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Preliminary phytochemical screening and antioxidant activity of petroleum-ether and hydroalcoholic extracts of the leaves of *strobilanthes barbatus*.

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ABSTRACT

Strobilanthes barbatus commonly known as Suruli mammuni belonging to the family acanthaceae is found in the hills in tropical Asia of which 150 species of this family are distributed in the Indian subcontinent. The important characteristic feature of members in this family is that they show a phenomenon called MASTING i.e. synchronous production of seed at long intervals by a population of plants. Strict masting only occurs in species that are monocarpic in which individuals of the species only reproduce once during their life time, and then die. The extraction was carried out by cold extraction using two solvents such as petroleum-ether and hydro alcohol according to the increasing order of polarity. The qualitative phyto-chemical screening showed the presence of Phytosterols, triterpenoids and saponins in petroleum-ether extract and Saponins and tannins in hydro alcoholic extract. Antioxidant study was performed by DPPH method and Nitric Oxide scavenging method in the above two extracts and comparative study was also done. The study was performed by taking various concentrations of the two extracts ranging from 12.5, 25, 50, 100, 200 µg/ml. The results showed a positive response as compared with the standard ascorbic acid. The comparative study of antioxidant activity in the two extracts shows that petroleum-ether extract shows better antioxidant activity than hydro alcoholic extract.

Keywords: *Strobilanthes barbatus*, acanthaceae, masting, Petroleum-ether, Hydro alcohol, DPPH method, Nitric oxide scavenging activity.

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INTRODUCTION

Plants stands as an infinite resource for drug development, novel chemotypes and pharmacophores and scaffolds for amplification into efficacious drugs for a multitude of disease indications and other valuable bioactive agents¹. Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal values and around 5000 species have specific therapeutic value. Plants especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the plants drugs include Vinblastine, Vincristine, Taxol, Podophyllotoxin, Morphine, Codeine, Aspirin, Atropine and Artemisinin. On the other hand the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance².

Free radicals and related species have attracted a great deal of attention in recent years. They are mainly derived from oxygen (ROS) and nitrogen (RNS) and are generated in our body by various endogenous systems, exposure to different physicochemical conditions or pathophysiological states. Free radicals can adversely alter lipids, proteins and DNA and have been implicated in ageing and a number of human diseases. Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations³. The damage produced by the free radicals may be prevented by antioxidants. An antioxidant is any substance that when present at low concentrations significantly delays or prevents oxidation of cell content like proteins, lipids, carbohydrates and DNA. The body itself has developed several endogenous antioxidant systems to deal with the production of ROI. These systems can be divided into enzymatic and nonenzymatic groups. The enzymatic antioxidants include superoxide dismutase (SOD), catalase etc. The non-enzymatic antioxidants include lipid soluble vitamins, vitamin E, and vitamin C etc⁴.

The term plietesial has been used in reference to perennial monocarpic plants “of the kind most often met with in the Strobilantheae” (a sub tribe of Acanthaceae containing *Strobilanthes* and allied genera) that usually grow gregariously, flower simultaneously following a long interval, set seed, and die. It flowers once in seven years. The genus has around 250 species, of which at least 46 are found in India. *Strobilanthes barbatus* belongs to the family acanthaceae is mainly found in the regions of Kerala, Karnataka, Tamilnadu and Maharashtra. It is a gregarious shrub

which grows up to 3-4m height with quadrangular stem, elliptic-lanceolate leaves and with glandular hairy fruit. Flowering occurs in the month of September-December⁵.



Figure1: Leaves of *Strobilanthes barbatus*

The work on chemical composition of leaves revealed the presence of Phytosterols, triterpenoids, tannins and saponins. The present study was carried out to determine the phytoconstituents present in the leaves and to evaluate the antioxidant activity of the petroleum-ether extract and hydro- alcoholic extract of the leaves of *Strobilanthes barbatus*. Antioxidant study was performed by means of two methods; DPPH method and Nitric Oxide Scavenging method.

