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Phytochemical investigations and antibacterial activity of *Salacia oblonga* Wall ethanolic extract

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Abstract

Salacia oblonga Wall, a medicinally important plant, belonging to the family *Celastraceae*, is a large woody climber distributed in southern India and Sri Lanka. In the present investigation, ethanol extracts of *S. oblonga* were prepared from aerial and root parts of the plant in the presence and absence of HCl and antibacterial activity was tested. Both aerial and root extracts exhibited pronounced activity against human pathogens. The MIC and MBC values ranged from 0.078-1.25 mg/ml and 0.156 -2.50 mg/ml, respectively. GC-MS profile of aerial and root extracts displayed the presence of 11 and 6 compounds. The present investigation demonstrated that ethanolic extracts of *S. oblonga* have potential antibacterial activity against human pathogens and could serve as a source for the development of new age antimicrobials.

Key words: *Salacia oblonga*, MIC, MBC, GC-MS

Introduction

Plants have the capability to synthesize a wide range of phytochemicals that possess significant biological functions. Many of these compounds, have beneficial effects on human health and can be used to treat diseases (Prasanabalaji *et al*., 2012; Mahesh *et al*., 2008). Plants act as a source for an assortment of chemicals that can be isolated from various parts like the bark, leaves, roots, seeds and fruits (Suvarchala *et al*., 2012). Several useful plant based drugs have been recognized with traditional uses (Fransworth *et al*., 1985). Plant extracts and their constituents have been known to exhibit biological activities, mainly antimicrobial (Iscan *et al*., 2002; Arunkumar *et al*., 2012; Sokovic *et al*., 2009; Kanatt *et al*., 2008) and antioxidant (Seun-Ah *et al*., 2010; Patel and Jasrai, 2012).

Salacia oblonga Wall belonging to the family *Celastraceae*, is a woody climber, distributed in southern regions of India and Srilanka. The aerial and root parts of *S.oblonga* have been used extensively in Ayurveda and traditional Indian medicine for the treatment of diabetes (Matsuda *et al*., 1999). The root bark of *S.oblonga* is also used in the treatment of gonorrhoea, rheumatism and skin diseases (Anonymous *et al*., 1972; Chopra *et al*., 1956; Karunanayake *et al*., 1984). Our study reports for the first time, the metabolite composition of ethanolic aerial and root extracts of *S. oblonga* and its antibacterial activity.

Materials and Methods

Plant material

S.oblonga plants were collected from the western ghats, India, and authenticated by Dr.Sidda Mallaya, Research officer, Regional Research Institute, Bangalore, India.

Extract preparation

The shade dried plants were separated into aerial and root parts and ground into a fine powder, using an electric blender. The phytochemicals were extracted in ethanol in presence and absence of HCl with the help of a soxhlet apparatus. The extracts were concentrated, using a rotavapor and were stored at -20ºC for further use.

Microbial culture collection

The microbial cultures (*Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae* and

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Pseudomonas aeruginosa) were obtained from IMTECH, Chandigarh, India and other test microorganisms (*Salmonella typhimurium, Enterobacter cloacae, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis* and *Listeria monocytogenes)* were collected as clinical isolates from Global Hospitals, Hyderabad, India. The bacteria were cultured on Mueller Hinton (MH) Agar and activated in MH broth at 37ºC 24 h before experimentation. The bacterial cultures were maintained on MH agar and were subcultured every fortnight.

Antimicrobial assay

The antimicrobial activity of plant extracts was investigated by agar well diffusion method (Perez *et al*., 1990). The Mueller-Hinton agar (MHA) was poured onto the petriplates with an inoculum size of 10^6 colony forming units (c.f.u)/ml of bacteria. The wells were made in the MHA plates with the help of a borer (8mm). The extracts at a concentration of 1 mg/ml were used for evaluating the antibacterial activity. A broad spectrum antibiotic, amikacin at a concentration of 50 µg/ml was used as a positive control, whereas the solvent and solvent along with HCl served as negative control. The extracts at neutral pH (7.0) and acidic pH (5.5) were analyzed for the activity. The plates were incubated overnight at 37ºC for allowing bacterial growth. After incubation, the zones of inhibition observed around the wells (including the well diameter) were measured and tabulated for each of the test microorganism. All the experiments were performed in triplicate.

