Original Article

Evaluation of Antimicrobial (Antibacterial and Antifungal) Potential of Root Extracts of *Spilanthes acmella* Murr. – An Important Medicinal Plant

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Abstract

Plants produce many secondary metabolites which constitute an important source of antimicrobial, anticancer and pharmaceutical activities. Plant extracts which inhibit the pathogenic microorganisms have potential use as therapeutic drugs. *Spilanthes acmella.* (Family : Asteraceae) is an important medicinal plant with major chemical compound spilanthol which is having many bioactive properties. The present study has been under taken to evaluate the antimicrobial potential of root extract of *S.acmella*. In the present study, the methanolic extract of roots of *S.acmella* was tested for its antibacterial and antifungal activity by Agar cup bioassay method.. The antibacterial activity of root extract of *S.acmella* was studied against gram positive bacteria *Bacillus subtili (MTCC 2394), Staphylococcus aureus* (MTCC 96) and gram negative bacteria *Escherichia coli* (MTCC 443*), Pseudomonas aeruginosa* (MTCC 741). The various concentrations of methanolic root extract (500, 1000, 1500, 2000 µg) were tested and high activity was observed at 2000 µg. Among the test organisms, *Pseudomonas aeruginosa* showed highest zone of inhibition (3.3cm) followed by *E.coli* (2.8cm). Lesser activity was seen against *Bacillus subtilis* (2.6cm) and *Staphylococcus aureus* (2.1 cm). The antifungal activity of root extract of *S.acmella* was tested against five different fungal species i.e. *Macrophomina phaseolina* (MTCC 257), *Rhizoctonia solani MTCC 4633), Fusarium oxysporum (MTCC 1755), Alternaria alternate (MTCC 2724) and Colletotrichum capsicii* (MTCC 3414). The various concentrations of methanolic root extract (500, 1000, 1500, 2000 µg) were tested and maximum activity was observed at 2000 µg. All the five organisms were inhibited by the test solution with varying sensitivity. Among all, high inhibition zones were observed in *Fusarium oxysporum* (2.8 cm) followed by *Colletotrichum capsicii* (2.5cm), *Rhizoctonia solani* (2.2cm.). The *Alternaria alternata* and *Macrophomina phaseolina* showed low inhibition zones of 2.0 cm and 1.8 cm respectively.

Keywords: *Spilanthes acmella,* Roots, Antimicrobial Activity, Antibacterial, Antifungal, Agar Cup Bioassay

Introduction

Plants have been used for thousands of years as food, to treat health disorders and prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities.

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Numerous plant compounds or extracts which have been discovered through the research and clinical investigation systems are used in mainstream medicine today.

The plant chemical constituents are not only used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds[1]. The usage of medicinal plants is popularly known as herbalism, a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Plant drugs are derived either from the whole plant or from different organs like leaves, stem, bark, roots, flower, seeds etc.

In recent years, there is a growing attention for discovery of new antimicrobials of plant origin. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Incidents of epidemics due to such drug resistant micro-organisms are now becoming global problem posing enormous public health concerns. This is due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases. Also majority of synthetic antibiotics are highly toxic at their optimum dosage level[2,3]. This has necessitated the discovery of new antimicrobial drugs of plant origin.

Plants possess many chemicals with various biological properties and has a great potential in developing broad spectrum antibiotics. There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants[4,5,6,7]. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. Thus bioactive evaluation of the plant products will result in identification of new antimicrobial agents which can be developed as drugs for the treatment of many ailments.

In the present investigation, we have selected *Spilanthes acmella* Murr. which is commonly known as toothache plant, an important medicinal plant belonging to family Asteraceae. It is widely distributed in tropical and subtropical regions of the world. It has been reported to possess various biological activities like antipyretic, antidiuretic, anti-inflammatory, antioxidant, immune-modulatory, hepato-protective, anticancer and anti-toothache[8]. The plant has been found to produce important secondary metabolites like spilanthol, scopoletin, myrecene, α amyrin, β amyrin etc. The active chemical component is spilanthol, an alkamide which is present in roots and all aerial parts of the plant[9]. Spilanthol has high industrial demand for its use in pharmaceutical, cosmetic and toothpaste industry.

