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INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF PSORALEA CORYLIFOLIA LEAF AND CALLUS EXTRACTS AGAINST MULTIDRUG RESISTANT PERIODONTAL DISEASE CAUSING MICROORGANISMS

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ABSTRACT

Some specific microorganisms have been identified as etiologic agents of periodontitis. The main goal of treatment is to control the spread of infection. Depending on the extent of the gum disease, treatment varies. Therapeutic antibiotic along with surgery are the most opted form of treatment. But, due to high costs of antibiotics, the disastrous side effects and emergence of multi drug resistant strains of the pathogenic bacteria, a safe alternative is being investigated scientifically in this study.

The *in vitro* antibacterial activity of *Psoralea corylifolia* leaf and its corresponding callus extracts were studied against pathogenic bacteria causing periodontitis. Leaves and corresponding calli were extracted using petroleum ether, chloroform, acetone, methanol and distilled water. Among the five solvents used, leaf and callus extracted in methanol were found to be more effective against pathogenic bacteria. *Psoralea corylifolia* extracts should be further studied to determine the bioactive compounds as well as to understand the possible mechanisms of action and evaluate its toxicity looking towards a pharmaceutical employment.

Keywords: *Psoralea corylifolia* callus, extract, periodontal, multidrug resistance

INTRODUCTION

More than 700 bacterial species or phylotypes, of which over 50% have not been cultured, have been detected in the oral cavity. Species belonging to the genera Gemella, Granulicatella, Streptococcus, and Veillonella are commonly found. There is a distinctive predominant bacterial flora of the healthy oral cavity that is highly diverse and is site and subject specific. (Aas et al. 2005)

The bacteria, *Porphyromonas gingivilis, Bacteroides forsythus, Prevotella intermedia, Camphylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Actinobacillus actinomycetemcomitants, Treponema* and *Eubacterium* species are the major components of dental plaque, which is host associated biofilm. Persistent plaque deposition causes inflammation of the gingiva which is called as gingivitis. When gingivitis is not treated, it can advance to periodontitis. In periodontitis, gums pull away from the teeth and form spaces called periodontal pockets that become infected; this may eventually cause loss

of teeth. The main goal of treatment is to control the spread of infection. Depending on the extent of the gum disease treatment varies. Therapeutic antibiotic along with surgery are the most opted form of treatment.

In the indigenous health care delivery system, numerous plant species and natural products derived from plants are used to treat diseases of infectious origin. Due to emerging antibiotic resistant infections, considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for prevention and cure of different human diseases since they are safe and effective. Studies have attempted to shed light on the antibacterial activity of some indigenous medicinal plants. Nonetheless, the investigations have primarily been restricted to screening only. In order to promote herbal drugs there has to be an evaluation of therapeutic potentials of drugs (Geyid et al, 2005). The medicinal plant *Psoralea corylifolia* is widely used by the traditional medicinal practitioners for the treatment of infectious diseases and is put to systematic scientific investigation in this study.

The Fabaceae family comprises of many species with biological activities. Isopsoralidin, a new crystalline material has been obtained from the seeds of *Psoralia corylifolia*. Raffinose has also been isolated. The seed oil is anti-staphylococcal at 0.5 ug/ml. The antibacterial activity of this plant has been reported. (Yin et al, 2004; Newton et al, 2000; Newton et al, 2002)

Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in traditional system of medicine is justified. The development of plant cell cultures is an important strategy for bioprospection of natural products. Thus, the large scale production *in vitro* of bioactive compounds or extracts used as antibacterial should be encouraged due to their scientific, economical or ecological importance.

1. MATERIALS AND METHODS:

1.1 Plant Collection and Processing:

Leaves of *Psoralea corylifolia* were collected from the urban fringe area of Nagpur city (M. S., India). Voucher specimen is deposited with the Department of Botany; RTM Nagpur University, Nagpur, India. The leaves were washed under running tap water and air dried under shade. After 15 days the dried leaves were macerated in a mixer grinder to yield a fine powder which was sieved to yield particle size of 50-150mm. This dried powder (50g) was extracted in a Soxhlet apparatus using 100ml of petroleum ether (60-80 ^{0} C), chloroform (61^0 C), methanol (78.5^0 C) and water (80 ^{0} C)(Mukherjee P, 2002). The extracts obtained were dried and stored in sealed tubes at 4 ^{0}C. The methanolic extracts were found to be more potent than the other solvent counterparts and hence used in this study (Moon et al. 2006; Moon et al. 2009)

1.2 Clinical Isolates

Clinical isolates of *Porphyromonas gingivilis, Prevotella intermedia, Camphylobacter rectus and Actinobacillus actinomycetemcomitants* were obtained from swabs from periodontal pockets which is pathological deepening of the gingival sulcus. The bacterial cultures were maintained on Nutrient Agar (Himedia, Mumbai) at 4^O C and subcultured every two weeks.