Minimum inhibitory concentration* (MIC) *and minimum bactericidal count* (MBC) *of Salacia oblonga extracts

The minimum inhibitory concentration (MIC) was determined by broth macrodilution method (Chattopadhyay *et al*., 1998a). Two fold serial dilutions of the crude extracts, with appropriate antibiotic as control were prepared in Mueller–Hinton broth (Chattopadhyay *et al*., 1998b). A direct suspension of microorganisms was prepared in saline water from a 24 h old suspension in Mueller–Hinton broth. The turbidity of the suspension was adjusted to match 0.5 McFarland standard (Mc Farland *et al*., 1907), using a spectrophotometer (Shimadzu UV 2450 UV-Vis Spectrophotometer) at 625 nm, which corresponds to 2.4×10^8 c.f.u/ml. For broth dilution tests, 0.1 ml of the standardized suspension of bacteria (10^8 c.f.u/ml) was added to each tube followed by addition of the extracts with varying concentration ranging from 0-2000 µg/ml and further incubation at 37ºC. The lowest concentration of the tube which did not show any visible growth after macroscopic evaluation was considered as MIC. MBC was taken as the lowest concentration that did not show any visible growth after two fold dilution with plain MH broth.

Statistical Analysis

The data obtained from the results were calculated as mean ± standard deviation in triplicate. The data were compared by least significant difference (LSD) test, using Statistical Analysis System (Ver.9.1)

GC-MS *analysis*

For GC-MS analysis, the samples were injected into an HP-5MS capillary column (30 m length x 250ìm dia. x 0.25ìm film thickness), Agilent Technologies, USA GCMS model, consisting of 6890 N Gas Chromatograph coupled with 5,973 insert MSD (Mass Selective Detector). The injector was set at 250ºC and the detector at 280ºC. The stepped temperature program-was as follows: held at 50ºC for 2 min and then, from 50 to 280ºC at the rate of 10ºC/min, held for 5 min. The total run time was of 30 min. The GC-MS interface temperature was at 280ºC and the injection volume was 1µl. The solvent was injected in a split less mode. The MS scan range was from 35 - 6,000 Da. Compound identification was obtained by comparing the retention times with that of the spectral data obtained from NIST library of the corresponding compounds.

Result and Discussion

Search for antimicrobials from natural sources has received much attention and identifying the compounds from plants that act as suitable antimicrobial agents in place of synthetic compounds has become imperative in order to avoid drug resistance. Phytochemicals isolated from the plants serve as effective agents in the control of microorganisms (Kelmanson *et al*., 2000; Ahmad and Beg, 2001). Many studies have been done on isolation of antimicrobial compounds from the plant extracts (Guleria and Kumar, 2006; Zakaria *et al*., 2007). Hence isolation of the novel compounds with antimicrobial activity from the medicinal plants has great potential.

In the present study, antimicrobial activity of *Salacia oblonga* ethanol aerial and root extracts was investigated against human pathogens. Antimicrobial activity was evaluated by measuring the zone of inhibition exhibited by aerial and root ethanolic extracts, following the agar well diffusion method (Figure 1 and Table 1).

Both the extracts have shown varying degree of antibacterial activity towards pathogens. Among the tested pathogens, aerial neutral extract has shown maximum inhibition against *Salmonella typhimurium*, whereas aerial acidic, root acidic and neutral extracts, have shown highest inhibition against *Staphylococcus aureus*. In addition, acidic extracts showed better antibacterial activity in comparison to neutral extracts. *S. oblonga* ethanol root acidic extracts were found to have maximum activity towards most of the pathogens, followed by aerial acidic and neutral extracts. However, inhibition zones of less than 9mm were observed for the following organism *Staphylococcus epidermidis, Listeria monocytogenes* and *Enterobacter aerogenes*.

