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Research Article

IN VITRO STUDIES ON ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF *SCAEVOLA TACCADA*

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ABSTRACT

Medicinal plants find an enormous application in developing antimicrobial drugs that can substitute synthetic microbicides. The current study was carried out to investigate the antibacterial, antifungal activities and phytochemical components of leaf extracts of *Scaevola taccada*. The leaves were extracted using solvents of varying polarities. Among various solvent extracts studied, methanol leaf extract showed the highest antibacterial activity and hence was used for further analysis. The extract also showed significant antifungal activity. The phytochemical screening of the extract indicated the presence of carbohydrates, proteins and phenols.

Keywords: *Scaevola taccada*, Antibacterial, Antifungal, Phytochemical screening.

INTRODUCTION

Plants constitute the basis of traditional medicine systems that have been in existence for number of years and continue to provide mankind with new therapeutics. Many plant extracts and essential oils isolated from plants have been shown to possess biological activity in vitro and in vivo. This justifies research on plant based medicine and focuses on the characterization of antimicrobial activity of these plants[1,2]. Medicinal uses of plants range from the application of the roots, barks, stems, leaves and seeds to the use of extracts from the plants[3]. These plant extracts are a source of many potent and powerful drugs.

Scaevola taccada has been used in various traditional medicines. The crushed fruit of *S.taccada* was used by early settlers to treat tinea[4]. The leaves were used for indigestion. They were also used to cure headache[5]. In addition there are also reports indicating the use of leaf decoction and the flesh of the seeds as a contraceptive[6]. The juice from ripe fruit has been used to treat sores and infected eyes whereas a combination of juices from ripe fruits and stem has been used as a remedy for bites and stings[7]. A mixture of pounded root bark with salt is used for cuts and skin diseases[8]. In Indonesia the root is used as an antidote when poisonous fish and crabs are consumed[5].

The present study determines the antibacterial and antifungal activities through in vitro methods and investigates the phytochemical constituents in the leaf extract of *Scaevolus taccada.*

MATERIALS AND METHODS

Plant collection and identification

Fresh plants of *Scaveolus taccada* were collected from the fields located in Coimbatore, India and identified by the Department of Plant Biology and Plant Biotechnology, Meenakshi College for Women, Chennai, India.

Maintenance of culture

Bacterial pathogens were maintained on nutrient agar. The bacterial strains used in this study were obtained from Microbial Metabolite Lab culture collection, Centre for Advanced Studies (CAS) in Botany, University of Madras, Guindy campus, Chennai, India. Bacterial strains used in this study were *Vibrio cholerae, Klebsiella pneumoniae, Salmonella typhi and Shigella sonnei.* Fungal strains of *Fusarium oxysporum, Fusarium solani, Rhizoctonia solani* and *Odium monilioides* were used for the antifungal activity assays.

Preparation of *S. taccada* leaf extract

The leaves were washed, shade dried, ground into powder and stored in room temperature.

Direct extraction with different solvents

Direct extraction with hexane, ethyl acetate and methanol following the method of Eloff [9] was used as an extraction method for the purpose of preliminary screening of the *S. taccada* leaves.

In this method, finely ground material (5 gm) was extracted with 50 ml of hexane, ethyl acetate and methanol in conical flask in shaking condition. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/ml solutions ready for further analysis.

Well diffusion assay

Agar diffusion (Well diffusion) assay[10] is used widely to determine the anti-bacterial activity of crude extract. The technique works well with defined inhibitors. Nutrient agar prepared was poured in the Petri dish. 24 hours growing culture (*V.cholerae, K.pneumoniae, S.typhi* and *S.sonnei*) were swabbed on it. The wells (10mm diameter) were made by using cork borer. The different concentrations (250, 500, 750, 1000µg) of the crude extracts were loaded in the wells. The plates were then incubated at 37^{0}C for 24 hours. The inhibition diameter was measured.

