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Comparative Account of Antimicrobial Activities of Leaf and Leaf Callus Extracts of *Psoralea corylifolia* L.

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ABSTRACT

Antimicrobial activity of leaf and leaf callus extract of *Psoralea corylifolia* was studied using different solvent like chloroform, acetone, ethanol and water against two-gram (+) ve and two-gram (+) ve bacterial strains like *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and fungal strains *Aspergillus niger* and *Penicillium chrysogenum*. The antimicrobial activity was determined by disc diffusion method. Out of the four-extract used, acetone and ethanol extracts were found to be highly active in both leaf and leaf extracts. It is also found that the zone of inhibition was much higher in callus extracts, when compared to that of leaf extracts.

Keywords: *Psoralea corylifolia, callus* extract, Pseudomonas *aeruginosa, Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*

INTRODUCTION

Psoralea corylifolia L., of the family Fabaceae, a much-branched annual herb, it is a native to India and Iraq. It is found as a weed in waste places in Madhya Pradesh, Chattisgarh, Uttar Pradesh, Uttaranchal, Rajasthan, Gujarat and Andhra Pradesh. Traditional practitioners use this plant as a laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic.[1] As medicinal plants represent a rich source of antimicrobial agents and plant origin herbal medicines represent one of the most important fields of traditional medicine all over the world. Herbal remedies can be made from complete plant parts or mostly from the leaves, roots, bark, seeds, flowers of various plants and they are applied topically, vocally or through inhalation. The importance of medicinal plants to both individual and societal health is greater. These plants' medicinal usefulness comes from their bioactive phytochemical components, which have defined physiological effects on the human body. Alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds, and many others are among the most significant bioactive phytochemical components. In the present time, drug resistance in microbes is a very serious problem and hence, herbal medicines are symbolizing safety in contrast to the synthetics that are regarded as unsafe to human and environment. There are varied methods of medicines like Ayurveda, Homeopathy and Unani, which utilize plant materials for many potent and powerful drug productions. Presently, Aurveda considered as a vital system of medicine and governed the worldwide recognition and having non-toxic substances. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The present work is an attempt for the pharmacological screening of the leaf of medicinal plant

Psoralea corylifolia L. and its calli.

MATERIALS & METHODS

Plant Material: *Psoralea corylifolia* L., an erect annual herb grows up to 30-180cm in height. Stem and branches grooved, studded with conspicuous glands and with a few appressed and spreading white hairs. Leaves simple, broadly elliptic, inciso-dentate, rounded and mucronate at the apex, sparingly clothed with white hairs on both surfaces, base cunneate, rarely rounded, main nerves 5, springing from the base, and 4-6 pairs of lateral nerves higher lip from the midrib, petioles 1cm long, hairy and gland-dotted, stipules lanceolate, persistent. Flowers close, in dense axillary solitary 10-30-flowered racemes, peduncles 1-2 in. long, hairy, pedicels very short. Calyx is long, hairy outside, the upper teeth linear lanceolate, the lower ovate, twice as long as the upper. Corolla is bluish purple, nearly twice as long as the calyx, standard orbicular, clawed, and glabrous. Pods are long, ovoid-oblong, somewhat compressed, closely pitted, mucronate, black, and glabrous. Seed smooth, one and adhering to the pericarp.

Extraction Procedure: The leaves of *Psoralea corylifolia* L., were collected from Dandeli, Uttara Kannada District, Karnataka State, Southern India. The leaves were dried under shade and made in to coarse powder using an electrical grinder. Callus induction by inoculating young leaf segments in MS medium supplemented with 20 µm 2,4 –D by standard tissue culture technique. Callus harvested after two months, dried at 40 ^{0}C in oven for 24 hrs. and made in to fine powder. The powder was subjected for successive extraction with chloroform, acetone, ethanol and water using Soxhlet apparatus separately. The extracts were dried and dissolved in DMF (Dimethyl formamide) solution and screened for antimicrobial activity.

Preliminary Phytochemical Screening: The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The preliminary phytochemical analysis was carried out by following procedures[2]:

Test for Alkaloids: A small portion of the extract is stirred with few drops of 1% Hydrochloric acid and filtered. The filtrate is treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins: One ml of extract is diluted with 20ml of distilled water and shaken vigorously for 15 min formation of stable foam indicates the presence of saponin

Test for Tannins: Development of blue green color in the extract when treated with ferric chloride indicates the presence of tannins.