MATERIALS AND METHODS

Plant Material

The whole plant of *Strobilanthes barbatus* belonging to family acanthaceae was collected from the place kuruppunthara, kottayam district, Kerala. The herbarium sheet for the plant with some general information were prepared and duly authenticated by Dr. Jomy Augustine, Head of the department of botany, St. Thomas college pala, kottayam district, kerala. The leaves were dried in shade and powdered to coarse powder and then kept in air tight bottle until use.

Extraction and Isolation

The leaves of *Strobilanthes barbatus* were washed and dried in shade for 7 days. The dried leaves were powdered into coarse powder and kept in air tight bottle. Extraction was carried out using two solvents. The solvent such as petroleum-ether and hydro-alcohol were selected on the basis of their increasing order of polarity. The principle followed in the extraction process was cold maceration. About 150 gm of the plant powder was taken. The coarse powder was soaked

first in petroleum-ether for 7 days with intermittent shaking at room temperature. After 7 days it was filtered and the filtrate was concentrated in a rotary evaporator. The marc was then soaked in hydro alcoholic solvent for 7 days with intermittent shaking and then filtered and the filtrate was concentrated in a rotary evaporator. Then they were subjected for phytochemical screening for the detection of various phytoconstituents.

PRELIMINARY PHYTOCHEMICAL SCREENING.^{6, 7, 8}

A portion of residue from each extract was subjected for preliminary phytochemical screening for the detection of the presence of various phytoconstituents.

ANTI-OXIDANT ACTIVITY

***In-vitro* Antioxidant activity by DPPH Method.⁹**

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Procedure

Different volumes (1.25-10 μ l) of plant extracts were made up to 40 μ l with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control.

% inhibition = (Absorbance of control - Absorbance of sample / Absorbance of control) x 100

The result was compared with the standard ascorbic acid.

***In-vitro* Antioxidant activity by Nitric Oxide Scavenging Activity.¹⁰**

Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitro prusside (5mmolL⁻¹) in phosphate buffered saline pH 7.4, was mixed with different concentration of the extract (125-2000 μ g mL⁻¹) prepared in methanol and incubated at 25°C for 150 minutes. A control without the test compound, but an equivalent amount of methanol was taken. After 150 minutes, 1.5mL of the incubated solution was removed and diluted with 1.5mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphthyl ethylene diamine dihydrochloride was measured at 540nm and the percentage scavenging activity was measured with reference to the standard..

% inhibition = (Absorbance of control - Absorbance of sample / Absorbance of control) x 100

The result was compared with the standard ascorbic acid.