2.3 Inoculum Preparation

Stock cultures of clinical isolates were maintained at 4 ^{0}C on nutrient agar slants. A working bacterial inoculum was prepared by inoculating a loop full of the clinical isolate into a 3 ml sterile nutrient broth tube and incubated at 37 ^{o} C for 24 hours. The turbidity was matched with 0.5 Mc Farland's Nephelometer Standard. (WHO, 1983; NCCLS, 2000). Dilutions to the tube were done with sterile nutrient broth to get a cell density corresponding to 2 x 10^6 CFU/ml.

2.4 Media

Nutrient Agar (M001), Agar Agar Type I (RM666), Mueller Hinton Agar No. 2 (M1084) and Nutrient broth (M002) were procured from Hi-Media, Mumbai. The preparation of media was done strictly according to the manufacturer's instructions.

2.5 Antibiotic discs

Commercially available standard antibiotic discs were obtained from Hi-Media, Mumbai. The abbreviations and strength are given in brackets. The antibiotic discs used were Amoxycillin (Ac-30 mcg), Ampicillin (A-10 mcg), Chloramphenicol (C-30 mcg), Erythromycin (E-15 mcg), Penicillin-G (P-10 mcg), Kanamycin (K-30 mcg), Tetracyclin (T-30 mcg), Cephalexin (Cp-30 mcg), Ciprofloxacin (Cf-5 mcg), Co-trimoxazole (Co-25 mcg), Gatifloxacin (Gf- 5mcg), Norfloxacin (Nx-10 mcg), Ofloxacin (Of- 5mcg), Pe-floxacin (Pf-5 mcg), Sparfloxacin (Sc-5 mcg) and Streptomycin (S-10 mcg).

2.6 Antibiotic sensitivity test

The antibiotic sensitivity of the clinical isolates was studied by Bauer-Kirby disc diffusion method. (Bauer et al 1966). A sterile non-toxic cotton swab was dipped into the inoculum tube and rotated firmly against the upper inside wall of the tube to express excess fluid. This swab was now used to streak the entire agar surface of the plate three times turning the plate 60 ^{o} between each streaking. Five antibiotic discs were placed aseptically on each plate with enough spacing. All the plates were incubated at 37 ^{o}C for 18-24 hours. After incubation, plates were examined for zone of inhibition. Zones were measured and recorded as sensitive, resistant or intermediate referring the zone size interpretive chart. (NCCLS, 2002)

2.7 Activity Testing of Methanolic Extracts of Plant

A suspension (0.1 ml) of the test organisms from the 18 hour cultures was thoroughly mixed with 20 ml of sterile Mueller Hinton Agar maintained at 45-50^{o} C. The seeded M.H. Agar is poured in presterilized petri plates and set aside. After solidification, the seeded agar was punched with a flamed (sterile) 10mm cork borer in order to obtain a well of 10mm diameter in the center of the petri plate. 100 ul of the methanolic plant extract is loaded into the well accurately with a micropipette (with presterilized tips) to obtain concentration of 10, 30, 50 and 100mg/ml. The petri plates were delicately handled and kept in refrigerator for 30 minutes and then at room temperature for 30 minutes which facilitated diffusion of the plant extract. The petri-plates were then incubated at 37^{o} C for 24 hours. (Perez et al. 1990) The zone of inhibition was measured with HiAntibiotic ZoneScale, HiMedia, Mumbai. 2% MeOH and sterile distilled water were used as negative controls.

2.8 Callus induction and maintenance

Leaves form plantlets of *P. corylifolia* were washed in fresh tap water. The leaves were washed with 1mg/ml $HgCl_2$ (40 min) and sterilized by stirring in 0.5mg/ml sodium hypochlorite solution (20 min) followed by three successive washes of sterile distilled water. These leaves were wrapped in perforated aluminium foil and then incubated in dark at 26+/- 1^{0} C for 24 hours. Leaf explants were inoculated on Murashige and Skoog (MS) medium supplemented with 2.0ppm 2-iso Pentenyladenine (2-iP) and 0.5ppm NAA at 25° C and 26° C. Callus was cultured at 3000 lux light intensity provided for 14 and 16 hours per day followed by a dark period of 10 and 8 hours respectively at pH 5.8 (Moon et al. 2010). Two month old callus derived from the leaf explant was weighed and dried by wrapping them in perforated aluminum foils and keeping at 40^{O}C for 72 hours in an oven. The dried weight was recorded. This dried callus was ground into fine powder and methanol soxhletion yielded the extract which was cooled to room temperature. This was filtered through Whatmann No. 1 filter paper and the filtrate was utilized in this study at various concentrations of 10, 30, 50 and 100 mg/ml.

3. RESULTS AND DISCUSSION

The pattern of resistance obtained after performing antibiotic sensitivity tests is shown in respective tables. The clinical isolates were found to be resistant to one or more than one antibiotic. Table I.

