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Phytochemical screening, antibacterial and free radical scavenging effects of *Artemisia nilagirica, Mimosa pudica* and *Clerodendrum siphonanthus* –An *in–vitro* study

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ABSTRACT

Objective: To evaluate methanolic extracts of leaves of *Artemisia nilagirica*, *Mimosa pudica and Clerodendrum siphonanthus* for phytochemical analysis, antibacterial activity and free radical scavenging activity. **Methods:** Antibacterial activity was performed by disc diffusion method against two gram positive and four gram negative strains. Free radical scavenging potential was evaluated using total antioxidant activity (thiocyanate method) and diphenyl–picryl–hydrazyl (DPPH) assay. **Results:** Results of the present study showed that *Clerodendrum siphonanthus* exhibited significant antibacterial effect against *Klebsiella pneumoniae* (30 mm), *Proteus mirabilis* (16 mm), *Salmonella typhi* (16 mm), *Staphylococcus aureus* (12 mm), *Escherichia coli* (11.5 mm) and *Bacillus subtilis* (10 mm). *Mimosa pudica* and *Artemisia nilagirica* showed good antibacterial effects. *Clerodendrum siphonanthus* was found to be extremely effective in scavenging lipid peroxide (IC_{50} 8 mg/mL) and DPPH radicals (IC_{50} 7 mg/mL), whereas *Artemisia nilagirica* and *Mimosa pudica* showed moderate activity. Phytochemical analysis of these plants revealed presence of tannins, alkaloids, flavanoids, terpenoids and glycosides. **Conclusions:** This study showed that *Artemisia nilagirica*, *Mimosa pudica* and *Clerodendrum siphonanthus* may serve as a potential agent for new therapeutics.

1. Introduction

According to the World Health Organization, 80% of Asian and African population still depends on traditional medicine for primary health care. Globally, India has been acknowledged as a major resourceful area in traditional medicine. The primary benefits of using plant derived medicine are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment. Many commercially proven drugs used in modern medicine are from traditional medical plants, with ethnobotanical and ethnomedical knowledge[1]. Antimicrobial substances present in tissues of higher plants have long been regarded as important factors in the resistance of higher plants to various bacteria. Hence, researchers have always felt the need for scientific screening of the plants, which may help the pharmacologists and phytochemists. In drug discovery, random screening as a tool in identifying new biologically active molecules has been the most productive. Free radicals are generated by both internal (cellular respiration *etc*) and external (alcohol, pollution, smoking *etc*) sources. These free radicals can damage all cellular macromolecules (proteins, carbohydrates, lipids and nucleic acids) and attribute to cancer and atherosclerosis[2]. However, these radicals are controlled by antioxidants, which can safely interact and terminate the chain reaction before vital molecules are damaged. There are several enzyme systems (catalase, superoxide dismutase *etc*) within our body that scavenge free radicals. In addition, micronutrients such as vitamin E, beta–carotene and vitamin C from dietary sources can act as antioxidants[3,4].

Worldwide, more than hundreds of plants are used as traditional medicine for the treatment of bacterial infections and other diseases[5,6]. Although many have been treated

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by conventional pharmaceutical approaches, there is growing interest in the use of natural products by the general public. In the present study, three medicinal plants namely *Artemisia nilagirica* (Asteraceae)(*A. nilagirica*), *Mimosa pudica* (Fabaceae)(*M. pudica*) and *Clerodendrum siphonanthus* (Lamiaceae)(*C. siphonanthus*) were selected. Traditionally, these plants have been acknowledged to reduce inflammation and also to treat lung diseases, cough and cold[7]. The objective of the study is to evaluate the phytochemical analysis, antibacterial and free radical scavenging activities of the selected medicinal plants.

2. Materials and methods

2.1 Plant materials and extraction

Leaves of *A. nilagirica*, *M. pudica* and *C. siphonanthus* were collected from Nilgiri district and Chenglepet, Tamil Nadu, India, in April 2010, which was authenticated by the Department of Plant Biology & Biotechnology, Loyola College, Chennai, India. A voucher specimen was deposited. Leaves of *A. nilagirica*, *M. pudica* and *C. siphonanthus* were shade dried, powdered and used for the extraction. About 1 kg of dry powder was taken in an aspirator bottle to which 3 l of methanol was added for the extraction. This was shaken occasionally for 48 h and the extract was filtered with Whatman filter paper No.1. This procedure was performed thrice and all the extracts were decanted and pooled. The extracts were filtered before drying, using Whatman filter paper No. 2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40℃ [8].

2.2. Bacterial inoculum preparation

Gram positive bacterial (such as *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121) and Gram negative bacterial (such as *Escherichia coli* MTCC 443, *Klebsiella pneumoniae* MTCC 109, *Proteus mirabilis* MTCC 425, *Salmonella typhi* MTCC 531) cultures were obtained from Microbial Type Culture Collection, Chandigarh, India. Bacterial inoculums were prepared in Mueller Hinton Broth (Himedia) and maintained for 24 h at 37 ℃. The cell suspensions were diluted with sterile MHB to provide an initial cell count of about 10^6 CFU/mL.