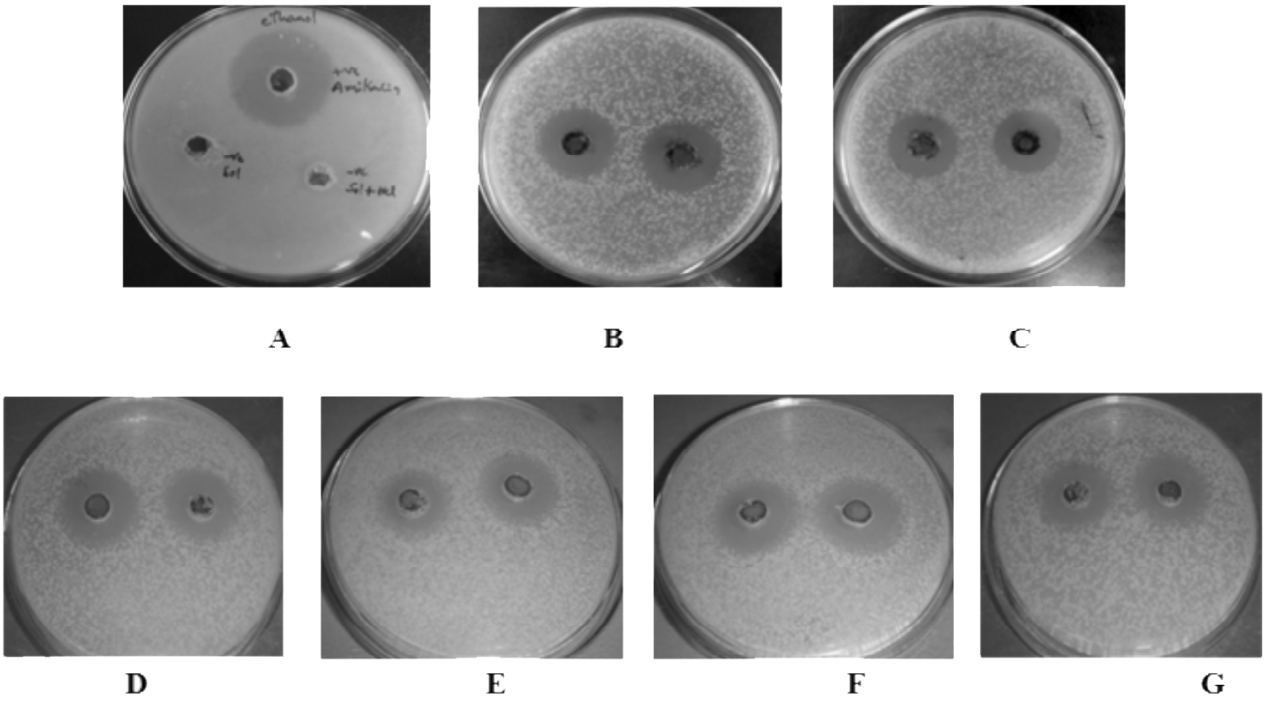

Figure 1: Zones of inhibition exhibited by ethanol aerial and root (neutral and acidic) extracts of *S. oblonga* towards pathogens A: Amikacin (positive control), ethanol solvent neutral and acidic (negative control): ethanol acidic and neutral aerial (B-D) and root (E-G) extracts against *E. cloaceae, S. aureus , P.aeruginosa,* respectively.

