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Research Article

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Antimicrobial evaluation of methanolic extract from flower of *Securinega leucopyrus* (AEFSL): A medicinal approach

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ABSTRACT

Methanolic extract of Securinega leucopyrus flowers was evaluated for their in-vitro antimicrobial activity. The plant was collected from moist region of Wainganga River, Bhandara (Maharashtra), India. Securinega leucopyrus is extensively used as medicine in Indian folklore for treatment of various infectious diseases. The antimicrobial activity of the plant extracts against various strains of bacterial species was tested for the zone of inhibition using the disc-diffusion assay, minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) values. This extract showed profound inhibitory action on the growth of bacterial strains of some species of genera belonging to Acinetobacter, Bacillus, Brevundimonas, Brucella, Enterobacter, Escherichia, Micrococcus, Pseudomonas, Staphylococcus and Xanthomonas.

Keywords: Antimicrobial activity; Methanolic extract; Infectious diseases; Minimal inhibition concentration; Minimal bactericidal concentration.

INTRODUCTION

Higher plants have always been a source of new medicinal drugs [1]. The number of plants investigated in previous studies is still small. Biological or pharmacological screening of such species shows a wide gap in study and a space is left with researchers to critically analyze the properties of such an extensive range of plants [2]. Among 2,50,000-5,00,000 plant species, only few are phytochemically tested. Thus, modern chemists are astonished towards the unidentified species looking forward as a hope for finding new therapeutics [3-4]. On a global basis it was estimated that 130 drugs that are in use belong originally or made synthetically taking initial lead components from such sources [5].

Medicinal plants signify a wealthy source of antimicrobial agents [6]. Whole plant extracts sometimes serves as medicine in many parts of the world. On the other hand, different parts of plant like root, stem, flower, fruit, twigs etc. are also used for extraction to develop novel drug molecules [7-8].

Considering the enormous availability and potentiality of such plants, studies are going on not only at local level but also on industrial scale globally for antimicrobial drugs with reference to antibacterial and antifungal properties. Following examples depicts the clear picture of the world investigations undertaken to screen the local flora for antibacterial and antifungal activity from *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Ziziphus mauritiana and Withania somnifera. Acacia nilotica*, belongs to the family Mimosaceae, decoction of its bark is used as gargle and pods in urino-genital diseases. *Sida cordifolia* belongs to family Malvaceae, its root is used in nervous disorders,

coryza, and cardiac diseases. *Tinospora cordifolia* belongs to family Menispermaceae, its stem is used as an ingredient for ayurvedic preparations in general debility, dyspepsia, fevers and urinary infections. Also its root is a powerful emetic and used for visceral obstruction; its watery extracts are used in leprosy. *Withania somnifera* belong to the family Solanaceae and its plant parts are used in hiccup, cough, dropsy, rheumatism and gynecological disorder and as a sedative in senile debility. It is also beneficial in inflammatory circumstances, ulcers [16] and scabies as external application. Leaves of this plant are used as febrifuge and applied to lesions, painful swellings and sore eyes. *Ziziphus mauritiana* belongs to the family Rhamnaceae. Its fruits are used as tonic in medicine. They form one of the ingredients of 'Joshanda', a medicine used in chest troubles. Its kernel is used as sedative and to stop vomiting. It is also employed as an antidote to aconite-poisoning and in abdominal pain; its seeds are given in diarrhea. Leaves with catechu (*Areca catechu*) used as an astringent and considered diaphoretic. [9].

The Indian subcontinent has known for its biodiversity. Specifically in India, our state Maharashtra comes under hot spot biodiversity zone among twenty five global biodiversity hot spots with nearly 4,000 species of flowering plants. Gondia and Bhandara district of Maharashtra has large plant diversity. Plant for this study, *Securinega leucopyrus*, is commonly available in this part of country. This plant [10] belongs to the family Euphorbiaceae, commonly known as *Ghat-bor* in Marathi. It is unexplored for antimicrobial activity against various bacterial strains. We have endeavored for the first time to study antimicrobial activity of this species.

EXPERIMENTAL SECTION

Plant Collection

The aerial part of the plant *Securinega leucopyrus* was collected from the moist region near Wainganga River, Bhandara (Maharashtra), India. The sample of plant was identified by senior plant taxonomist, Dr. S. U. Borkar, Department of Botany, Govt. Institute of Science, Nagpur (Maharashtra), India. The specimen was submitted to the herbarium of the department with authentication code, IoSc./Bot.Sci/03/16/2016-17/121, (Figure-1).

Figure-1: *Securinega leucopyrus* plant

Extraction

Recently collected aerial parts (fruits) of the plant *Securinega leucopyrus* were washed, shade dried under room temperature for duration of three weeks. The dried plants solid was grinded to a coarse powder.