Test Microorganisms

The bacterial and fungal microorganisms selected were obtained from Microbial Type Culture collection (MTCC), IMTECH, Chandigarh. The strains are maintained and tested on Nutrient agar for bacteria and Potato Dextrose Agar (PDA) for fungi. The antibacterial activity of root extract of *S.acmella* was studied against gram positive bacteria *Bacillus subtili (MTCC 2394), Staphylococcus aureus* (MTCC 96) and gram negative bacteria *Escherichia coli* (MTCC 443*), Pseudomonas aeruginosa* (MTCC 741). The antifungal activity of root extract of *S.acmella* was tested against five different fungal species i.e. *Macrophomina phaseolina* (MTCC 257), *Rhizoctonia solani MTCC 4633), Fusarium oxysporum (MTCC 1755), Alternaria alternate (MTCC 2724) and Colletotrichum capsicii* (MTCC 3414).

Bacillus subtilis is a gram positive ubiquitous bacterium commonly recovered from water, soil, air, and decomposing plant residue. The bacterium produces an endospore that allows it to endure extreme conditions of heat and desiccation in the environment. It has been associated with outbreaks of food poisoning[10].

Staphylococcus aureus is a Gram-positive spherically shaped bacterium, *Staphylococcus aureus* is also responsible for food poisoning and achieves this by generating toxins in the food, which is then ingested[11]. Although *S. aureus* usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning.[12.13]

Escherichia coli is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms[14]. Most strains are harmless, but some are pathogenic, causing serious food poisoning in their hosts[15].

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, aerobic–facultatively anaerobic, rod-shaped bacterium that can cause disease in plants and animals, including humans.[16,17] *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses like hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. According to the World Health Organization *P. aeruginosa* poses one of the greatest threats to humans in terms of antibiotic resistance.[18]

Macrophomina phaseolina is a plant pathogen fungus that causes damping off, seedling blight, collar rot, stem rot, charcoal rot, basal stem rot, and root rot on many plant species like peanut, cabbage, pepper, chickpea, soybean, sunflower, sweet potato, alfalfa, sesame, potato, sorghum, wheat, and corn, among others.[19]

Pathogenic strains of *F. oxysporum* have been studied for more than 100 years. The host range of these fungi is broad and includes animals, ranging from arthropods to humans,[20] as well as plants. It infects plants like tomato, tobacco, legumes, cucurbits, sweet potatoes, banana and other herbaceous plants.[21] *F. oxysporum* generally produces symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting and damping-off.[22]

Rhizoctonia solani is a ubiquitous soilborne necrotroph that inflicts damage on a wide range of economically important crops. Symptoms on diverse hosts include seed rot, root rot, hypocotyl rot, crown rot, stem rot, limb rot, pod rot, stem canker, black scurf, seedling blight, and pre- and post-emergence damping off.[23]

Colletotrichum capsici is a species of fungus and plant pathogen which causes leaf blight on *Chlorophytum borivilianum*, basil, chickpea and pepper as well as dieback in pigeon pea and anthracnose in poinsettia.[24]

Alternaria alternata is a fungus causing leaf spots, rots, and blights on many plant parts, and other diseases. It is an opportunistic pathogen on over 380 host species of plant. It can also cause upper respiratory tract infections[25] and asthma in humans with compromised immunity.[26]

Limited study has been done to evaluate antibacterial and antifungal activity of root extracts of *S.acmella*. Hence this study has been undertaken to evaluate the antimicrobial potential of *Spilanthes acmell.* The *in vitro* antibacterial and antifungal activity was tested by Agar cup bioassay method. It is an important method to test the ability of plant extract to inhibit the bacterial and fungal growth.

Methodology

This study was done in 2016 and duration taken for study was 1 year.

