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Original Article

# ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *SOPHORA INTERRUPTA* BEDD

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**Abstract**

In the present study we report the antimicrobial and antioxidant activities of leaf extracts of *Sophora interrupta*. Different solvent extracts were prepared using methanol, acetone and hexane. Methanolic extract of *S. interrupta* (MES) showed effective antimicrobial activity against both bacterial and fungal pathogens compared with acetone extract of *S. interrupta* (AES) and hexane extract of *S. interrupta* (HES). MES formed maximum inhibition zone against bacteria *B. subtilis* (8.2 mm) and fungus *Candida albicans* (7.3 mm). AES showed maximum inhibition zone against bacteria *S. aureus* (6.8 mm) and fungus *Aspergillus niger* (6.4 mm). HES displayed maximum inhibition zones of 4.3 and 4.6 mm against bacteria *B. subtilis* and fungus *C. albicans*. Both MES and AES also showed effective antioxidant activity by showing radical scavenging activity against DPPH and $H_2O_2$ free radicals. Fourier transform infra red (FTIR) spectrum analysis revealed that the secondary metabolites present in the extract such as terpenoids, saponins, flavonoids, polyphenolics and proteins were responsible for the bioactivities of *S. interrupta*.

**Keywords** *S. interrupta,* Methanolic extract, Antimicrobial activity, Antioxidant activity and FTIR

## INTRODUCTION

In recent years, management of diseases by plants and its derivatives gaining importance due to side effects posed by synthetic drug candidates. The World Health Organization (WHO) has estimated that 70% of world's population depends on traditional medicine for their primary health care needs, and this traditional medicine involves the use of plant extracts and their active components [1]. Increasing trends of microbial resistance to antibiotics and various chronic and degenerative pathologies of humans caused by reactive oxygen species (ROS) have triggered the search for bioactive compounds from plants with alternative mechanisms of action to counteract pathogenic microbes and natural antioxidants capable of protecting the body against oxidative stress and free radical-induced damage [2,3]. The plant kingdom harbours enormous amounts of therapeutic agents that have diverse applications in the pharmaceutical, nutraceutical and agrochemical industries. The active principles responsible for the therapeutic effects of medicinal plants are phytochemicals, usually secondary metabolites, including but not limited to alkaloids, steroids, flavonoids, terpenoids and tannins [4].

During stress more reactive oxygen species (ROS) such as superoxide anion radicals, hydroxyl radicals and hydrogen peroxide are produced in the body than enzymatic antioxidants such as glutathione peroxidase, superoxide dismutase (SOD) and catalase and non-enzymatic antioxidants such as glutathione, tocopherol (vitamin E), ascorbic acid (vitamin C), carotenoids, and flavonoids. This imbalance leads to cell damage [5, 6]. A lack of antioxidants, that can quench reactive free radicals, leads to degenerative diseases (Shahidi et al., 1992), including inflammation, cances, cardiovascular and neurodegenerative disorders. It is believed that, plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [7,8].

*Sophora interrupta* belongs to the family Fabaceae (Leguminaceae, Papilonaceae) which is commonly called as Edwariamadarasapatna. There are more than hundreds of species belongs to this family which have various pharmacological activities such as anti-cancer, anti-inflammatory, antispasmodic, antibacterial. From the preliminary phytochemical studies it was identified that it has constituents like alkaloids, flavonoids, glycosides, phenols, carbohydrates and proteins [7-9].

The present study reports the antimicrobial and antioxidant activity of the leaf extracts of *S. interrupta*.

