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# Antimicrobial and antioxidant activity of Smilax perfoliata Lour.

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## ABSTRACT

The medicinal plants have catered to healthcare needs of man since the beginning of human civilization. It is, therefore, of great interest and significance to assess the potential of plants as antimicrobials and antioxidants such that their use in folk medicine is substantiated with scientific data and their use may be continued for the benefit of man and of the society as a whole. Smilax perfoliata Lour. is widely used in traditional cuisine by many tribes of North- east India. The present study was conducted to evaluate the antimicrobial and antioxidant activity of the leaves of Smilax perfoliata in order to validate its use in traditional cuisine and medicine. The ethanol extract of S. perfoliata leaves showed the presence of- alkaloids, flavonoids, phenolics and tannins, steroids, cardiac glycosides, anthraquinone glycosides and saponins. In addition, the plant inhibited bacteria and fungi and also exhibited antioxidant activity. The present work substantiates the biological activities of the plant and therefore, encourages its use as per the available ethnobotanical knowledge. Further, studies are required to isolate and identify the bioactive principle (s) from the plant.

Keywords: Smilax perfoliata, Antimicrobial activity, Antioxidant activity, IC<sub>50</sub>

## INTRODUCTION

The medicinal plants have catered to healthcare needs of man since the beginning of human civilization. Pathogenic microorganisms have claimed and continue to claim the lives of millions worldwide and hence, adversely affect the global economy. Modern day synthetic drugs and antibiotics fail to effectively counteract infectious diseases due to development of drug- resistance in the causative microorganims. This situation is further compounded by the severe side-effects of these synthetic drugs. The researchers, therefore, have resorted to search for antimicrobial compounds from natural sources, especially, plants. These natural antibiotics have been in use in the form of ethnomedicine and are bio-compatible. In addition to natural antimicrobials, natural antioxidants, of late, have attracted the interest of the research community for use in foods and medicinal materials to replace synthetic antioxidants which are being restricted due to their carcinogenicity. Antioxidants are known to play an important role in maintaining human health by antagonizing the ill-health effects of the reactive oxygen species (ROS) and free radicals generated during metabolic processes. It is, therefore, of great interest and significance to assess the potential of plants as antimicrobials and antioxidants such that their use in folk medicine is substantiated with scientific data and their use may be continued for the benefit of man and of the society as a whole.

*Smilax* (Family - Smilacaceae) is a large genus of climbing shrub distributed in tropical and temperate regions of the world. *Smilax perfoliata* Lour. is found in various parts of India and has tuberous rhizomes [1]. It is a robust more or less strongly armed climber. Stem is used as toothbrush to strengthen the gums. Tender shoot is taken in curries and is useful as blood purifier [2]. Roots and stems are used as anticancer, anti-dysenteric and in urinary complaints [3]. The plant is also widely used in traditional cuisine by many tribes of North- east India. The present study was conducted to evaluate the antimicrobial and antioxidant activity of the leaves of *Smilax perfoliata* in order to validate its use in traditional cuisine and medicine.

## MATERIALS AND METHODS

#### **Chemicals and Equipment:**

All the chemicals used in the study were of analytical grade. Dimethyl Sulfoxide (DMSO), Ciprofloxacin and 2, 2-Diphenyl-1- picrylhydrazyl (DPPH), and other general purpose laboratory chemicals and reagents were procured from Merck Specialities Pvt. Ltd, Mumbai, India. Microbiological media were obtained from HiMedia, Mumbai, India.

#### **Preparation of Plant Extracts:**

The leaves were collected locally and processed. The cleaned and shade dried material was ground into fine powder using electric blender. Plant extract was prepared by cold maceration method. Dried powder was extracted by macerating in ethanol (1: 20 w/v) for 48 hours with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper into pre-weighed beakers and was dried in a rotatory vacuum evaporator (IKA RV 10 Digital) until a constant dry weight of each extract was obtained. The residues were stored aseptically at 5°C for further use.