2.3. Disc diffusion method

Antibacterial activity was carried out using disc diffusion method[9]. Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (Himedia, Mumbai). The test cultures (100 μL of suspension containing 10^8 CFU/mL) were swabbed over the solidified media and the plates were further allowed to dry for 10 min. Sterile discs were loaded with methanolic extracts of *A. nilagirica*, *M. pudica* and *C. siphonanthus* (5 mg/disc). The loaded discs were placed on the surface of the medium and were left to diffuse for 30 min at room temperature. Negative control was prepared using respective solvents. Streptomycin (15 μg/disc) was used as positive control. The plates were incubated at 37 ℃ for 24 h. The zone of inhibition was recorded in millimeters and the experiment was performed thrice.

2.4. Determination of Total Antioxidant Activity

The antioxidant activities of *A. nilagirica*, *M. pudica* and *C. siphonanthus* were determined using the thiocyanate method[10]. Four different concentrations (5, 10, 25 and 50 mg/mL) of the extracts were prepared in methanol and were added to linoleic acid emulsion (2.5 mL, 40 mM, pH 7.0) and phosphate buffer (2 mL, 40 mM, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.280 4 g linoleic acid with 0.280 4 g Tween-20 as an emulsifier in 50 mL 40 mM phosphate buffer[11]. The final volume was adjusted to 5 mL using 40 mM phosphate buffer at pH 7.0, after homogenization. The mixed samples were then incubated at 37oC in a glass flask for 60 h, to accelerate the oxidation process. One milliliter of the incubated sample was removed after 12 h, to which 0.1 mL 20 mM $FeCl_2$ and 0.1 mL 30% ammonium thiocyanate were added. The absorbance of this was measured at 500 nm, using a spectrophotometer (DU640i, Beckman), with BHA as the reference compound. To eliminate the solvent effect, the control sample, which contained the same amount of solvent added to the linoleic acid emulsion in the test sample and the reference compound, was used. All data reported are the average of triplicate analysis. Percentage inhibition of lipid peroxide generation was calculated using the following formula.

$$\%\ \text{Inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

2.5. DPPH radical scavenging activity

In this assay, 1 mL of varying concentrations (5, 10, 25, and 50 mg/mL) of the methanolic extracts of *A. nilagirica*, *M. pudica* and *C. siphonanthus* was mixed with 1 mL of methanolic solution of DPPH (0.2 mM). The mixture was vortexed and incubated for 30 min. The optical densities of the solutions were measured at 517 nm using Hitachi 2050 spectrophotometer, using BHA as the standard reference[12].

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

2.6. Phytochemical analysis

Phytochemical analysis (flavonoids, terpenoids, steroids,

alkaloids, tannins and glycosides) was performed by following Edeogal Method[13].

2.7. Statistical analysis

Comparison between the control and the extract treated groups was analyzed by SPSS software package, Version 11.5 with Student *t*-test. $P<0.05$ was considered to be significant.

3. Results

3.1. Antibacterial assay

The methanol extracts of *A. nilagirica*, *M. pudica* and *C. siphonanthus* were screened against two gram positive and four gram negative bacteria. *C. siphonanthus* exhibited significant antibacterial effect against *Bacillus subtilis* (10 mm), *Staphylococcus aureus* (12 mm), *Klebsiella pneumoniae* (30 mm), *Proteus mirabilis* (16 mm), *Escherichia coli* (11.5 mm) and *Salmonella typhi* (16 mm). The highest zone of inhibition was recorded against *Klebsiella pneumoniae*. *M. pudica* and *A. nilagirica* inhibited the bacterial growth (Table 1).

3.2. Total antioxidant activity

Total antioxidant activities of *A. nilagirica*, *M. pudica* and *C. siphonanthus* were determined using the ammonium thiocyanate method. *C. siphonanthus* (IC_{50} 7 mg/mL) was found to be effective in scavenging lipid peroxide radicals. *M. pudica* (IC_{50} 10 mg/mL) scavenged the generated free radicals significantly at concentrations >10 mg/mL (Figure 1). Moderate activity was observed in *A. nilagirica* (IC_{50} 22 mg/mL).

Table 1.
Antibacterial activity of the methanol extracts of *A. nilagirica*, *M. pudica* and *C. siphonanthus*.

