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Research Article

IN VITRO ANTIFUNGAL ACTIVITY OF VARIOUS EXTRACTS OF LEAF AND STEM PARTS OF *SOLENA AMPLEXICAULIS* (LAM.) GANDHI

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ABSTRACT

Objective: To evaluate the antifungal properties of different alcoholic and aqueous extracts of leaf and stem parts of *Solena amplexicaulis* (Lam.) Gandhi.

Methods: *In vitro* antifungal activity was determined by using agar disc diffusion technique and the Minimum Inhibitory Concentration (MIC) was determined by using broth dilution method against nine fungal species.

Results: Among the four organic solvent and aqueous extracts, methanol extract exhibited higher antifungal activity but their effectiveness was varied. The activity was compared with the standard drug, tetracycline. The methanolic extract of stem showed significantly higher antifungal activity than the leaf part. In MIC the inhibitory effects of methanol extract of leaf and stem parts were determined to be ranging between 300 and 500µg/mL and 300-600µg/mL respectively.

Conclusion: The results clearly prove that the plant extracts have therapeutic potential for the treatment of various infectious diseases caused by fungi.

Keywords: *Solena amplexicaulis,* Antifungal activity, MIC, Disc diffusion method.

INTRODUCTION

Traditional medicine is an important source of potentially useful compounds for the development of chemotherapeutic agents. The Indian flora offers variety of plants having medicinal properties. These plants can be exploited to find out effective alternative to synthetic drugs[1]. In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and many side effects[2]. Biologically active compounds from natural sources have always been a great interest for scientists working in infectious diseases[3]. Therefore, there are increasing interests in using natural antimicrobial compounds. The antimicrobial research is geared towards the discovery and development of novel antimicrobial agents. Many plant species different families of angiosperms have been reported to show antimicrobial activity[4].

Solena amplexicaulis (Lam.) Gandhi. (Cucurbitaceae) is commonly called as creeping cucumber. The traditional healers are using the tubers, leaves and seeds of this plant for the treatment of spermatorrhoea, thermogenic, appetizer, cardiotonic, diuretics, haemorrhoids and invigorating[5]. The whole plant is a potential source of natural antioxidant[6,7] antidiabetic[8] and antibacterial agents[9]. The leaves have good antiinflammatory activity. Hence it is recommended for the treatment of inflammation, skin lesions and skin diseases[10]. Crude leaf juice is used to cure jaundice[11]. The fresh stem is externally used to promote the conception[12]. Unripe fruits are eaten to strengthen the body[13] and the decoction of the root is taken orally to cure stomachache[14] in folklore medicinal practices.

The aim of this current investigation is to evaluate the *in vitro* antifungal activity of different alcoholic and aqueous extracts of leaf and stem parts of *S. amplexicaulis* and to determine Minimum Inhibitory Concentration (MIC) of appropriate alcoholic extract of these parts against certain fungus.

MATERIALS AND METHODS

Plant material

The fresh leaf and stem parts of *S. amplexicaulis* were collected from a scrub jungle in Madukkarai, Coimbatore district, Tamil Nadu, India. Collected plant materials were washed thoroughly in tap water, shade dried and then homogenized to fine powder and stored in air tight bottles.

Preparation of extracts

About 50g of powdered plant materials were extracted (50g/250ml) in a soxhelt extractor for 8 to 10 hours, sequentially with the alcoholic solvents *viz.,* hexane, benzene, chloroform and methanol, and water. Then the extracts were evaporated to dryness.

Source of fungal strains

Nine fungal strains were tested for antifungal properties of the leaf and stem parts of the study plant which include *Aspergillus fumigatus, A. niger, Candida albicans, Paecilomyces lilacinus, Trichoderma viride, Verticillium lecanii, Mucor* sp., *Fusarium* sp. and *Penicillium* sp. All these fungal strains were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore and they were maintained at 4°C on potato dextrose agar slants for further use.