#### Qualitative phytochemical analysis:

Qualitative phytochemical analysis was performed for alkaloids, saponins, flavonoids, phenols and tannins, sterols, cardiac glycosides and anthraquinone glycosides by following the standard methods [4-6].

## In vitro Antibacterial Activity Assay:

#### Test organisms

The ethanol extract of the plant was screened against 8 bacterial strains, four of which were Gram positive and four Gram negative. The test organisms were *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96, *Proteus mirabilis* MTCC 1429, *Bacillus cereus* MTCC 430, *Escherichia coli* MTCC 739, *Salmonella enterica* serv. *typhi* MTCC 3917, *Pseudomonas aeruginosa* MTCC 1688, *Staphylococcus epidermidis* MTCC 435 and *Candida albicans* MTCC 3017. The test strains were obtained from the IMTECH, Chandigarh, India.

#### **Preparation of inoculum**

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of culture from the stock to test tubes of nutrient broth and incubating for 24 hours at 37°C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 0.5 McFarland standard which corresponded to a cell density of  $10^6$  CFU mL<sup>-1</sup>.

#### Antibacterial susceptibility test

The agar well diffusion method [7, 8] was used to determine the antimicrobial activity of the extract. *In vitro* antibacterial activity was screened by using nutrient agar obtained from Himedia (Mumbai). The plates were prepared by pouring 25 ml of molten media into sterile petri-plates (diameter 100 mm). The plates were allowed to solidify at room temperature and 100  $\mu$ L inoculum suspensions was spread uniformly with the help of a sterile glass spreader after which four 6 mm diameter wells were bored into the medium with the help of a sterile glass well borer. The plant extract was dissolved in DMSO to obtain a final concentration of 200 mg/mL and 100  $\mu$ L of it was loaded into one of the wells. The extract was allowed to diffuse for 45 minutes at room temperature, after which the plates were transferred for incubation at 35°C. After 24 hours of incubation, the inhibition zones around the wells were measured. The experiment was performed in triplicate and mean and standard deviation were calculated. Ciprofloxacin (10  $\mu$ g/ml) was used the standard drug while DMSO and distilled water were used as negative control.

#### In vitro Antioxidant Activity Assay:

The free radical scavenging activity of the extract was measured in vitro by 1, 1- diphenyl-2-picrylhydrazyl (DPPH) assay [9]. A 0.30 mM solution of DPPH was prepared in DMSO and 1 mL of this solution was added to 3.0 mL of the different concentrations of the plant extract dissolved in DMSO. The mixture was incubated at room temperature for 30 min and the absorbance was measured at 517 nm against blank (SHIMADZU UV-1800 Spectrophotometer, Japan). Reaction mixture without test sample served as control. The percentage scavenging was calculated by the following equation:

% inhibition = 
$$\frac{Absorbance_{Control} - Absorbance_{Test} \times 100}{Absorbance_{Control}}$$

 $IC_{50}$  was defined as the concentration of the extract required to achieve 50% inhibition of DPPH radicals. The experiment was performed in triplicate and  $IC_{50}$  was expressed as mean  $\pm$  standard deviation. Ascorbic acid was used as the standard.

#### RESULTS

The ethanol extract of *S. perfoliata* leaves showed the presence of important secondary metabolites like- alkaloids, flavonoids, phenolics and tannins, steroids, cardiac glycosides, anthraquinone glycosides and saponins (Table 1).

The extract exhibited antibacterial activity against six out of the eight bacterial strains and also inhibited the fungi-*C. albicans* (Table 2). The zone of inhibition ranged from  $10 \pm 0 \text{ mm}$  (*S. epidermidis*) to  $13 \pm 1 \text{ mm}$  (*P. mirabilis* and *B. cereus*). Maximum activity was observed against *P. mirabilis* and *B. cereus*. The negative control did not inhibit the test strains.

The extract was observed to scavenge DPPH free radicals in a concentration dependent manner (Figure 1). IC<sub>50</sub> for the ethanol extract of *S. perfoliata* leaves was obtained to be 110.35  $\pm$  8.35 µg/mL while that for ascorbic acid was observed to be 4.19  $\pm$  0.01 µg/mL.