Table 1 : Antimicrobial activity of *S. oblonga* aerial and root (neutral and acidic) ethanol extracts against pathogenic bacteria by agar well diffusion method

Pathogenic organism	Zone of inhibition(mm)[a]				Antibiotic
	Aerial neutral[c]	Aerial acidic[d]	Root neutral[e]	Root acidic[f]	(Amikacin)
Bacillus subtilis (+)	10.53±1.33	12.67±0.49	12.23±0.20	14.83±0.72	33.90±0.79
Enterococcus faecalis (+)	NI[b]	NI	NI	NI	31.66±0.58
Staphylococcus aureus (+)	15.13±0.32	17.77±0.49	18.07±0.21	18.63±0.59	28.66±0.47
Staphylococcus epidermidis (+)	NI	NI	NI	NI	26.33±0.58
Listeria monocytogenes (+)	NI	NI	NI	NI	27.50±0.50
Escherichia coli (-)	12.10±0.10	14.24±0.49	14.00±0.72	15.87±0.15	31.16±0.58
Klebsiella pneumoniae (-)	15.70±0.36	16.13±0.42	15.70±0.36	17.52±1.14	26.33±0.58
Pseudomonas aeruginosa (-)	15.13±0.32	15.93±0.67	16.40±0.36	18.03±0.75	24.33±0.76
Salmonella typhimurium (-)	15.97±0.15	16.43±0.67	16.17±0.15	17.50±0.75	27.16±0.76
Enterobactor aerogenes (-)	NI	NI	NI	NI	29.66±0.58
Enterobactor cloacae (-)	15.24±0.49	16.47±0.50	15.97±0.42	18.30±0.52	28.50±0.86

[a]Inhibitory zones in mm, including diameter of the well (8.0mm); mean ± standard deviation of three replicates.

[b]No inhibition or inhibition zones was less than 9 mm.

[c]Aerial neutral; [d]Aerial acidic; [e]Root neutral; [f]Root acidic.

Table 2 : MIC and MBC of *S. oblonga* aerial and root parts (acidic and neutral) ethanol extracts against pathogenic bacteria

Pathogenic organism	Ethanol extracts mg/ml								Antibiotic (Amikacin)	
	AN[a]		AA[b]		RN[c]		RA[d]			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus subtilis	1.25	2.50	0.625	2.50	1.25	2.50	0.31	1.25	0.078	0.156
Enterococcus faecalis	NT	NT	NT	NT	NT	NT	NT	NT	0.018	0.039
Staphylococcus aureus	0.31	0.625	0.156	0.31	0.156	0.31	0.078	0.156	0.078	0.156
Staphylococcus epidermidis	NT[e]	NT	NT	NT	NT	NT	NT	NT	0.156	0.31
Listeria monocytogenes	NT	NT	NT	NT	NT	NT	NT	NT	0.078	0.156
Escherichia coli	1.25	1.25	0.625	1.25	0.625	1.25	0.31	0.625	0.005	0.01
Klebsiella pneumonia	0.31	0.625	0.31	0.625	0.31	0.625	0.156	0.31	0.078	0.32
Pseudomonas aeruginosa	0.31	0.625	0.31	0.625	0.31	0.625	0.156	0.31	0.039	0.078
Salmonella typhimurium	0.31	0.625	0.156	0.31	0.31	0.625	0.156	0.31	0.078	1.25
Enterobacter aerogenes	NT	NT	NT	NT	NT	NT	NT	NT	0.078	0.156
Enterobacter cloacae	0.31	0.625	0.31	0.625	0.31	0.625	0.156	0.31	0.078	0.156

[a]Aerial neutral; [b]Aerial acidic; [c]Root neutral; [d]Root acidic; [e] Not tested.

Table 3: Composition of ethanol aerial and root (neutral and acidic) extracts of *S. oblonga* by GC MS analysis.