Antifungal assay

An inoculum of each of the fungal strains was suspended in 5ml potato dextrose broth and incubated at 37°C for 2 days. The antifungal activity was tested by the disc diffusion assay[15]. For this, the inoculum was spread over potato dextrose agar medium with sterile glass spreader. Small circular paper discs (5mm diameter) impregnated with known amount of each extract was placed upon the surface of the inoculated plates separately. The plates were kept at room temperature for absorption of extract in the medium and then incubated at 37°C in the incubator for 24 to 48 hrs. The antifungal activity was evaluated by measuring the diameter of inhibition zone[16]. Tetracycline was used as positive control and Dimethyl sulfoxide (DMSO) was used as negative control. Triplicates were maintained for all experiments.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined through the broth dilution method[17]. Fungi were first grown in the potato dextrose broth for 24 hrs and then the inoculum was diluted for five times (10^{-5}dilution) because to control its vigorous growth. Then each test tube was added with 1800µl of potato dextrose broth and eight different concentrations of methanolic leaf and stem extracts (100 to 800µg/mL) separately followed by inoculation of 200µl of respective fungi and kept at 37°C for 24-48hrs. The tubes were examined for visual turbidity and compared with positive control (tetracycline) and negative control (DMSO).

Statistical analysis

For *in vitro* antifungal activity of the extracts, the results were recorded as mean±standard deviation (SD) (n = 3) and subjected to one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test using SPSS (version 9, SPSS Inc., Chicago, USA). P<0.05 was chosen as the criterion for statistical significance.

RESULTS

Antifungal assay

The results of antifungal activities of the leaf and stem parts of *S. amplexicaulis* are given in Tables 1 and 2. Among the five extracts tested, methanol extract had greater antifungal potential followed by chloroform extract and then the other extracts. The maximum zones of inhibition were observed for methanolic extract against the fungi, *Aspergillus niger* (13mm) and *Mucor* sp., (13mm) exhibited by leaf extract. The methanolic stem extract highly controlled the colonial growth of *Candida albicans* and *Trichoderma viride* (zone of inhibition 20mm each). The positive control, tetracycline showed high degree of inhibition against the fungi *Aspergillus fumigatus* (35mm) and *Penicillium* sp. (35mm). Negative control (DMSO) showed no zone of inhibition. Further, it was noted that the methanolic stem extract showed greater antifungal activity than that of the leaf extract. It may be due to the presence of rich variety of appropriate secondary metabolites in the stem than leaf that can interfere the growth of fungi. The leaf extract of hexane control only *Candida albicans* (11mm) and water extract inhibit the growth of only the *Penicillium* sp.(12mm) only. On the other hand the stem extract of hexane and water showed very less inhibitory activity.

Table 1: Antifungal activity of the leaf extract of *Solena amplexicaulis* on certain fungi

Plant Extracts	Diameter of the inhibition zone (mm)								
	AF	AN	CA	PL	TV	VL	M sp.	F sp.	P sp.
Control*	35±0.9^{a}	40±0.4^{a}	10±0.5^{a}	30±0.5 ^{a}	28±0.6 ^{a}	30±0.9 ^{a}	26±0.8 ^{a}	20±0.8 ^{a}	35±0.6 ^{a}
Hexane	-	-	11±0.5^{ab}	7±0.7^{b}	8±0.8^{b}	-	-	-	-
Benzene	-	6±0.6^{b}	8±0.3^{c}	8±0.8^{bc}	10±0.7^{c}	7±1.2^{b}	8±1.0^{b}	-	7±0.8^{b}
Chloroform	6±0.7^{b}	6±1.2^{b}	8±1.2^{c}	9±0.8^{bd}	7±1.1^{bd}	8±0.5^{bc}	-	7±0.8^{b}	-
Methanol	11±0.8^{c}	13±1.1^{c}	7±1.0^{cd}	7±0.6^{b}	6±0.5^{be}	10±0.9^{d}	13±0.8^{c}	11±0.4^{c}	11±0.8^{c}
Water	-	6±0.9^{b}	-	6±1.3^{b}	-	-	7±0.6^{b}	-	12±0.9^{cd}

Values are expressed as mean±SD of three parallel measurements.

Means followed by different letter(s) in columns are significant to each other at 5% level according to DMRT.

*Tetracycline, AF-*Aspergillus fumigatus,* AN-*A. niger,* CA-*Candida albicans,* PL-*Paecilomyces lilacinus,* TV-*Trichoderma viride,* VL-*Verticillium lecanii,* M sp.-*Mucor* sp., F sp.-*Fusarium* sp. and P sp.-*Penicillium* sp.