## MATERIALS AND METHODS

### Antimicrobial activity

Antibacterial activity of the leaf extracts was checked against *Staphylococcus aureus, Bacillus subtilis* (Gram+ve bacteria) and *Klebsiella pneumonia, Escherichia coli* (Gram-ve bacteria) by employing disc diffusion assay according to the method of Cruickshank et al (1968) [10] . 2.8% of nutrient agar (NA) medium was prepared by dissolving 28 g of NA in 1000 ml of sterile distilled water. Autoclaved the NA medium and poured in sterile petridishes. Petridishes were transferred to laminar air flow (LAF) and the medium was allowed to solidify. After solidifying the medium, 200 µl of bacterial inoculum was spreaded on the medium. Paper discs were made using Whatman no.1 filter paper. Each NA plates consisting of three sterile paper discs. One disc containing 50 µl of leaf extract, second disc comprises solvent DMSO was used as negative control and third disc with standard antibiotic (Streptomycin) was used positive control.

Antifungal activity of the leaf extracts was checked against *Aspergillus niger, Candida albicans* and *Candida nonalbicans*. 2.4 % of potato dextrose agar (PDA) medium was prepared by dissolving 24 g of PDA in 1000 ml of sterile distilled water. Autoclaved the PDA medium and poured in sterile petridishes. Petridishes were transferred to laminar air flow (LAF) and the medium was allowed to solidify. After solidifying the medium, 200 µl of fungal inoculum was spreaded on the medium. Paper discs were made using Whatman no.1 filter paper. Each PDA plates consisting of three sterile paper discs. One disc containing 50 µl of leaf extract, second disc comprises solvent DMSO was used as negative control and third disc with standard fungicide (Voriconazole) was used positive control.

### Antioxidant activity

Antioxidant activity of the leaf extracts was checked by free radical scavenging assays which including DPPH and $H_2O_2$ radical scavenging assays.

### DPPH radical scavenging assay

DPPH (2, 2 diphenyl 2 picryl hydrazyl hydrate) radical scavenging activity of different extracts was determined according to the method described by rammohan et al [11]. DPPH reacts with antioxidant that can donate hydrogen, and reduce DPPH. The change in color (from violet to light yellow) was measured by UV-Vis spectrometer at 517 nm. The solution of DPPH in methanol ($6 \times 10^{-5}$ M) was prepared. 3 ml of the DPPH solution was mixed with 1 ml of methanolic solution containing plant extract at different concentrations (50, 100, 150, 200 and 250 µl/ml). The samples were incubated in the dark for 30 min at room temperature and the decrease in absorbance was measured. The experiment was repeated thrice. Ascorbic acid was used as standard. Radical scavenging activity (RSA) was calculated by using following equation % Inhibition = $[(A_C - A_S)/A_C] \times 100$, Where $A_C$ = absorption of control; $A_S$ = absorption of test solution. IC50 values were determined using linear regression coefficient.

**Hydrogen peroxide ($H_2O_2$) radical scavenging assay**

The ability of the different leaf extracts of *S. interrupta* to scavenge $H_2O_2$ was determined according to the method of Ruch et al (1989) [12]. A solution of $H_2O_2$ (40 mM) was prepared in phosphate buffer (pH 7.4). Leaf extracts at different concentrations (25, 50, 75, 100 and 150 µl/ml) were added to $H_2O_2$ solution (0.6 mL, 40mM). This solution was incubated in the dark for 15 min and the absorbance was measured at 230 nm against a blank solution containing the phosphate buffer without $H_2O_2$. Ascorbic acid was used as standard positive control. The experiment was repeated thrice. Radical scavenging activity (RSA) was calculated and IC50 values were also determined.

**RESULTS AND DISCUSSION**

**Antimicrobial activity**

Antibacterial activity of the different extracts of leaves of *S. interrupta* including methanolic extract (MES), acetone extract (AES) and hexane extract (HES) was checked against Gram+ve (*S. aureus, B. subtilis*) and Gram-ve bacteria (*K. pneumonia, E. coli*). MES showed very good antibacterial activity against all the tested bacteria. MES formed effective inhibition zones with the diameter of 8.2, 7.5, 6.8 and 6.1 mm against *B. subtilis, S. aureus, E. coli* and *K. pneumonia* respectively. AES also showed good antibacterial activity and formed the inhibition zones of 6.8, 6.3, 5.8 and 5.1 mm respectively against *S. aureus, K. pneumonia, E. coli* and *B. subtilis*

respectively. HES showed very less activity against all the tested bacteria with inhibition zones of 4.3, 4.1, 3.8 and 3.2 against *B. subtilis, S. aureus, E. coli* and *K. pneumonia* respectively (Figure. 1).

