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Antibacterial Principles of *Salacia oblonga* WALL Extracts against Drug Resistance Pathogens

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Abstract: ***Salacia oblonga Wall containing vital phytoconstituents viz. mangiferin, salacinol, kotanolol etc has been in used since long in the treatment of diabetes, inflammation and burn wounds. The antimicrobial activity of S. oblonga aerial and root parts has been tested against drug resistant pathogens staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae. Ethyl acetate extracts of S. oblonga parts have shown significant growth inhibition. Further the extract was fractionated by thin layer chromatography (TLC) and silica gel column chromatography. The active fractions from the chromatography displayed growth inhibition against drug resistant pathogens. Our study provides baseline information for the potential use of plant extracts to fight against drug resistant human pathogen.***

Keywords: *Salacia oblonga*, antibacterial activity, chromatography

1. Introduction

Antibiotic resistance is the capability of a microorganism to resist the effects of antimicrobial agents. From past 50 years, excessive usage of antibiotics has led to the appearance of bacterial resistance and also to the transmission of resistance genes among pathogenic microorganisms. *S.aureus*, *P. aeruginosa* and *K. pneumoniae* are some important drug resistant pathogens exhibiting resistance to a number of commercial antibiotics.

Medicinal plants are rich source of drugs of modern medicine, pharmaceutical intermediates and chemical entities for synthetic drug (Hammer et al., 1999). Plant derived substance have recently gained great interest due to an array of applications (Baris et al., 2006). Increasing rate of antibiotic resistance of microorganisms necessitates the development and research on new antimicrobial agents or resistance modifiers. Compounds derived from medicinal plants have increased widespread interest in the search of alternative antibacterial agents because of the discernment that they are safe and have a long history of use in folk-medicine for the treatment of infectious diseases (Guarrera et al., 2005).

Salacia oblonga Wall is an important medicinal plant that has been extensively used in traditional Indian ayurvedic medicine as a liver tonic, anti-inflammatory agent, anodyne amenorrhea, diabetes and treatment of wounds. The root bark extracts are used for itches, asthma, thirst and ear diseases (Li *et al*., 2008; Chawla *et al*., 2013). The important phytocostitutents of *S.oblonga* include salcinol, mangiferin, kotanlol, with proven biological activities *viz* nephroprotection, antimutagenic, anti-inflammatory and antimicrobial (Ismail et al., 1997; Chawla *et al*., 2013). The present study was undertaken to evaluate the antibacterial activity of *S. oblonga* extracts against drug resistant pathogens viz., *S. aureus, K. pneumonia* and *P. aeruginosa.*

2. Materials and Methods

Extract preparation

S.oblonga plants were collected from the Western Ghats, India. The shade dried plants were separated into aerial and root parts and ground into a fine powder, using an electric blender. The phyto-chemicals were extracted in Ethyl acetate with the help of a soxhlet apparatus. The extracts were concentrated, using a rotavapor and were stored at -20ºC for further use.

Culture collection

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were procured from microbial type culture collection (MTCC), IMTECH, Chandigarh, India. The bacteria were cultured on Mueller Hinton Agar (MHA) and activated in MH broth at 37ºC 24 h before experimentation. The bacterial cultures were maintained on MH agar and were subculture 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 served as negative control. 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 the extracts. All the experiments were performed in triplicate.

MIC and MBC

The minimum inhibitory concentration (MIC) was determined by the broth dilution method (Chattopadhyay *et al*., 1998). Twofold serial dilutions of the crude extracts, with appropriate antibiotic (Amikacin mg/ml) as +ve control were prepared in Mueller–Hinton broth. A direct suspension of microorganisms was prepared in saline from a 24 h old suspension of Mueller–Hinton broth. The suspension turbidity was adjusted to match 0.5 McFarland standard which corresponds to 2.4×10^8 cfu/ml. For broth dilution tests, 0.1 ml of the standardized suspension of bacteria (2.4×10^8 cfu/ml) was added to each tube at a final concentration of 0.005–5.12 mg/ml, and incubated at 37 ^{0}C. MBC was taken as the lowest concentration that did not show any visible growth after two fold dilution with plain MH broth.

Column chromatography

The column chromatography (450x30mm) was carried out by using 60-120 mesh size silica gel to elute individual components from the *S. oblonga* ethyl acetate root extract, exhibiting maximum antimicrobial activity. EtOAc with constant bed volume and size was maintained. The matrix was equilibrated using hexane (200ml) and compounds eluted using a mobile phase of hexane and EtOAc. The resulting fractions were collected separately, which were analyzed for the antimicrobial activity (Cao *et al*., 2013).