Establishment of Plant Material

S.acmella seeds were obtained from Medicinal and Aromatic Plants Research Station, Rajendranagar, Hyderabad, sown in soil in pots and the plants were maintained in the Botanical Garden in the University **(Fig. 1**).The root material to test the antimicrobial activity was collected from these plants.

Figure 1: Establishment of *Spilanthes acmella* plants in pots in the Botanical Garden

Preparation of Plant extract

The roots collected from field grown plants were washed thoroughly, shade dried and ground to a course powder. Soxhlet apparatus was used and the method given by James et al. was followed.[27] The powdered material (50 grams) was extracted with 500 mL Methanol at 65°C in a Soxhlet apparatus. Solvent was recovered under reduced pressure to obtain crude extracts. The extract was made into different concentrations (500, 1000, 1500, 2000 µg) that were tested for its antibacterial and antifungal activity against the test organisms and employed in antimicrobial bioassay.

The incubation time for antibacterial activity was 24 hours at 37°C and for antifungal activity it was 48 hours at 25°C.

Results

Antibacterial Activity of Root Extract

Root extract, at various concentrations (500, 1000, 1500, 2000 µg) were tested for its antibacterial activity against the test organisms. All the test solutions exhibited antibacterial activity. The zone of inhibition increased with increase in concentration of root extract from 500 to 2000 µg. Less inhibition zones were observed at lower concentrations of extract i.e 500 to 1500 µg. However below 500 µg concentration of root extract did not show any activity. Among all the concentrations, maximum activity was observed at 2000 µg concentration of extract **(Table 1).**

Table 1. Antibacterial activity of root extracts of field grown plants of *S.acmella*

Test Bacteria	Concentration of root extract in µg			
	500	1000	1500	2000
	Zone of inhibition in cms			
B.subtilis	1.1	1.6	2.3	2.6
S.aureus	1.0	1.3	1.9	2.1
P.aeruginosa	1.6	2.1	2.6	3.3
E.coli	1.3	2.6	2.8	2.8

The root extract was tested for its antibacterial activity against four different bacterial species i.e. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

The antibacterial activity of different species was compared at 2000 µg concentration of root extract. Among all, the activity of plant extract was found to be highest in *Pseudomonas aeruginosa* with zone of inhibition 3.3 cm followed by *E.coli* (2.8 cm). Lesser activity was seen against *Bacillus subtilis* (2.6 cm) and *Staphylococcus aureus* (2.1 cm) **(Fig. 2 and Plate 1).**

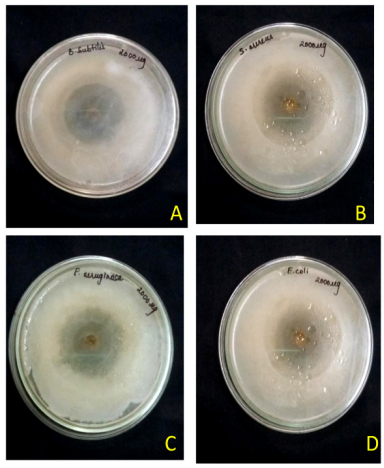

Plate 1: Antibacterial activity of Root extract of *S. acmella*

Antifungal Activity of Root Extract

Effect of various concentrations (500, 1000, 1500, 2000 µg) of *S.acmella* root extract was tested on different fungal species. The antifungal activity was observed in all the concentrations tested, but below 500 µg concentration of root extract did not show any activity. Less inhibition zones were observed at lower concentrations of extract i.e 500 to 1500 µg. The zone of inhibition increased with increase in concentration of root extract from 500 to 2000 µg and the maximum activity was observed at 2000 µg concentration of extract.