Poison plate method

Antifungal activity of leaves was determined by food-poisoned technique[11]. Extract concentration (1000µg) was mixed with sterilized PDA (Potato Dextrose Agar) medium in separate flasks, and transferred into Petri plates. The media was allowed to solidify. The seven day old fungal culture disk (*Fusarium oxysporum, Fusarium solani, Rhizoctonia solani* and *Odium monilioides)* of 10 mm diameter was taken and inoculated to the center of Petri plates containing leaf extracts in aseptic condition. PDA medium, without leaf extracts served as control. All plates were incubated at 28 + 2°C and radial growth of colony was measured after seven days of incubation. Each test was performed in triplicate.

Phytochemical screening of *S. taccada* crude extract

The different qualitative chemical tests were performed for establishing the profile of the given extract for its chemical composition. The following tests were performed on the extract to detect various phytoconstituents present in it.

Detection of alkaloids[12]

Solvent free extract (50mg) was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows:

Mayer's test[12]

To a few ml of the filtrate, a drop of Mayer's regent was added by the sides of the test tube. Absence of white creamy precipitate indicated the test as negative.

Detection of carbohydrates and glycosides[13]

The extract (100mg) was dissolved in 50ml of water and filtered. The filtrate was subjected to the following tests:

Fehling's test

One ml of the filtrate was boiled on water bath with 1ml each of Fehling's solution I and II. A red precipitate indicated the presence of sugar.

Benedict's test

To 0.5ml of the filtrate, 0.5ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A red precipitate indicated the presence of sugar.

Detection of glycosides

50 mg of the extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following test:

Borntrager's test[12]

To 2ml of the filtrate hydrolysate, 3ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicated the presence of glycosides.

Detection of saponins

Foam test[14]

The extract (50mg) was diluted with distilled water and made up to 20ml. The suspension was shaken in a graduated cylinder for 15 minutes. Absence of 2cm layer of foam indicated the test as negative.

Detection of proteins and amino acids[15]

The extract (100mg) was dissolved in 10ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to following tests:

Millon's test[16]

To 2ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicated the presence of proteins.

Biuret test[17]

An aliquot of 2ml of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

Detection of phenolic compound

Ferric chloride test[18]

The extract (50 mg) was dissolved in 5ml of distilled water. To this, a few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

RESULTS AND DISCUSSION

Medicinal plants have always had an important place in the therapeutic armoury of mankind[19]. They form the backbone of traditional medicine and the beneficial activity of plant extract on human welfare is due to the presence of bioactive compounds in the extract[20]. Some of these compounds may exert their activity by acting as antimicrobial agents. Almost all the antibacterial agents, isolated from the plants are aromatic or saturated organic compounds, they are most often obtained initially by ethanol or methanol extraction[21, 22, 23].

The present study utilizes three different solvents *viz*. hexane, ethyl acetate and methanol to determine the most suitable solvent for extracting effective antimicrobial compounds from the leaves of *S.taccada*. The methanol extract showed effective inhibitory action from 500µg concentration onwards and it was maximum at 1000µg as shown in Fig. 1. The extract was effective against all the four bacterial strains namely *V.cholerae, K.pneumoniae, S.typhi* and *S. sonnei* with zone of inhibition of 11 mm, 15mm, 17mm and 16mm respectively. The effect was maximum against *S.typhi.* The ethyl acetate extract was effective against all the four bacterial strains, only from 750µg concentration onwards, the highest being against *K.pneumoniae* (Fig. 2). The hexane extract showed minimum inhibitory effect compared to the other extracts in which the maximum was against *K.pneumoniae* (Fig. 3). The methanol extract effectively inhibited all the test pathogens when compared with ethyl acetate and hexane extracts. The methanol extract was chosen for further studies because of its high inhibitory activity.

From the poison plate method, it was inferred that the methanolic leaf extract exhibited inhibitory effects against all the four fungi, *Fusarium oxysporum, Fusarium solani, Rhizoctonia solani* and *Odium monilioides* (Fig. 4).