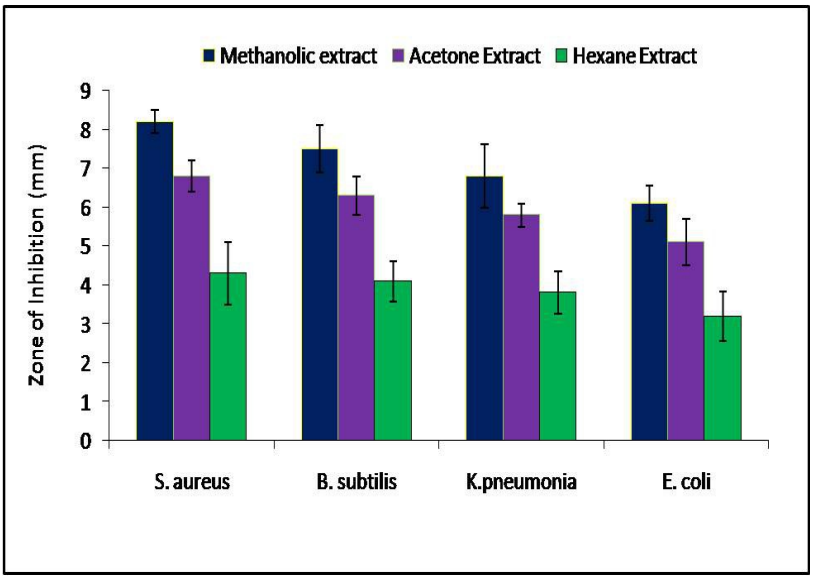


**Figure. 1 Antibacterial activity of leaf extracts of *S. interrupta***

Antifungal activity of the different extracts of leaves of *S. interrupta* was checked against *Aspergillus niger, Candia albicans* and *Candida nonalbicans*. MES formed effective inhibition zones with the diameter of 6.8, 7.3 and 6.8 mm against *A. niger, C. albicans* and *C. nonalbicans* respectively. AES also showed good antifungal activity and formed the inhibition zones of 6.4, 5.8 and 6.1 mm against *A. niger, C. albicans* and *C. nonalbicans* respectively. HES showed less activity with inhibition zones of 3.8, 4.6 and 4.1 mm against *A. niger, C. albicans* and *C. nonalbicans* respectively. MES and HES showed highest inhibitory activity against *C. albicans* while AES showed highest inhibitory activity against *A. niger* (Figure. 2) Antimicrobial activity of the leaf extracts of *S. interrupta* could be due to terpenoids, flavonoids proteins and polyphenolic compounds present in the leaf extract which were confirmed by FTIR analysis. Terpenoids especially triterpenoids could play an important role in the antimicrobial activity as they can easy enter inside the bacteria by rupturing cell membrane. After entering inside they interact with important enzymes required for cellular metabolism.