Compound group	Compound name	Ethanol extracts (RC[a])			
		AN[b]	AA[c]	RN[d]	RA[e]
Alkanes and alkenes		11.84	13.38	36.95	23.01
	Cyclotrisiloxane, hexamethyl	—	—	36.95	23.01
Aliphatic esters		9.56	23.78	—	65.36
	Silicic acid diethyl bis(trimethylsilyl) ester	—	—	—	65.36
	Carbonic acid, 2,2,2-trichloroethyl-undec-10-enyl ester	—	23.78	—	—
	Trichloroacetic acid, undec-10-enyl ester	7.75	—	—	—
Ketones		48.57	25.85	—	—
	2-p-Nitrophenyl-Oxadiazol-1,3,4one-5	44.72	—	—	—
	Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl	—	25.85	—	—
Aliphatic amine		—	18.99	—	—
	2,3-Dimethylamphetamine	—	18.99	—	—
Aliphatic alcohol		12.77	—	—	—
	Phytol	11.24	—	—	—
Benzene ring		15.27	—	36.92	—
	Benzeneethanol α,α,β-triphenyl	15.27	—	—	—
	Benzo [h] quinoline, 2,4-dimethyl	—	—	14.00	—
	Benzene 1,1',1''.1'''-(oxydimethylidyne) tetrakis-	—	—	22.92	—
Phenols		1.99	—	26.12	11.63
	Pyridine-3-carboxamide.oxime. N-(2-trifluromethylphenyl)	—	—	26.12	—
	5-Methyl-2-phenylindolizine	—	—	—	11.63

[a]Relative area percentage (peak area relative to the total peak area percentage);
[b] Aerial neutral; [c] Aerial acidic; [d] Root neutral; [d] Root acidic

The MIC was taken as the lowest concentration of the test substance that prevents visible growth of microorganism. MIC was studied at different concentrations, ranging from 0.0097 to 5 mg/ml. The data obtained from MIC and MBC were shown in Table 2. The ethanol aerial and root (neutral and acidic) extracts have shown prominent MIC values which is in the range of 0.078 mg/ml - 1.25 mg/ml against all the pathogens and the MBC values were in the range of 0.156 - 2.5 mg/ml. The MIC and MBC values obtained against *Staphylococcus aureus* were on par with the standard antibiotic amikacin. All the extracts have shown more or less the same values of MIC and MBC against all pathogens.

Ethanol aerial and root (neutral and acidic) extracts were subjected to GC-MS analysis. In the ethanol aerial extract, 11 compounds have been detected, whereas in the ethanol root extract, 6 compounds were identified (Table 3). Ethanol aerial neutral extract exhibited the presence of tetraethyl silicate, trichloroacetic acid, undec - 2 - enyl ester, phytol, benzeneethanol α - α - β - triphenyl and 2-p-Nitrophenyl-Oxadiazol-1,3,4one-5 in higher quantities. Root extracts, displayed 6 compounds among which, silicic acid diethyl bis (trimethylsilyl) ester and cyclotrisiloxane hexamethyl were present in higher quantities. Presence of higher quantity of these compounds might be responsible for the biological activity of the extracts against human pathogens. Hema *et al*., (2011) reported the antibacterial activity of phytol from the extracts of *Murraya koenigii*. In the present study with the ethanolic aerial neutral extract, phytol was found to be a major constitutent.

The evolution and spread of antibiotic resistance with development of new drug resistance strains of disease causing organisms is a great concern for global community. Effective treatment of disease is dependent on the development of novel pharmaceuticals. In our study, ethanol extracts obtained with different parts, displayed varying degree of antibacterial activity against various human pathogens. GC/MS analysis, exhibited presence of some of the phytochemicals in higher concentrations. Till now only small percentages of plants have been explored for their phytochemical analysis and pharmacological studies. Borkotoky *et al*. (2013) have reported that secondary metabolite *viz*., alkaloids, flavonoids, tannins and terpenoids might be responsible for their antimicrobial activity. Earlier workers have reported that antimicrobial activity of plant extracts can be attributed to the secondary metabolites which exert their activities through different mechanisms (Pahlaviani *et al*., 2013). Alkaloids have toxic effects on the foreign cells whereas, tannins have been found to form irreversible complexes with proline rich proteins (Pahlaviani *et al*., 2013). As the plant products contain a multitude of compounds, it is difficult for the organism to develop resistance through a single mechanism.

Conclusion

Present study has shown antibacterial activity of *S. oblonga* aerial and root ethanol extracts against different human pathogens. Presence of different group of phytochemicals in this plant, may become a source of bioactive substances for the medicinal uses. Further, fractionation is needed to isolate active components present in the extracts, followed by pharmacological evaluation.

Conflict of interest

The authors declare no conflict of interest.

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