Bacteria	Zone of inhibition (mm)			
	A. nilagirica (5 mg/disc)	*M. pudica* (5 mg/disc)	*C. Siphonanthus*(5 mg/disc)	*Streptomycin* (30 μg/disc)
Bacillus subtilis	12.0	16.0	10.0	15.0
Staphylococcus aureus	12.0	15.0	12.0	15.0
Klebsiella pneumoniae	16.0	20.0	30.0	16.0
Proteus mirabilis	12.0	11.0	16.0	15.0
Escherichia coli	11.5	12.0	11.5	15.0
Salmonella typhi	15.5	14.5	16.0	15.0

Experiments were performed in triplicates.

Table 2.
Phytochemical analysis of *A. nilagirica*, *M. pudica* and *C. Siphonanthus* methanolic extracts.

Plant	Flavonoids	Terpenoids	Steroids	Alkaloids	Tannins	Glycosides
A. nilagirica	+	+	−	+	+	−
M. pudica	+	−	−	+	−	+
C. Siphonanthus	+	−	+	−	−	+

+ = Present − = Absent.

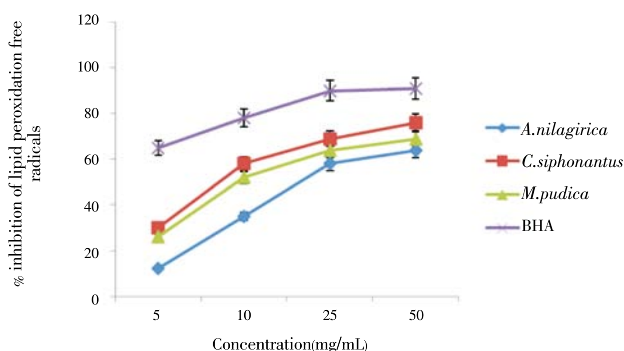

Figure 1. Scavenging activity of BHA (control), *A. nilagirica*, *M. pudica* and *C. Siphonanthus* on lipid peroxidation (n = 3).

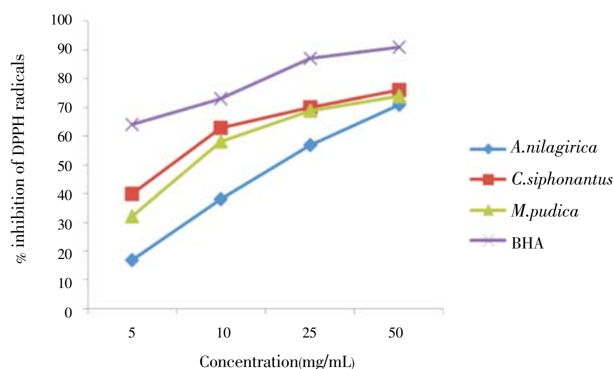

Figure 2. Scavenging activity of BHA (control), *A. nilagirica*, *M. pudica* and *C. Siphonanthus* on DPPH free radicals (n = 3).

3.3. Inhibition of DPPH radical

C. siphonanthus (IC_{50} 7 mg/mL) and *M. pudica* (IC_{50} 9 mg/mL) showed significant free radical scavenging activity generated by DPPH. Since more than 50% of DPPH radical inhibition is considered to be significant, the inhibition was observed from 10 mg/mL (Figure 2).

3.4. Phytochemical analysis

Results of phytochemical analysis of *A. nilagirica*, *M. pudica* and *C. Siphonanthus* are shown in Table 2.

4. Discussion

Phytotherapy is based on the active principles contained in plants. Plant derived natural products such as flavonoids, terpenoids, glycosides, steroids *etc* have received considerable attention in recent years due to their diverse pharmacological properties including antibacterial, hepatoprotective and antioxidant activities. In the present study, *C. siphonanthus* and *M. pudica* effectively inhibited the tested bacterial strains, particularly the gram negative bacteria. Moreover, the inhibition of these extracts is found to be higher than the inhibition of the standard streptomycin, against the selected microorganisms. Phytochemical analysis of these plant extracts showed the presence of flavonoids, terpenoids, steroids and carbohydrates. In the previous findings, flavonoids were found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to form a complex with extracellular soluble protein and the bacterial cell wall[14].

Result of lipid peroxidation assay significantly scavenged the generated radicals. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation[15,16]. In the present study, *C. siphonanthus* revealed the presence of phenolic compounds. *C. siphonanthus* and *M. pudica* scavenged the generated DPPH radicals significantly. In DPPH assay, the generated radicals are reduced by the donation of protons by the extracts, leading to the color change from purple to yellow, which can be quantified by its decrease in absorbance at 517 nm. DPPH is a stable radical; this radical becomes unstable when it receives electrons from reducing agents. *C. siphonanthus* and *M. pudica* act as reducing agents, exhibiting antioxidant property. These antioxidant mechanisms play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infections and degenerative diseases[17].

The present study indicates that *C. siphonanthus* possesses good antibacterial effect and is extremely active in scavenging the DPPH radicals. It also shows significant reducing power with an inhibitory action on lipid peroxidation. Further study is needed to examine the exact active principle responsible for this biological activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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