Table 1: Antibiotic sensitivity of control micro-organisms

		Zone of inhibition in mm diameter															
Sr.no	**Control Micro-organisms**	**A**	**Am**	**C**	**E**	**P**	**K**	**T**	**Cp**	**Cf**	**Co**	**Gf**	**Nx**	**Of**	**Pf**	**Sc**	**S**
1	Porphyromonas gingivilis	18	22	24	19	17	19	22	15	33	24	29	32	32	30	32	12
2	Prevotella intermedia	32	33	26	27	22	26	27	29	28	29	19	19	26	26	32	15
3	Camphylobacter rectus	20	22	25	22	25	22	22	16	32	20	26	28	26	22	30	12
4	Actinobacillus actinomycetemcomitants	18	20	21	20	18	17	20	14	22	18	20	20	30	30	30	16

Clinical isolates of *Porphyromonas gingivilis* which show resistance to commonly used antibiotics like Amoxycillin, Penicillin, Cephalexin, Streptomycin etc., when treated with leaf MeOH extract of *Psoralea corylifoli* and corresponding callus show a zone of inhibition of 24 mm and 20 mm diameter at 100 mg/ml concentration thereby suggesting the potential of callus to produce the bioactive phytochemical responsible for antibacterial activity. Table II

Table 2: Antibacterial activity of Psoralea corylifolia leaf extracts and callus extracts

Sr.no	**Test Micro-organisms**	**Control 1& 2**	**Type of extract**	**Diameter of zone of inhibition in mm; Concentration in mg/ml**							
				Extract of leaf *P. corylifolia*				**Extract of callus of *P. corylifolia* leaf**			
				10	**30**	**50**	**100**	**10**	**30**	**50**	**100**
1	*P. gingivilis*	nil	Methanol	12	13	14	24	11	12	13	20
			Aqueous	Nil	Nil	Nil	Nil	Nil	nil	Nil	nil
2	*P. intermedia*	nil	Methanol	20	26	28	30	18	23	26	28
			Aqueous	14	20	19	24	Nil	nil	Nil	nil
3	*C. rectus*	nil	Methanol	14	20	21	27	15	18	19	21
			Aqueous	Nil	Nil	Nil	Nil	Nil	nil	Nil	nil
4	*A. actinomycetemcomitants*	nil	Methanol	20	22	23	29	15	19	20	22
			Aqueous	Nil	14	20	26	Nil	nil	11	13

In this study, the clinical isolates of *Prevotella intermedia* show resistance against most commonly used antibiotics such as Amoxycillin, Co-trimoxazole, Cephalexin, Ampicillin, Kanamycin, Penicillin etc. Interestingly, the MeOH plant extracts show commendable activity against multi-drug resistant *Prevotella intermedia* strains. Worth mentioning is the extraordinary activity shown by both the methanolic leaf extract and its corresponding callus methanolic extract at all the concentrations Table III.

Table 3: Antibacterial activity of stress induced Psoralea corylifolia callus growth by changing temperature of incubation

Temperature (in degree celsius)	Zone of inhibition in mm diameter			
	P. gingivilis	*P. intermedia*	*C. rectus*	*A. actinomycetemcomitants*
25	12	14	Nil	20
26	11	12	Nil	16

Callus not obtained at temperature 27, 28 , 29 and 30 degrees Celsius

The clinical isolates of *Camphylobacter rectus* show resistance to more than one antibiotic as clearly seen from the resistance pattern in Table IV. *P. corylifolia* leaf MeOH extracts is effective through 30 mg/ml to 100mg/ml concentration while the methanolic callus extract is effective at all the concentrations for most of the clinical isolates tested.

Table 4: Antibacterial activity of stress induced Psoralea corylifola callus growth by changing pH of media

pH	Zone of inhibition in mm diameter			
	P. gingivilis	*P. intermedia*	*C. rectus*	*A. actinomycetemcomitants*
5.5	nil	Nil	Nil	Nil
5.6	nil	Nil	Nil	Nil
5.7	nil	Nil	Nil	Nil
5.8	12	14	Nil	20
5.9	nil	Nil	Nil	Nil
6	nil	Nil	Nil	Nil

The clinical isolates of *Actinobacillus actinomycetemcomitants* show a common resistance pattern for Am, A, P, and Cp. The *P. corylifolia* MeOH callus extract showed great potential as an antibacterial agent as compared to the MeOH leaf extracts at all the concentrations tested. Table V.

Table 5: Antibacterial activity of stress induced Psoralea corylifolia callus growth by changing photoperiod

Photoperiod (light in hours)	Zone of inhibition in mm diameter			
	P. gingivilis	*P. intermedia*	*C. rectus*	*A. actinomycetemcomitants*
8	Nil	Nil	Nil	Nil
10	Nil	Nil	Nil	Nil
12	Nil	Nil	Nil	Nil
14	12	14	Nil	20
16	11	11	Nil	14
18	Nil	Nil	Nil	Nil

4. CONCLUSION

From the results of antibacterial screening of MeOH leaf extracts and its corresponding callus extract used in this study, it is clear that *P. corylifolia* exhibits significant antibacterial activity. Further research in this study focuses on the isolation of bioactive phytochemicals and also inducing the callus to produce higher concentrations of bioactive phytochemicals which are responsible for the antibacterial activity and to combat the multi-drug resistance shown by the human pathogenic bacteria.

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