The preliminary phytochemical screening of *S. taccada* revealed the presence of proteins, phenols, carbohydrates and glycosides significantly. Alkaloids and saponins were completely absent as presented in Table1.

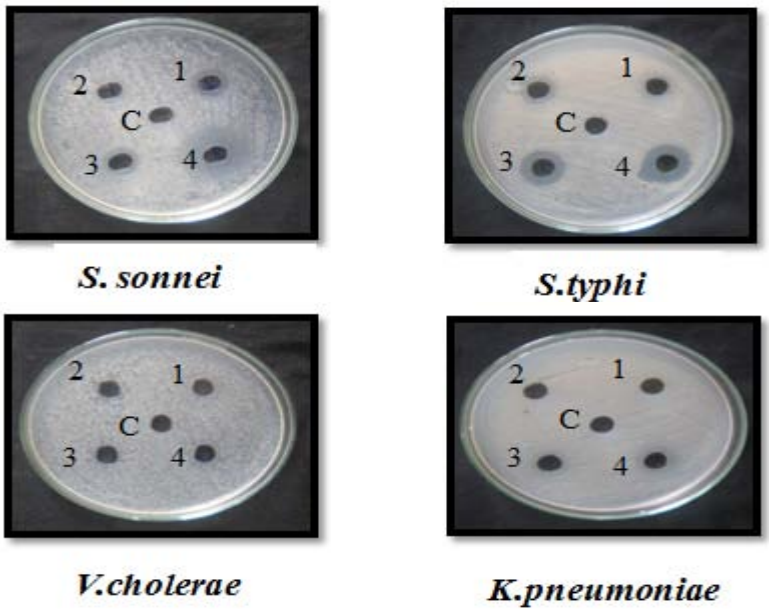

Fig. 1: Anti-bacterial effect of methanol extract of *S.taccada* leaves by Well diffusion assay. C-Control, 1-250 µg, 2-500 µg, 3-750 µg, 4-1000 µg extract.

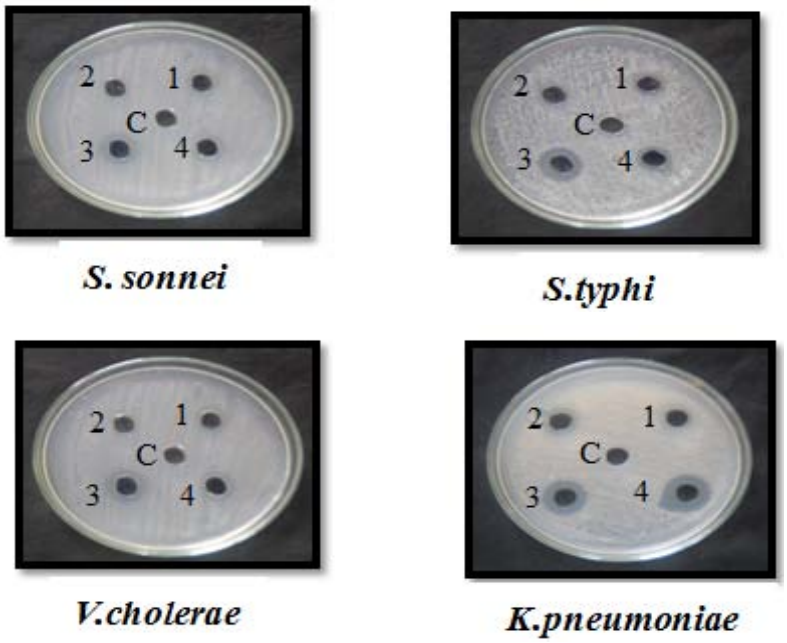

Fig. 2: Anti-bacterial effect of ethyl acetate extract of *S.taccada* leaves by Well diffusion assay. C-Control, 1-250 µg, 2-500 µg, 3-750 µg, 4-1000 µg extract.