The root extract was tested for its antifungal activity against five different fungal species i.e. *Macrophomina phaseolina, Rhizoctonia solani, Fusarium oxysporum, Alternaria alternata* and *Colletotrichum capsicii.*

The antifungal activity of different species was compared at 2000 µg concentration of root extract **(Table 2).** All the five organisms were inhibited by the test solution with varying sensitivity. Among all, high inhibition zones were observed in *Fusarium oxysporum* (2.8 cm) followed by *Colletotrichum capsicii* (2.5 cm), *Rhizoctonia solani* (2.2 cm). The *Alternaria alternata* and *Macrophomina phaseolina* showed low inhibition zones of 2.0 cm and 1.8 cm respectively **(Plate 2 and Fig. 3).**

Table 2. Antifungal activity of root extracts of *S.acmella*

Test Fungi	Concentration of root extract in µg			
	500	1000	1500	2000
	Zone of inhibition in cms			
F. oxysporum	1.2	1.5	2.3	2.8
M.phaseolina	0.8	1.0	1.4	1.7
R.solani	1.4	1.7	2.0	2.2
C.capsici	1.6	2.0	2.2	2.5
A.alternata	1.0	1.4	1.7	2.0

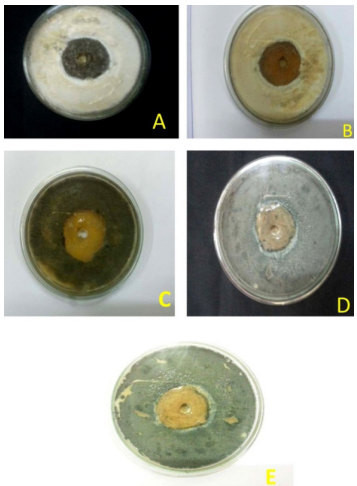

Plate 2: Antifungal activity of *S.acmella* root extract against different fungal species

A - *Fusarium oxysporum* B - *Colletotrichum capsici*

C - *Rhizoctonia solani* D - *Alternaria alternata*

E - *Macrophomina phaseolina*

Discussion

Earlier studies reported the antibacterial and antifungal activity of various species of *Spilanthes*. In *Spilanthes calva*, methanolic root extracts (100 mg/ml) showed inhibition against *Enterococcus faecalis*[28] and *Streptococcus mutans*[29]. *Spilanthes mauritiana* root and flower extracts has also shown to exhibit good antibacterial activity against various bacteria[30]

Spilanthes acmella fractions from the chloroform and methanol extracts of aerial plant parts inhibited the growth of many bacteria and fungi.[31] This antimicrobial activity may be due to the presence of spilanthol in them which is in accordance to studies done by Molina-Torres et al., 2004 who studied the antifungal and bacteriostatic activities of spilanthol and other alkamides from the roots of *Heliopsis longipes*[32].

In the present evaluation for antibacterial activity, among all, maximum activity was observed at 2.0 mg/l of extract in *Pseudomonas aeruginosa* with zone of inhibition 3.3cm followed by *E.coli* (2.8 cm). Similarly, high antibacterial activity was reported from the flower extract of *S.acmella*[33].

In the present evaluation for antibacterial activity, the maximum activity was observed at 2000 µg concentration, with high inhibition zones in *Fusarium oxysporum* (2.8 cm) followed by *Colletotrichum capsicii* (2.5 cm), *Rhizoctonia solani* (2.2 cm) and *Alternaria alternata* (2.0 cm).Similarly in a study, *S.acmella* flower head extracts has shown high inhibition zones against various fungi and largest inhibition zones were observed against *Fusarium oxysporum, Fusarium monoliformis* followed by *Aspergillus niger* and *Aspergillus parasiticus*[33]. In another study, *S. calva* was found to have antifungal activity against the fungi like *Fusarium oxysporum* and *Trichophyton mentagrophytes*[34].

Conclusion

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by many microbes including the resistant ones. The evaluation of *S.acmella* root extract has inhibited the different species of bacteria and fungi. Hence, it can be exploited for the development of plant based antimicrobials which holds immense potential for pharmaceutical applications. The activity of *S.acmella* root extract against bacteria and fungi clearly indicates the presence of potential antimicrobial properties which can be developed as broad-spectrum antibiotics. This study enables the use of *S.acmella* root extracts as a new choice for the treatment of infectious bacterial and fungal diseases in future.

Source of Funding: Self

Conflict of Interest: Not Applicable

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