3. Results and Discussion

Antimicrobial Assay

In the present study antimicrobial activity of *Salacia oblonga* wall extracts was evaluated by agar well diffusion method against drug resistant pathogens viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Different solvents of *S. oblonga* extracts were evaluated for antibacterial activity, ethyl acetate extracts have shown superior activity compared to the other solvents. The aerial and root parts were separated and analyzed for activity. Extracts displayed good antimicrobial activity against drug resistant pathogens. Root and aerial extracts displayed growth inhibition 20± mm and 15.12± mm against *S. aureus*, 19.8± mm and 19.4± against *Klebsiella pneumonia* and 17.8± mm and 18.17± mm against *Pseudomonas aeruginosa* respectively. Root extracts have shown better growth inhibition (20 mm) against *S.aureus* compared to the aerial extracts (15.12 mm). In case of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* root extracts displayed slightly higher growth inhibition compared to the aerial extracts.

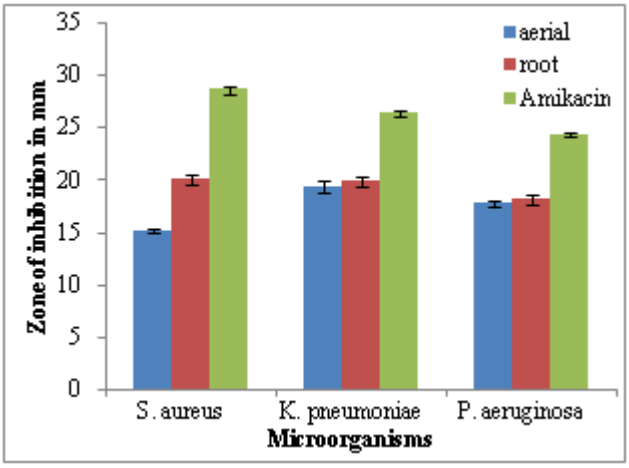

Figure 1: Antimicrobial activity of *S. oblonga* ethyl acetate extracts against drug resistance pathogens.

MIC and MBC

The ethyl acetate extracts exhibited exceptionally low values of MIC and MBC tested in range of 0.005 to 5.12 mg/ml. *S. oblonga* aerial extracts displayed MIC and MBC values 0.64 and 1.28 mg/ml, root extracts displayed 0.08 and 0.16 mg/ml against *S.aureus* which were comparable to standard antibiotic. Aerial and root extracts exhibited similar MIC and MBC values 0.08 and 0.16 mg/ml against *K. pneumonia*. Aerial extracts have shown MIC and MBC values 0.32 and 0.64 mg/ml respectively and root extracts displayed MIC and MBC 0.16 and 0.32 mg/ml respectively against *P. aeruginosa* (Table 1).

Table 1: MIC and MBC values of the extracts (EA-ethyl acetate aerial, ER- ethyl acetate root) against drug resistant pathogens

Pathogens	Extracts (mg/ml)				Amikacin (mg/ml)	
	EA		ER			
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	0.64	1.28	0.08	0.16	0.08	0.16
K. pneumoniae	0.08	0.16	0.08	0.16	0.08	0.32
P. aeruginosa	0.32	0.64	0.16	0.32	0.04	0.08

S. oblonga extracts displayed antimicrobial activity against different drug resistance pathogens. Antimicrobial properties of *S. oblonga* and its mode of action may depend on several factors viz., disruption of the membrane structure and function, disruption of the DNA/RNA synthesis and function, interference with intermediate metabolism (proteins), induction of coagulation of cytoplasmic content and interfere with intracellular communications (Radulovic et al 2013). In order to obtain information and characterization of the active components present in the crude extracts of *S. oblonga*, various chromatographic techniques were applied. TLC was used initially for separation of compounds and mobile phase was optimized for better resolution.

Silica gel column chromatography

EtOAc root extract exhibiting maximum antimicrobial activity was subjected to silica gel chromatography. The extract was introduced into the column and partitioned into different fractions with the help of a mobile phase

containing hexane and ethyl acetate in the ratio of 95:5ml. Once the fractions were obtained, they were pooled together based on the similar thin layer chromatography (TLC) patterns, the activity of each fraction was checked independently. The obtained fractions were sub fractionated into smaller fractions and evaluated for their activity against the pathogenic bacteria species. Among the tested fractions, one fraction in particular demonstrated excellent activity against all three pathogens.

4. Conclusion

Present study confirms the antibacterial activity of *S.oblonga* extracts against drug resistance pathogens. However, further investigations are needed to identify the active compounds from the extract.

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