About 150 gm powdered sample was exposed to hot percolation in a Soxhlet apparatus using methanol, at a temperature range of 40-80^{0}C. The alcoholic extract of fruits of *Securinega leucopyrus* (AEFSL) was concentrated to a dry mass on water bath and kept in desiccator [11].

Preliminary Phytochemical Test

Methanolic extract obtained by the above method from *Securinega leucopyrus* (AEFSL) was exposed to qualitative tests for the identification of numerous secondary metabolites present in fruit of plant by the standard processes [12].

Antimicrobial activity

Microorganisms

A Total of 25 bacterial species, 56 strains of 24 bacterial species were used in this study (Table 1). Microorganisms were provided by Department of Microbiology, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India. Identification of the assessment organisms were confirmed by Microbial Identification in Biotechnology Research division, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India

Disc-diffusion assay

The dried plant extract was dissolved in the same solvent (methanol) to a final concentration of 30 mg/ml and sterilized by filtration by 0.45 µm Milli pore filters. Antimicrobial tests were evaluated by disc-diffusion method

[13] using 100 µl of suspension containing 108 CFU/ml of bacteria spread on nutrient agar (NA), Sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively.

The discs (6mmin diameter) were impregnated with 10 µl of the extracts (300µg/disc) at the concentration of 30 mg/ml and located on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (10 µg/disc), sulbactam (30 µg) + cefoperazona (75µg) (105µg/disc), and/or netilmicin (30 µg/disc) were used as positive reference standards to conclude the sensitivity of one strain/ isolate in each microbial species tested.

The inoculated plates were incubated at 37^0C for 24 hour for clinical bacterial strains. Plant associated microorganisms were incubated at 27^0C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Every test in this experiment was repeated twice.

Micro dilution assay

The minimal inhibition concentration (MIC) values were also calculated for the microorganisms which were determined as sensitive to the methanol extract of *Securinega leucopyrus* in disc diffusion assay.

The inocula of microorganisms were prepared from 12 hour broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The *Securinega leucopyrus* extract dissolved in 10% dimethyl sulfoxide (DMSO) were first diluted to the highest concentration (500µg/ml) to be tested, and then serial twofold dilutions were made in a concentration range from 7.8-500 µg/ml in 10 ml sterile test tubes containing nutrient broth. MIC and minimal bactericidal concentration (MBC) values of *Securinega leucopyrus* extract against bacterial strains isolates were determined based on a micro-well dilution method [14] and described with some modifications as follows.

The 96-well plates were prepared by dispensing into each well 95µl of nutrient broth and 5µl of the inoculum. A100µl from *Securinega leucopyrus* extract primarily prepared at the concentration of 500µg/ml was added into the first wells. Then, 100 µl from their successive dilutions was transferred into six consecutive wells. The last well containing 195 µl of nutrient broth lacking compound and 5 µl of the inoculum on each strip was used as negative control. The final volume in each well was 200 µl. Maxipime (Bristol-Myers Squibb) at the concentration range of 500–7.8 µg/ml was arranged in nutrient broth and used as standard drug for positive control. The plate was protected with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for20 seconds and then incubated at suitable temperatures for 24 hour. Microbial growth was estimated by absorbance at 600 nm using the ELx 800 universal micro plate reader (Bio Tech Instrument Inc.) and confirmed by plating 5 µl samples from clear wells on NA medium. The extract confirmed in this study was screened two times against each organism.

The MIC is defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. MBCs were determined by plotting 5µl samples from clear wells onto NA plates without plant extract. The MBC is the concentration at which there was no microbial growth.

RESULTS AND DISCUSSION

In the present work, the antimicrobial compounds from the flowers of *Securinega leucopyrus* were executed against broad range of microorganisms on the ground of disc-diffusion and micro dilution assay.

This helps to detect quantitatively their potency by the presence or absence of inhibition zones and zone diameters (Tables 1), MIC and MBC values (Table 2).

The results shows that the methanol extract has inhibition effect on the growth of total 25 bacterial species, 56 strains of 24 bacterial species which are *Acinetobacter calcoaceticus, Bacillus amyloliquefaciens, Bacillus atrophaeus, Bacillus cereus, Bacillus lentimorbus, Bacillus licheniformis, Bacillus macerans, Bacillus megaterium, Bacillus pumilus, Bacillus sphaericus, Bacillus substilis, Brevundimonas diminuta, Brucella abortus, Enterobacter agglomerans, Enterobacter pyrinus, Escherichia coli, Kocuria varians, Leclercia adecarboxylata, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella typhimurium, Staphylococcus aureus, Staphylococcus epidermis, Xanthomonas campestris* (Tables 1).

However, the negative control had shown no activity against any bacterial varieties evaluated. Maximal inhibition zones, MIC and MBC values for the microorganisms sensitive to the methanol extract of *Securinega leucopyrus* were in the range of 7–17 mm, 31.25–250 µg/ml and 62.5–500 µg/ml, respectively (Tables 1 &2).