Test for Phenols: Phenol test. Small quantity of extract is diluted with 5% ferric chloride solution. Development of intense color indicates the presence of phenols.

Test for Steroids and Triterpenes

Lieberman–Burchard test- The extract is treated with 50% sulphuric acid and a few drops of acetic anhydride are added. The development of reddish-brown ring indicates the presence of steroids.

Salkowski's test- A few drops of chloroform and few drops of concentrated sulphuric acid was added to the extract. Appearance of yellow color in the lower portion indicates the presence of triterpenes

Test for Flavonoids

Ferric chloride test- The extract is treated with few drops of 5% ferric chloride. The appearance of blackish green color indicates the presence of flavonoids.

Antimicrobial assay: The antimicrobial screening was done by using bacterial strains like *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and fungal strains *Aspergillus niger* and *Penicillium chrysogenum*. All the bacterial strains and fungal strains were obtained from the stock culture Department of Botany Bangurnagar Degree College Dandeli. The antimicrobial activity was determined by disc diffusion

method[3]. Three different concentrations of 25mg/ml, 20mg/ml and 15mg/ml respectively were prepared. Each sterile disc was loaded with 10µl of test extract to obtain effective concentrations as 250, 200 and 150 µg/disc and placed on the agar plates inoculated with respective microorganisms. The plates were kept for half an hour for pre-incubation diffusion. Then the plates were kept for incubation at 37°C for 24 hours for bacteria and 48 hours for fungi. At the end of incubation zones around the discs were measured in mm using Hi Antibiotic Zone scale. The study was performed in triplicate. Streptomycin disc was used as standard for bacteria and Nystain disc for fungi.

Determination of Minimum concentration:

The minimum inhibitory concentration was determined by serial dilution method [4]. Serial dilution of the extract was prepared in the test tubes containing peptone water as diluent. Fifty mg of the extract was dissolved in one ml of DMF which is further subjected for two-fold dilution. Totally 10 test tubes were maintained. The final concentration of the extract was now one half of the original concentration in each test tube. Each bacterial isolate was inoculated at 37°C for 24hrs. The tubes were then examined for the presence of growth considering turbidity as criterion. The highest dilution in each series that did not show turbidity and thus no growth was considered to be the MIC of the organism.

RESULT AND DISCUSSION

Table. 1, contains the phytochemical analysis of the leaf and leaf callus extract of *Psoralea corylifolia* L., which shows the presence of flavonoids and phenolic compounds. The antibacterial activity of *Psoralea corylifolia* L., leaf and leaf callus extract and the zone of inhibition in comparison with the standard used. In the present investigation, all the extracts have shown antimicrobial activity, even though their range of activity varied and the sensitivity of the microbes to different extracts. Significant enhancement has been observed in the corresponding callus extracts when compared to the leaf extracts. The activity can be positively correlated to the dose, as there is an increase in the zone of inhibition with increased dose. Highest zone of inhibition was observed in acetone extract at 250 ug/disk concentration, against all the tested microbial strains. Highest increase in the percent activity index was found in acetone extract at its highest tested concentration. All the strains were sensitive to streptomycin, while control (DMF) did not show any activity. In all, four different solvent extracts were tested each with three different concentrations. Among them, acetone extract is most active against all the tested microorganisms when compared to other extracts. The enhancement in the antimicrobial activity of callus extracts was observed in all the cases (Table 2). This enhancement in the activity might be due to the accumulation of active metabolites in the cell lines of callus cultures[5]. Similar enhanced activity of the callus extracts has been reported in *Bacopa monnier, Eclipta alba, Bixa Orellana, Solanum trilobatum, Alophyllus cobbe* and *Andrographis paniculata* by earlier workers [6,7,8,9,10,11,12,13].

Table-1 Phytoconstituents of *Psoralea corylifolia* L., leaf and callus extract

	Successive extracts							
Phytoconstituents	**Chloroform**		**Acetone**		**Ethanol**		**Water**	
	Leaf	**Callus**	**Leaf**	**Callus**	**Leaf**	**Callus**	**Leaf**	**Callus**
Alkaloids	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	+	+	-	+
Tannins	-	+	-	+	+	+	-	+
Phenolic compounds	-	-	+	+	+	+	+	+
Steroids/Triterpenes	-	-	-	-	-	-	-	+
Flavonoids	+	+	+	+	+	+	+	+

Table 2. Percentage inhibition of extracts of *Psoralea corylifolia L.*, when compared to control.