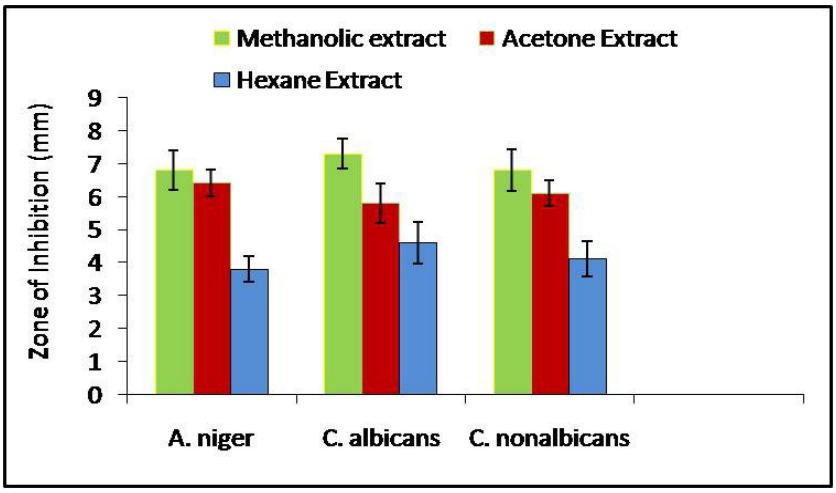


**Figure. 2 Antifungal activity of leaf extracts of *S. interrupta***

**Antioxidant activity**

Antioxidant activity of the different leaf extracts of *S. interrupta* including methanolic extract (MES), acetone extract (AES) and hexane extract (HES) was checked by employing DPPH and $H_2O_2$ radical scavenging assays. Both MES and AES showed effective radical scavenging activity against DPPH (Figure. 3a and b). While HES could not show any scavenging activities against DPPH radicals. Increase in the concentration of leaf extract increases the scavenging activity. MES and AES showed maximum inhibition activities of 54.38% and 51.45% at the respectively at the highest concentration. IC50 (inhibitory concentration at which 50% radicals are scavenged) values of MES and AES against DPPH radical were found to be 221.7 and 235.9 µl/ml respectively.

Free radical scavenging activity of the leaf extracts of *S. interrupta* was further confirmed by $H_2O_2$ radical scavenging assay. Both MES and AES showed effective free radical scavenging activity against $H_2O_2$ radical (Figure 4 a and b). MES and AES showed dosage dependant radical scavenging activity. HES was not showed any radical scavenging activity. MES and AES showed maximum radical scavenging activities of 277.3 and 358.34% respectively at highest concentration. IC50 values of MES and AES against $H_2O_2$ radical were found to be 277.3 and 358.34 µl/ml respectively. Radical scavenging activity of the leaf extracts of *S. interrupta* could be due to flavonoids and other polyphenolic compounds present in the leaf extracts. Free radicals like singlet oxygen, super oxides, peroxides causes oxidative stress which is linked to

many disorders such as inflammation, aging, atherosclerosis and neurodegenerative disorders. The radical scavenging activity of the leaf extract of *S. interrupta* proves the antioxidant activity of secondary metabolites present in *S. interrupta*