Extract	Alkaloids	Saponins	Flavonoids	Phenols and Tannins	Steroids	Cardiac glycosides	Anthraquinone glycosides
Ethanol	+	+	+	+	+	+	+

Table 1: Qualitative phytochemical analysis of extracts of S. perfoliata leaves

Tost Organism	Zone of Inhibition (in mm)					
Test Organism	Ethanol	Standard Drug				
Bacillus subtilis	11 ± 1	$28 \pm 1$				
Staphylococcus aureus	-	$28 \pm 1$				
Proteus mirabilis	$13 \pm 1$	$26 \pm 1$				
Bacillus cereus	$13 \pm 1$	$19 \pm 1$				
Escherichia coli	-	$27 \pm 1$				
Salmonella enterica serv.typhi	$11 \pm 0$	$27 \pm 1$				
Pseudomonas aeruginosa	$11 \pm 1$	$22 \pm 1$				
Staphylococcus epidermidis	$10 \pm 0$	$28 \pm 1$				
Candida albicans	$11 \pm 1$	$17 \pm 0$				
Results expressed as mean $\pm$ sd of three replicates						
- = No activity						

Table 2:	Antimicrobial	activity	of S.	perfoliata	leaves
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Figure 1: Antioxidant activity of S. perfoliata

#### DISCUSSION

Approximately 50, 000 people die every day worldwide due to infectious diseases [10]. Development of drug resistance, in the recent years, has rendered the current treatment strategies ineffective against most of the human pathogenic microorganisms. In order to meet up to the challenge of antibiotic resistance, researchers have resorted to medicinal plants in search of noble and effective drug molecules. Medicinal plants have been used extensively in

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traditional healthcare practices for their therapeutic potentials but substantial scientific data pertaining to their biological activities is still lacking. In the present study, the diffusion assay showed that the ethanol extract of S. perfoliata leaves was active against 3 Gram positive, 3 Gram-negative bacteria, and the fungus- Candida albicans. However, the plant did not showed inhibitory activity against S. aureus and E. coli. Various workers have already shown that Gram positive bacteria are more susceptible to plants extracts as compared to Gram negative ones [11, 12]. These differences have often been attributed to the differences in the bacterial cell wall. In Gram positive bacteria, the cell wall is single layered whereas in the Gram negative bacteria, it is multi-layered [13, 14]. The inhibitory activity of the plant extract was observed to be lower than the standard drugs, but still the extract in its crude form showed in broad spectrum activity by inhibiting both gram positive and gram negative bacteria and fungi. In addition to antimicrobial agents, natural products with antioxidant activity are used to aid the endogenous protective system. The antioxidants available in human diets are of great importance as potential protective agents to counteract oxidative damage to the body. In the present study, the ethanol extract of S. perfoliata showed promising antioxidant activity by scavenging DPPH radical. DPPH method is a rapid, simple and inexpensive method to measure antioxidant capacity of foods [15]. This assay has been used widely to quantify the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of complex biological systems. This method is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. The antioxidant and antimicrobial activity of S. perfoliata leaves may be due to the presence of various phytochemicals which are detected during the study. Phenolics, flavonoids and tannins are reported to exert a wide spectrum of biological effects such as- antioxidant and free radical scavenging activity and antimicrobial activity [16, 17, 18]. Alkaloids are reported to be antipasmodic, analgesic and also have bactericidal effects [18]. Many pharmacological activities such as antibiotic, antifungal, antiviral, hepatoprotective, antiinflammatory and anti-ulcer activities have been reported for saponins [19]. Steroids have been reported to exert analgesic properties [20] while cardiac and anthraquinone glycosides are reported to have antibacterial and antifungal activity[21, 22].

#### CONCLUSION

The present study has shown that *S. perfoliata* is a potent antimicrobial and antioxidant agent. Thus, the present work substantiates the biological activities of the plant and therefore, encourages its use as per the available ethnobotanical knowledge. Further, studies are required to isolate and identify the bioactive principle (s) from the plant.

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