Table-1: Antimicrobial activity of *Securinega leucopyrus* extracts (300µg/disc) against the bacterial strains tested based on disc-diffusion method

Microorganisms	Number of strains	Inhibition zone diameter around test disc		
		Methanol extract	NC[X]	Standard antibiotic discs[Y]
Acinetobacter calcoaceticus	2	9 mm/2	--	16mm (OFX)
Bacillus amyloliquefaciens	1	8 mm/1	--	27mm (SCF)
Bacillus atrophaeus	2	10 mm/2	--	21mm (SCF)
Bacillus cereus	2	14 mm/2	--	30mm (OFX)
Bacillus lentimorbus	1	10 mm/1	--	22mm (SCF)
Bacillus licheniformis	1	11 mm/1	--	(SCF)
Bacillus macerans	2	8-9 mm/2	--	26mm (SCF)
Bacillus megaterium	4	9-16mm/4	--	9mm (SCF)
Bacillus pumilus	2	11 mm/2	--	23mm(OFX)
Bacillus sphaericus	1	11 mm/l	--	18mm(OFX)
Bacillus substilis	4	10-18 mm/4	--	28mm(OFX)
Brevundimonas diminuta	1	9 mm/1	--	34mm (SCF)
Brucella abortus	10	9-14mm/10	--	12mm (SCF)
Enterobacter agglomerans	1	9 mm/1	--	12mm (OFX)
Enterobacter pyrinus	1	16 mm/1	--	NT
Escherichia coli	1	8 mm/1	--	(OFX)
Kocuria varians	1	12 mm/1	--	18mm (OFX)
Leclercia adecarboxylata	1	--	--	NT
Pseudomonas aeruginosa	3	11-16 mm/3	--	22mm (NET)
Pseudomonas putida	1	13 mm/1	--	24mm (SCF)
Pseudomonas syringae	2	10-13 mm/2	--	24mm (OFX)
Salmonella typhimurium	1	---	--	27mm (SCF)
Staphylococcus aureus	5	9-12 mm/5	--	22mm (SCF)
Staphylococcus epidermis	2	9 mm/2	--	NT
Xanthomonas campestris	4	12 mm/1	--	20mm (SCF)
Total 25 bacterial species		56 strains 8-18 mm/51 strains of 24 species		

[X]NC: negative control (Methanol).

[Y]OFX: Ofloxacin (10 µg/disc); SCF: sulbactam (30 µg) + cefoperazona (75 µg) (105 µg/disc); NET: netilmicin (30 µg/disc) were used as positive reference standard antibiotic discs (Oxoid); NT: not tested.

Table-2: The MBC and MIC values of *Securinega leucopyrus* methanol extract against the microorganisms tested in micro dilution assay

Microorganisms	Methanol extract MBC	Methanol extract MIC	Standard drug (maxipime)
Acinetobacter calcoaceticus	500	250	NT
Bacillus amyloliquefaciens	250	125	NT
Bacillus cereus	500	250	250
Bacillus lentimorbus	500	250	NT
Bacillus licheniformis	250	125	NT
Bacillus megaterium	125	62.5	NT
Bacillus macerans	62.5	31.25	NT
Bacillus pumilus	125	62.5	31.25
Bacillus substilis	62.25	31.5	125
Bacillus sphaericus	250	125	NT
Brevundimonas diminuta	500	250	NT
Escherichia coli	500	250	15.62
Micrococcus luteus	250	125	NT
Pseudomonas aeruginosa	500	125	NT
Pseudomonas syringae	500	125	31.25
Pseudomonas putida	250	125	125
Staphylococcus aureus	500	250	NT
Staphylococcus epidermidis	500	250	NT

MBC: minimal bactericidal concentration (µg/ml); MIC: minimal inhibition concentration (µg/ml); NT: Not tested.

This is the pioneering work which demonstrated that methanol extract of *Securinega leucopyrus* contains antimicrobial components with antibacterial effects.

In addition, these results confirmed by in previous studies which reveal that methanol is an improved solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents, such as water, ethanol, and n-hexane [15]

Our investigational data illustrated that there was no uniform response within or between the bacterial strains of the same species in terms of susceptibility to antimicrobial compounds in the methanol extract of *Securinega leucopyrus* (Tables 1 and 2). These kinds of differences in susceptibility between the microorganisms against antimicrobial substances in plant extracts may be elucidated by the differences in cell wall composition and/or inheritance genes

on plasmids that can easily be transferred among bacterial strains. The results suggest that methanol extract of the *Securinega leucopyrus* possess some significant organic compounds with antibacterial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases.

CONCLUSION

Natural antimicrobials are droolworthy source of attracting people towards plant diversity due to costly synthetic drugs with many side-effects. It is concluded from this study that plant like *Securinega leucopyrus* are good antimicrobials. Such explorations need to be carried out with further testing for its toxicity to human beings so that it takes the form of drug and become the pharma fact from laboratory leads.

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