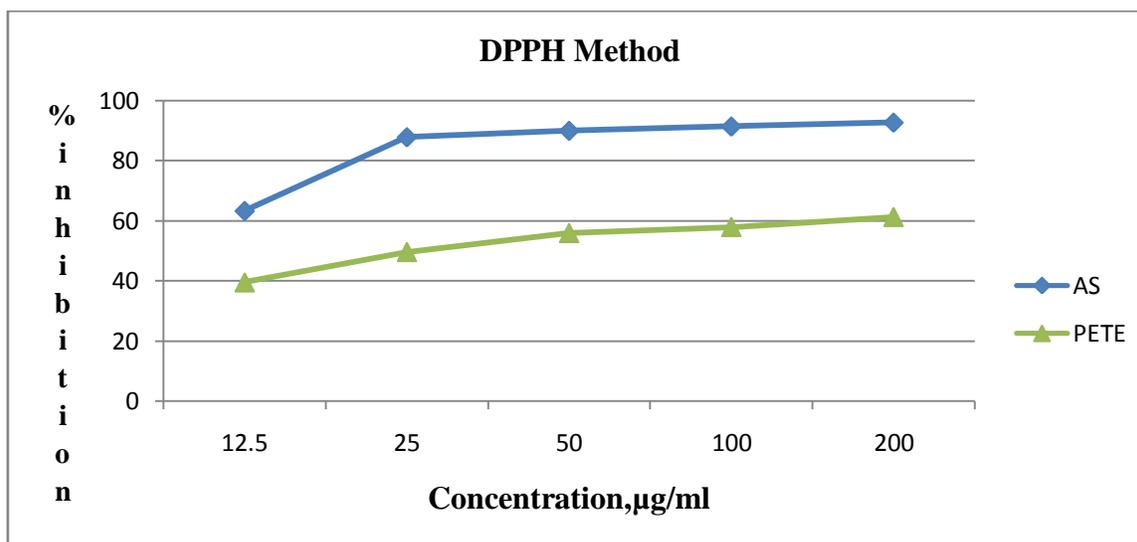
RESULTS AND DISCUSSION

Table 1:Results of the Phytochemical Screening of the two leaf extracts

Phytochemical Tests	Petroleum-ether Extract	Hydro alcoholic Extract
Alkaloids-	-	
Carbohydrates	-	-
Glycosides	-	-
Saponins	+	+
Terpenoids	+	+
Phytosterols	+	+
Tannins	-	-
Flavanoids	-	-
Proteins	-	-
Gums and mucilage	-	-
Resins	-	-

In-vitro* studies*Antioxidant Activity of Petroleum-ether Extract****DPPH radical scavenging activity**

The petroleum-ether extract (PETE) and the standard drug ascorbic acid (AS) were evaluated for their DPPH activity and results are given. The PETE showed a dose dependent activity and IC₅₀ value was calculated and compared with standard ascorbic acid. DPPH radical scavenging activity in terms of percent inhibition was found to be increased with enhancing the concentration of PETE. The inhibition was 39.45% (12.5µg/ml) and 61.26%(200µg/ml) for PETE, whereas the values were 63.28% (12.5 µg/ml) and 92.72% (200 µg/ml) for AS respectively. IC₅₀ value of PETE found to be 55.89µg/ml and IC₅₀ value of AS found to be 10.2 µg/ml.

**Figure 2: DPPH radical scavenging activity of AS and PETE**

Nitric Oxide Scavenging Activity

The PETE and the standard drug ascorbic acid (AS) were evaluated for their Nitric oxide Scavenging activity and results are given. The PETE showed a dose dependent activity and IC50 value was calculated and compared with standard ascorbic acid. Nitric oxide scavenging activity in terms of percent inhibition was found to be increased with enhancing the concentration of PETE. The inhibition was 25.25% (12.5 μ g/ml) and 60.28% (200 μ g/ml) for PETE, whereas the values were 48.40% (12.5 μ g/ml) and 93.85% (200 μ g/ml) for AS respectively. IC 50 value of PETE found to be 100 μ g/ml and IC50 value of AS found to be 13.75 μ g/ml.