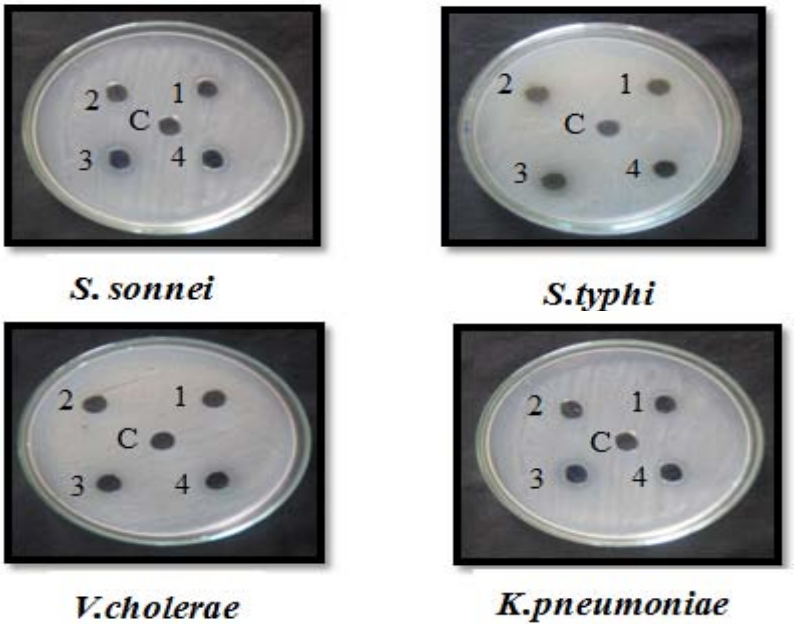

Fig. 3: Anti-bacterial effect of hexane extract of *S.taccada* leaves by Well diffusion assay. C-Control, 1-250 µg, 2-500 µg, 3-750 µg, 4-1000 µg extract.

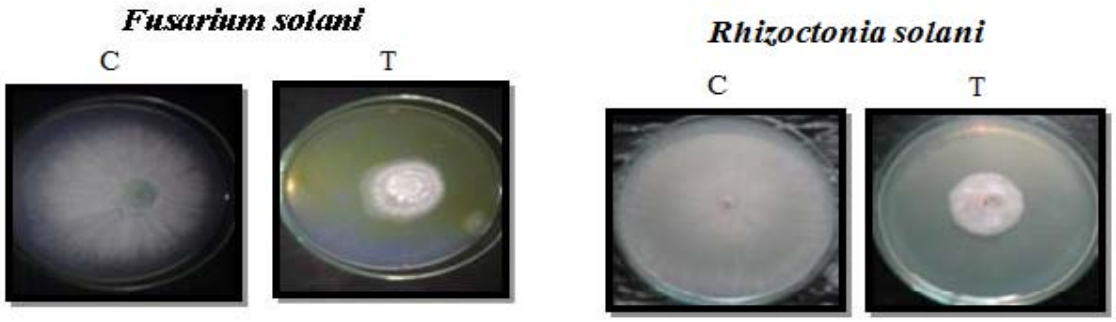

Fig. 4: Anti-fungal effect of methanol extract of *S.taccada* leaves by Poison plate method. C-Control, T-Test

Table 1: Phytochemical screening of methanol extract of *S.taccada* leaves

S. No.	Compound	Test	Result
1	Alkaloids	Mayer's test	-
2	Carbohydrates and glycosides	Fehling's test	++
		Benedict's Test	++
3	Glycosides	Borntrager's test	++
4	Saponins	Foam test	-
5	Proteins and amino acids	Biuret test	+++
		Millon's test	+++
6	Phenolic compounds	Ferric chloride test	++

- = Negative (absent), + = Positive (present)

Nowadays, medicinal plants are being sought for their medicinal value, as antioxidants and as antimicrobials. The results of the present investigation clearly indicate that the leaf extract of S.taccada exhibits maximum antibacterial and antifungal activity which can be used further for its medicinal properties. The study can be continued to explore the identity and structure of the bioactive compounds accountable for the observed pharmacological activities.

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