EXTRACT	CONC.[a]	Percentage Inhibition compared to control[b]											
		Bacillus subtilis		*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Escherichia coli*		*Aspergillus niger*		*Penicillium chrysogenum*	
		Leaf	Callus	Leaf	Callus	Leaf	Callus	Leaf	Callus	Leaf	Callus	Leaf	Callus
Chloroform	250	9.32 ±0.08	13.76 ±0.09	8.48 ±0.06	18.50 ±0.09	9.20±0.07	13.50±0.08	9.12 ±0.05	10.50±0.07	-	-	-	-
	200	8.40 ±0.09	12.40 ±0.08	8.10 ±0.06	17.20 ±0.08	8.50 ±0.09	12.20±0.08	8.00 ±0.09	9.40 ±0.08	-	-	-	-
	150	7.60 ±0.08	10.20 ±0.08	8.10 ±0.06	15.20 ±0.08	7.30 ±0.09	8.60 ±0.08	7.68 ±0.07	8.20 ±0.08	-	-	-	-
Acetone	250	12.32 ± 0.08	20.66 ±0.06	10.28±0.09	20.53±0.09	9.60±0.08	17.30±0.09	11.42 ±0.08	14.50±0.09	12.32 ± 0.08	20.66 ±0.06	9.32 ±0.08	13.76 ±0.06
	200	10.40 ±0.08	17.40 ±0.08	9.10 ±0.07	18.20 ±0.08	9.30 ±0.09	15.20±0.07	10.40 ±0.05	11.80±0.08	11.40 ±0.06	18.40±0.06	8.40 ±0.09	12.40 ±0.08
	150	9.40 ±0.09	13.20 ±0.07	8.40 ±0.06	15.46 ±0.08	8.30 ±0.09	11.60±0.06	8.58 ±0.08	9.50 ±0.08	10.40 ±0.08	17.40 ±0.08	7.60 ±0.08	10.20 ±0.08
Alcohol	250	8.56 ±0.06	12.26 ±0.08	8.32±0.09	12.14±0.12	10.20±0.08	14.30±0.06	9.22 ±0.05	11.50±0.09	10.20±0.08	14.30±0.06	8.56 ±0.06	12.26 ±0.08
	200	8.10 ±0.08	10.40 ±0.07	7.10 ±0.08	11.20 ±0.09	9.10 ±0.10	12.40±0.08	8.60 ±0.07	10.80±0.07	9.40 ±0.09	13.20 ±0.04	8.10 ±0.08	10.40 ±0.06
	150	7.40 ±0.07	9.20 ±0.08	7.20 ±0.08	9.46 ±0.08	7.30 ±0.07	10.50±0.06	7.58 ±0.07	8.64 ±0.08	8.10 ±0.08	10.40 ±0.06	7.40 ±0.07	9.20 ±0.08
Aqueous	250	10.50 ±0.06	12.96 ±0.08	9.22±0.09	12.80±0.12	9.560±0.08	10.60±0.16	9.52 ±0.08	12.50±0.09	-	-	-	-
	200	10.10 ±0.08	11.40 ±0.09	8.10 ±0.08	10.20 ±0.09	8.46 ±0.04	9.40±0.08	8.50 ±0.08	11.80±0.08	-	-	-	-
	150	9.10 ±0.07	9.40 ±0.08	7.50 ±0.08	8.46 ±0.05	7.80 ±0.08	8.40±0.06	8.18 ±0.08	8.63 ±0.08	-	-	-	-

[a] µg/disk [b]Values are mean of triplicates

CONCLUSION

The *Psoralea corylifolia* L., extracts of leaf and leaf callus extract tested against different bacteria showed inhibitory effect but varied with the organisms. Acetone extracts of leaf and leaf callus exhibited significant antibacterial activity against the tested bacteria. The presence of compounds like phenols, tannins, flavonoids, alkaloids, glycosides and triterpenes in the extracts might be responsible for the antimicrobial activity. Leaf callus extract proved to be superior to the antibacterial activity, since they are having more of these compounds than the normal plants. The present study opens a new era in correlating the Ayurveda and Siddha with modern microbiology. The promising result obtained in this study may lead to the development of a potential antibiotic from the leaf callus extract of *Psoralea corylifolia* L., *against* bacterial and fungal strains. The present study exhibited the antibacterial and antifungal effects of various callus extracts of *Psoralea corylifolia* L., is an indication of that the callus extract is beneficial in the treatment of these microorganisms.

Declaration by Authors

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