fabaceae is one of the most abundant plant of this genus and is rich source of alkaloids, glycosides, steroids, phenolic, flavanoids and tannins. Its biological constituents exhibit a variety of biological activities.

The *in-vitro* antimicrobial activity was carried out by Agar well diffusion method using different bacterial and fungal strains, which showed that the extract posses' prominent activity compared with that of standard. Zone of inhibition were found to be prominent in concentration of 100mg/ml for bacterial strain and fungal strains**.**

In Ayurvedic system of medicine, *Psoralea corylifolia* seeds are commonly used in various dermatological conditions. 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.

6. CONCLUSION

In the present study antibacterial and anti fungal activity of hexane and methanol extracts of *psoralea corylifolea* seed was confirmed.

In future, by isolating the various constituents of extract using different separation techniques such as preparative TLC, column chromatography etc, it would be possible to evaluate and study other activities of the plant. These isolated constituents in pure form would possess prominent anti inflammatory, antifungal and anti microbial activity and can be used in treatment of various skin disorders. This can lead to development of new potent compounds with significant activity.

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