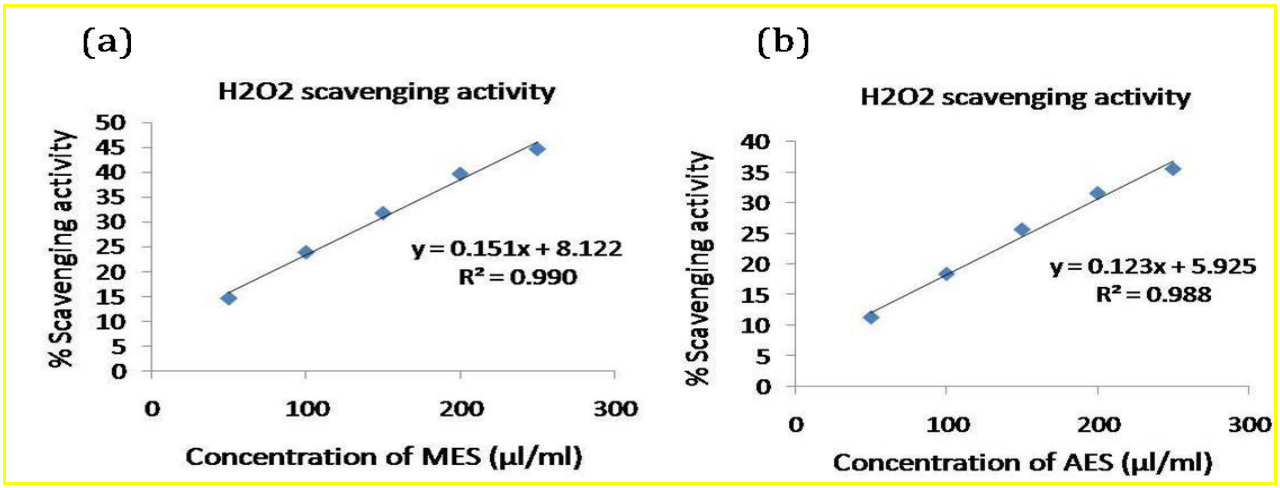


**Figure. 3 DPPH scavenging activity of (a) Methanolic extract and (b) Acetone extract of *S. interrupta***

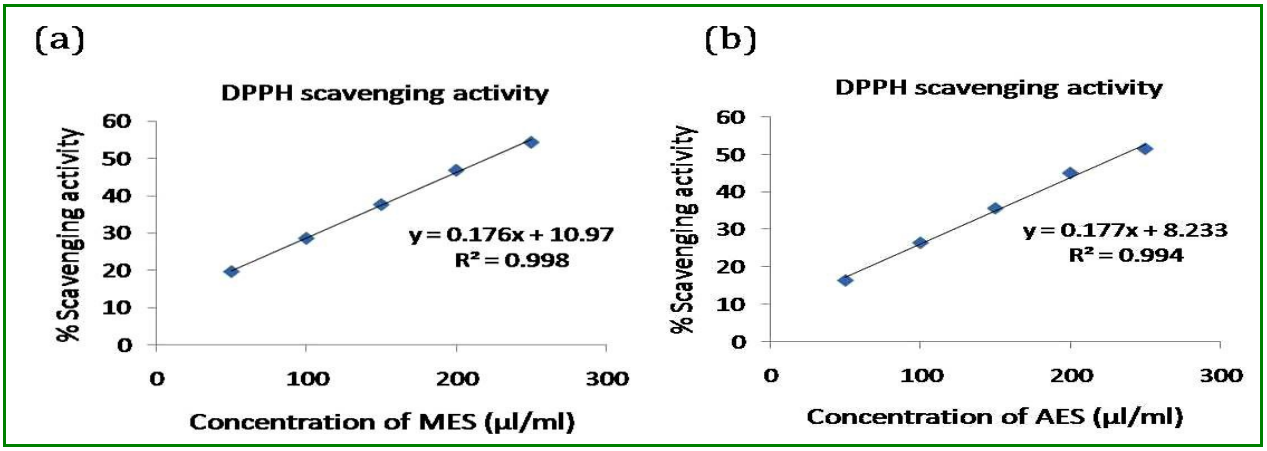


**Figure. 4 $H_2O_2$ radical scavenging activity of (a) Methanolic extract and (b) Acetone extract of *S. interrupta***

**FTIR analysis**

FTIR analysis of dry leaf powder of *S. interrupta* showed the peaks at 3222, 2921, 1610, 1312, 1030 and 815 $cm^{-1.}$ The peak at 815 $cm^{-1}$ is corresponding to the presence of aromatic functional groups in the leaf powder. The peak at 1030 $cm^{-1}$ is corresponding to the presence of hydroxyl

functional groups such as tri terpenoids, polyphenols and flavonoids in the leaf powder. The peak at 1312 $cm^{-1}$ is corresponding to the presence of carbonyl functional groups. The peak at 1610 $cm^{-1}$ is corresponding to primary amines of proteins. The peak at 2921 is corresponding to carboxy functional groups of proteins.

## CONCLUSION

In the present study we have evaluated the antimicrobial and antioxidant activity of leaf extracts of *S. interrupta*. Methanolic and acetone extracts showed effective antimicrobial activity against both bacterial and fungal pathogens. Hexane extract could not show effective antimicrobial activity. Similary Methanolic and acetone extracts showed effective antioxidant activity against DPPH and $H_2O_2$ radicals. FTIR analysis showed the presence of different secondary metabolites and proteins that were responsible for bioactivities of *S. interrupta*.

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