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Research Article

Study of Bactericidal Potency of *Smilax glabra* Rhizome

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ABSTRACT
Smilax glabra rhizome is well known traditional medicine and widely used throughout the world for its therapeutic use in a wide range of ailments. The present work was aimed to determine the antibacterial potential of this plant. The two Gram positive and two Gram negative bacteria were used to evaluate the antibacterial activity of methanol, chloroform, n-hexane and acetonitrile extract of the rhizome of *S.glabra* and it revealed that it inhibits the Gram positive organism namely *Staphyllococcus aureus* and *Bacillus subtilis* while Gram negative organism like *Escherichia coli* and *Salmonella typhi* were found to be resistant to all the extracts.

Keywords: *Smilax glabra* Rhizome, antibacterial, ailment, resistance.

INTRODUCTION

Among Asian countries India is blessed with the immense biodiversity which includes plants, animals, microbes etc. In the present era where scientists are more interested in synthesizing and increasing the number of new drugs, some scientists are also showing interest in plant based medicines. Extracts of plants belonging to 157 families have been reported to be microbiologically active. Medicinal plants have been used to cure a number of diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms [1]. Antibacterial properties of various plant parts like root, stem, leaves, seeds, flowers, fruits etc.have been well documented for some of the medicinal plants for the past two decades[2] . Increase in global antibiotic resistance of pathogenic bacteria, fungi and protozoa have geared up interest of researchers to investigate different sources for apparent antibiotics discovery. Emerging and re-emerging infections and diseases are continuously posing threat to human existence[3]. Plant contains many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, resins, phenol and flavonoids which are deposited in their specific part such as leaves, flowers, bark, seeds fruits, root etc[4]. In Asian continent the antimicrobial properties of plants is been increasingly reported from few decades.[5, 6]

The genus Smilax (Liliaceae) includes about 300 species and is widely distributed in tropical and temperate regions throughout the World, especially in East Asia and North America.[1]*Smilax glabra* rhizome is well known traditional Chinese medicine, which is used clinically. Rhizome of *Smilax glabra* has many pharmacological activities such as anticancer, antidiabetic and in the treatment of jaundice.

The study was therefore undertaken to evaluate the antimicrobial activity of *Smilax glabra* rhizome extracts (methanol, chloroform, n-hexane and acetonitrile) of *S.glabra* were prepared and evaluate for their antimicrobial activity against two Gram positive and two Gram negative bacteria.

MATERIALS AND METHODS

Plant material

Smilax glabra rhizome was obtained from the local market from Dadar, Mumbai and was identified by the expert taxonomist.

Chemical and Reagents

Standard antibiotics Ciprofloxin and clotrimazole were purchased from chemist. Analytical grade solvents purchased from Merck India were used in this study.

Preparation of Extract

The extracts of Smilax glabra rhizome powders were separately prepared in methanol, acetonitrile, chloroform and n- hexane (10 mg/ml). Briefly 10 milligrams of the plant powders was accurately weighed and suspended in 1 ml of organic solvents (methanol, acetonitrile, chloroform and n- hexane). The mixture was allowed to stand for 6 to 8 h and then filtered through Whatmann filter paper no.1. The filtrate was evaporated to dryness and the residue obtained was stored at 4^{o}C.

Microorganisms

The antimicrobial activities of the extracts were tested individually against Gram positive and Gram negative bacterial strains (Table 1). The bacterial strains were obtained from NCIM, (NCL) Pune and clinical isolates.

Bacterial susceptibility testing

In order to assess the antimicrobial activity of different extracts of *S.glabra* the residue was reconstituted in 0.5 ml

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Table 1: Test bacteria used to test the Bioassay

Sr. No.	Microorganism	Grams character	NCIM No.
1.	*Bacillus subtilis*	Gram positive	Clinical isolate
2.	*Staphylococcus aureus*	Gram positive	5021
3.	*Escherichia coli*	Gram negative	2256
4.	*Salmonella typhi*	Gram negative	Clinical isolate

Table 2: Antimicrobial Activity of *Smilax glabra*

Gram character	Organisms	Methanol	Chloroform	n-hexane	Acetonitrile
Gram positive	*S.aureus*	12 mm	10 mm	-	-
	B.subtilis	7 mm	-	8 mm	-
Gram negative	*E.coli*	-	-	-	-
	S.typhi	-	-	-	-

Key '–' = No inhibition; Zone of inhibition (mm) includes zone diameter of disc = 5 mm

of dimethyl sulphoxide (DMSO) it does not show any antimicrobial activity. Under sterile conditions, molten nutrient agar was prepared and poured into the petriplates. The plates were allowed to cool till the medium was solidified. The bacterial culture was inoculated on the surface of agar by sterile glass spreader then was allowed to dry. Small paper discs impregnated with test extracts were placed upon the surface of an inoculated plate. The plates were kept in the incubator at 37^0C for 24 h. The plates were then observed for any zones of inhibition surrounding the discs. The bioassay was done in triplicate and the average value was taken as zone of inhibition.[7] The zone diameter of the disc used for the study was 5 mm. The standard antibiotic amphicillin also tested against the test bacteria used in the study.

RESULTS AND DISCUSSION

The ability of the test substances to inhibit bacterial growth is indicated by the appearance of a zone of inhibition around the disc containing the test solution. After specified incubation period, the agar plates were examined for growth. First, the positive control without *Smilax glabra* rhizome extract was checked to ensure that each test strain was capable of providing adequate growth. The negative control was checked for the absence of growth thereby indicating the sterility of the medium. The remaining plates were examined for the presence or absence of growth.

The results of antibacterial assay clearly shows that the methanol extract of *S.glabra* showed the zone of inhibition against the Gram positive bacteria namely *S.aureus* (12mm) and *B.subtilis* (7mm) while chloroform extract gave activity against only *S.aureus* and n- hexane extract against *B.subtilis*.

In contrast to this the Gram negative bacteria were found to be resistance to all the solvent extract (Table-2). Solvents of varying polarity were used in preparing the extracts of *Smilax glabra* rhizome which showed varying effects on the test organisms. The standard antibiotic amphicillin 1000 µg/ml showed the inhibition against the test organisms. The presence of bioactive antibacterial compound in *S.glabra* was found in this order of potency methanol > chloroform > n-hexane.

Our study of antibacterial effect can be supported by the reported research work on medicinal plants which justifies the results as it was observed that Gram positive bacteria were more susceptible to inhibition by plant extract as compared to Gram negative bacteria .This may be due to the morphological differences between the two .[8]

In Gram negative organisms, the presence of lipopolysaccharides layer may prevent the diffusion of extract to the peptidoglycan layer of the cell wall leading to the résistance of these organisms towards most of the extracts.

The work done by previous researchers [9,10,11,12] reveals that the antimicrobial activity of the plant extracts is due to the presence of bioactive compound. Isolation of individual active compounds and finally subjecting them to trials, promises to open new avenues in the use of plants for therapeutic purpose.

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