Table 2: Antifungal activity of the stem extract of *Solena amplexicaulis* on certain fungi

Plant Extracts	Diameter of the inhibition zone (mm)								
	AF	AN	CA	PL	TV	VL	M sp.	F sp.	P sp.
Control*	35±0.9^{a}	35±1.3^{a}	16±1.1^{a}	35±0.7 ^{a}	28±0.5 ^{a}	30±1.1 ^{a}	25±1.3 ^{a}	20±1.5 ^{a}	35±1.1 ^{a}
Hexane	-	7±1.0^{b}	-	6±0.8^{b}	-	6±0.8^{b}	-	-	-
Benzene	-	7±0.3^{b}	7±1.1^{b}	7±1.2^{b}	9±0.9^{b}	6±0.7^{b}	-	-	-
Chloroform	8±1.5^{b}	7±1.1^{b}	8±0.8^{bc}	10±0.8^{d}	12±1.2^{c}	10±1.1^{c}	11±1.2b	8±0.6^{b}	8±0.5^{b}
Methanol	11±0.9^{c}	10±1.5^{c}	20±1.4^{d}	8±0.5^{cd}	20±0.2^{d}	9±1.1^{cd}	18±1.0^{c}	12±1.1^{c}	13±0.7^{c}
Water	-	-	7±0.5^{b}	-	7±1.5^{e}	6±1.2^{b}	7±1.1^{d}	8±1.0^{b}	-

Values are expressed as mean±SD of three parallel measurements.

Means followed by different letter(s) in columns are significant to each other at 5% level according to DMRT.

*Tetracycline, AF-*Aspergillus fumigatus,* AN-*A. niger,* CA-*Candida albicans,* PL-*Paecilomyces lilacinus,* TV-*Trichoderma viride,* VL-*Verticillium lecanii,* M sp.-*Mucor* sp., F sp.-*Fusarium* sp. and P sp.-*Penicillium* sp.

Table 3: Minimum inhibitory concentration (MIC) of methanolic extract of leaf and stem parts of *Solena amplexicaulis* on certain fungi.

Plant parts	Minimum inhibitory concentrations (µg/mL)								
	AF	AN	CA	PL	TV	VL	M sp.	F sp.	P sp.
Leaf	300	300	400	300	400	300	500	300	400
Stem	300	300	400	300	300	300	500	300	600

AF-*Aspergillus fumigatus,* AN-*A. niger,* CA-*Candida albicans,* PL-*Paecilomyces lilacinus,* TV-*Trichoderma viride,* VL-*Verticillium lecanii,* M sp.-*Mucor* sp., F sp.-*Fusarium* sp. and P sp.-*Penicillium* sp.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was defined as the lowest concentration of the extract inhibiting the visible growth of each microorganism[18]. Due to high antifungal activity of methanolic leaf and stem extracts, the MIC was determined for methanol extracts only (Table 3). The MIC values are ranging between 300 and 500µg/mL for leaf and 300 and 600µg/mL for stem extract against the tested fungi.

DISCUSSION

Plants can produce antifungal compounds to protect themselves from biotic attack that could be essential for fungi resistance[19]. Search of natural fungicide from the plant sources would definitely be a better alternative to the hazardous chemicals. Successful prediction of biological compounds from plant materials are largely dependent on the type of solvent used in the extraction procedure. This study revealed that generally the inhibitory activity is pathogen specific and depends on the solvent, concentration of the crude drug, temperature, plant parts used for the extraction of secondary metabolites and rate of diffusion[20]. The antifungal activity was higher in the methanol extracts against all the tested fungi. It may further indicates that the antimicrobial principles/chemical constituents which are either polar or non polar can be effectively extracted only through the organic solvent medium[21,22,23]. Many early studies also reported the effective inhibitory activity of

alcoholic solvents against the growth of the pathogenic fungi[24,25]. The hexane and water showed very less inhibitory effect which might be attributed to the extracting capacity of solvent and the concentration of the active ingredients in the extracts[26] and also most of the active ingredients are dissolved better in alcoholic solvents than in water[27]. Several workers have reported that water extracts do not have much activity against fungi[28,29,30]. Cucurbitaceae family contains more bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids[31] and also many early studies reported that the member of Cucurbitaceae showed more pronounced antifungal activity[32,33,34]. The overall results of the study revealed that the crude extract of the study plant contain certain constituents with significant antifungal property but their effectiveness varied.

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