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ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF STEM BARK OF *Schleichera oleosa* (Lour.Oken)

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ABSTRACT

The anti-bacterial and antifungal activities of ethanolic extract of stem bark of *Schleichera oleosa* (Lour.Oken) was screened by agar cup plate method using *Bacillus subtilis*, *Staphylococcus aureus* of gram positive; *Escherichia coli*, *Pseudomonas aeruginosa* of gram negative; and *Aspergillus niger* of antifungal strains. Ampicillin and Flucanazole were used as reference standard for bacteria and fungi organisms respectively. The study showed that, the ethanolic extract possesses significant antimicrobial activity against the selected bacteria and fungi when compared with the control and standard.

Keywords: Anti-bacterial, Antifungal and *Schleichera oleosa*.

INTRODUCTION

Schleichera oleosa (Lour.Oken) is a medium to large size tree belonging to the family Sapindaceae. It grows up to 20-40 meter in height, dioecious, deciduous, perennial often fluted trunk, with smooth, grey or pale brown bark, reddish inside, young branchlets terete, pubescent [1,2]. The leaves fall during December to January. The plant remain leafless for a short time and the new leaves in various shades of red, light and dark green appear during March to April. Flowering and fruiting takes place from the month of March and July [3,4]. The bark is used as astringent and antipyretic. It is also useful in pruritus, adenitis, ripening boils, nostalgia, arthralgia, malaria, inflammations and ulcers [1]. The paste of the bark (10gm.) along with water (one cup) is taken twice to cure menorrhoea [5]. The bark contains astringent principles, about 10% tannin, analgesic compound; lupeol, antitumour agents; betulin, betulinic acid and cyanogenetic glucoside. A dye and tannin are obtained from the bark utilised occasionally for tannin leather [3]. Raw fruits are pleasant, acidic taste and are eaten during summer; the aril is reported to possess cooling properties. Flowers are said to yield a dye, and are a source of nectar for honey bees [6]. Leaves contain crude protein, crude fibre, calcium, phosphorus and gallotannic acid. Powdered seeds are applied to wounds and ulcers of cattle to remove maggots [3]. Seeds contain cyanogenetic glucoside [7]. The pleasantly acid arillodes of the ripe seeds are eaten or roasted; whereas immature fruit is pickled, cooked young

leaves make a side dish [3]. The seed oil is bitter, sour, sweet, stomachic, anthelmintic, purgative, trichogenous and tonic. It is useful in burns, acne, dermatopathy, scald, trichopathy, cephalalgia and vitiated conditions of vata [1]. Kernel oil obtained from the seed stimulating the growth of hair [2]. The oil extracted from the seed called kusum oil is a valuable component of true Macassar oil used in hair dressing; it is also used for culinary and lighting purpose and in traditional medicine; it is applied to cure itching, acne, and other skin infections. In southern India it is used as a cooling bath oil [3]. It is used for external massage in rheumatism. The oil is a common adulterant of mustard oil. Seed and seed oil, presumably due to the presence of hydrocyanic acid, induce symptoms similar to irritant poisons, producing giddiness, dilation of pupils, and in fatal cases, syncope and death. Kusum oil could be utilised for soap manufacture and as a textile lubricant. It has a roughening action on the skin but has excellent cleansing and emulsifying properties. It is however a useful ingredient in soap charges [6]. Kernel oil contains Gadoleic acid, Oleic acid, Arachidic acid, Stearic acid, Palmitic acid, Behenic acid, cyanogenic compounds, 70% fat and 12% protein [8].

MATERIALS AND METHODS

Collection and Identification of plant material

The stem bark of *Schleichera oleosa* (Lour.Oken) was collected during February 2010 from the costal

regions of Kanyakumari District, Tamil Nadu. The specimen of the plant material (bark) was identified and authenticated by Mr.V.Chelladurai, Research officer-Botany, Central Council for Research in Ayurveda and Siddha (Government of India), Tirunelveli, Tamil Nadu.

Preparation of the extract

The stem bark of *Schleichera oleosa*. was dried under shade and made into coarse powder by hand operated mill. The powder was extracted with ethanol using cold percolation method [9]. The extract (dark red brown) obtained was used to screen its anti-bacterial and antifungal effects.

Anti-bacterial activity

In vitro antibacterial activity of ethanolic extract of stem bark of *Schleichera oleosa* was evaluated against microorganisms namely *Bacillus subtilis*, *Staphylococcus aureus* of gram positive; *Escherichia coli* and *Pseudomonas aeruginosa* of gram negative by agar cup plate method using nutrient agar medium and nutrient broth. Ampicillin was used as reference standard.

All the micro organisms were inoculated in the media and incubated at 37°C for four hours. It produces a turbid solution and then it was diluted with same media and compared with the standard. This level was equivalent to 3x10⁸ CFU/ml. The nutrient agar media was prepared, transferred to sterile petri dishes and allowed to solidify. A suspension of inoculum was added to media and swabs the entire surface of agar media. The inoculum was equally distributed in surface of the media by rotating the plate. The inoculated nutrient agar from each plate was punched with flame sterilized cork borer of size 5 mm diameter to get four cups. The extract of different concentrations and

standard were dissolved by DMSO and placed in each well separately under aseptic condition. Left the plates for one hour at room temperature as a period of pre-incubation diffusion to minimise the effects of variation in time between the applications of the different solutions. Then the plates were incubated at 37°C for 18 hours and observed for antibacterial activity [10,11]. The diameters of the zones of inhibition were observed and measured. The average area of zone of inhibition was calculated and compared with that of the standard.

In vitro antifungal screening

Evaluation of anti-fungal activity of ethanolic extract of stem bark of *Schleichera oleosa* was tested by agar cup plate method. The fungi employed for screenings was *Aspergillus niger*. Sabourauds dextrose agar-agar and Sabourauds dextrose broth were used as a medium. Flucanazole was used as reference standard drug. The stock solution of the reference standard (Flucanazole) and test (extract) were prepared by dissolving in DMSO to obtain required concentrations. The Sabourauds -dextrose-agar medium was sterilised by autoclaving at 121°C (15 lb/sq.inch) for 15 min. The Petri-plates, tubes and flasks plugged with cotton plugs were sterilised in hot air-oven at 150°C, for an hour. Transfer aseptically, molten Sabourauds dextrose-agar medium into each sterilized petri-plate. Inoculate with fungus, after solidification of the medium at room temperature. The plates were punched with flame sterilized cork borer of size 5 mm diameter to get four cups. The extract of different concentrations and standard were placed in each well separately under aseptic condition. The plates were incubated at 30-35°C for 48 hours [12]. After incubation the diameter of zone of inhibition was measured and tabulated.

Table 1. Zone of inhibition of ethanolic extract of stem bark of *Schleichera oleosa* against Gram positive Bacteria

S.No	Drug treatment	Concentration	Zone of inhibition(mm)	
			<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
1	<i>Schleichera oleosa</i>	3mg/well	8.16±0.54	9.16±0.55
2	<i>Schleichera oleosa</i>	5mg/well	15.5±0.5	17.16±0.54
3	Ampicillin	0.2 mg/well	23.5±0.54	28.66±1.21

Table 2. Zone of inhibition of ethanolic extract of stem bark of *Schleichera oleosa* against Gram negative Bacteria

S.No	Drug treatment	Concentration	Zone of inhibition(mm)	
			<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
1	<i>Schleichera oleosa</i>	3mg/well	8.5±0.5	10.66±0.81
2	<i>Schleichera oleosa</i>	5mg/well	12.83±0.65	17.66±1.36
3	Ampicillin	0.2 mg/well	20.83±0.91	31.5±2.07

Table 3. Zone of inhibition of ethanolic extract of stem bark of *Schleichera oleosa* against Fungi

S.No	Drug treatment	Concentration	Zone of inhibition (mm)
			<i>Aspergillus niger</i>
1	<i>Schleichera oleosa</i>	3mg/well	6.5±0.54
2	<i>Schleichera oleosa</i>	5mg/well	11.33±0.81
3	Ampicillin	0.2 mg/well	28.16±1.6

RESULTS AND DISCUSSION

The results of antibacterial screening of ethanolic extract of stem bark of *Schleichera oleosa* against Gram positive and Gram negative bacteria were presented in Table 1 and 2. *In vitro* antifungal screening of ethanolic extract of stem bark of *Schleichera oleosa* against fungal strain was tabulated in Table 3.

CONCLUSION

The study showed that, all the bacterial and fungal strains used in the screening were highly susceptible to the test extract. The extract found comparatively potent antimicrobial agent to ampicillin. It validates the traditional use of the plant against infectious diseases caused by pathogenic microorganisms.

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