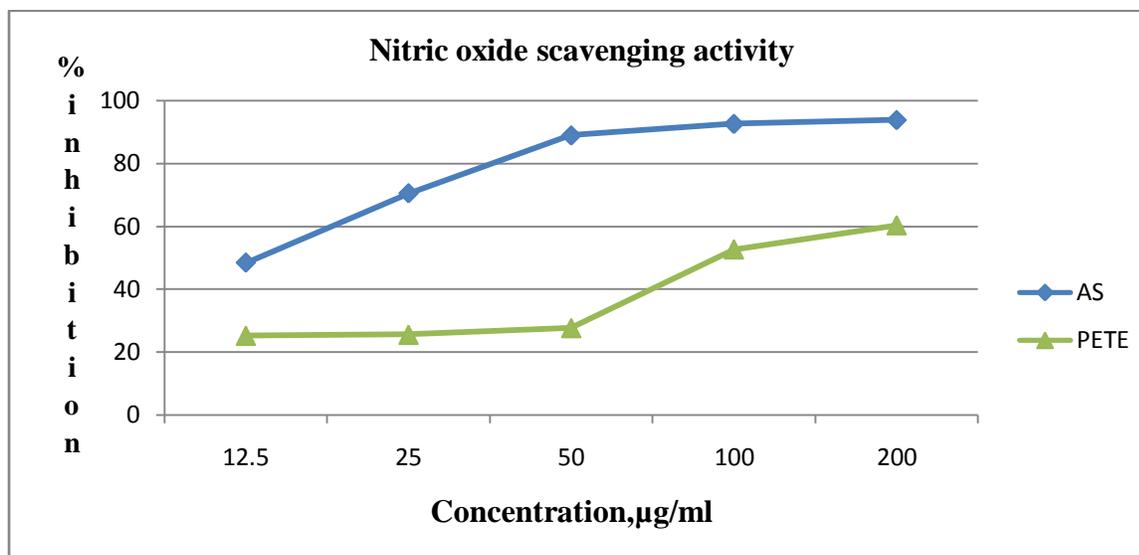


Figure 3: Nitric oxide scavenging activity of AS and PETE

Antioxidant Activity of Hydro alcoholic Extract

DPPH radical scavenging activity

The hydro alcoholic extract (HDAE) and the standard drug ascorbic acid (AS) were evaluated for their DPPH activity and results are given. The HDAE showed a dose dependent activity and IC50 value was calculated and compared with standard ascorbic acid. DPPH radical scavenging activity in terms of percent inhibition was found to be increased with enhancing the concentration of HDAE. The inhibition was 25.81% (12.5 μ g/ml) and 56.91% (200 μ g/ml) for HDAE, whereas the values were 63.28% (12.5 μ g/ml) and 92.72% (200 μ g/ml) for AS respectively. IC 50 value of HDAE found to be 75.39 μ g/ml and IC50 value of AS found to be 10.2 μ g/ml.

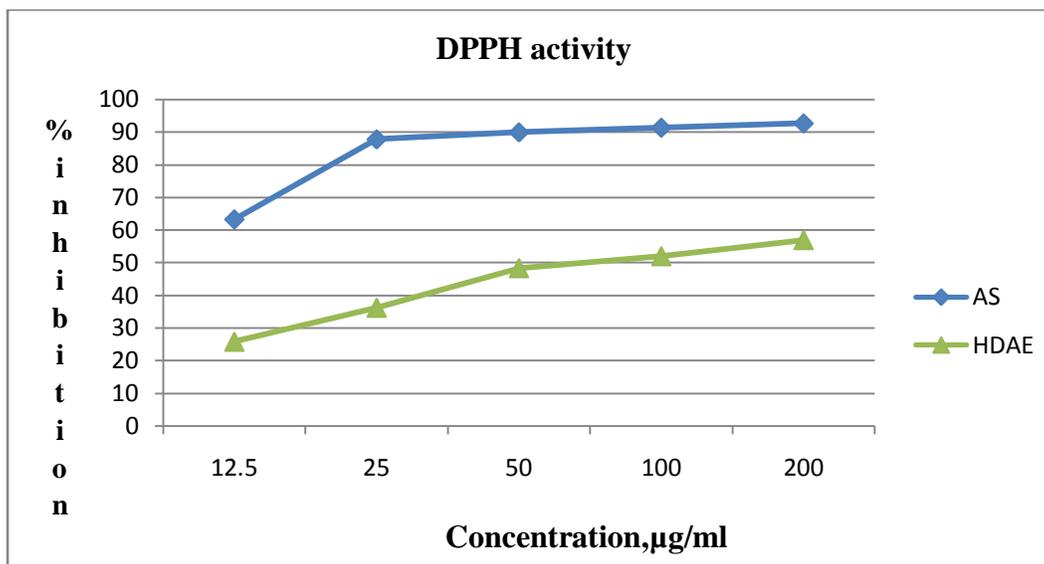


Figure 4: DPPH radical scavenging activity of AS and HDAE

Nitric Oxide Scavenging Activity

The HDAE and the standard drug ascorbic acid (AS) were evaluated for their Nitric oxide Scavenging activity and results are given. The HDAE showed a dose dependent activity and IC₅₀ value was calculated and compared with standard ascorbic acid. Nitric oxide scavenging activity in terms of percent inhibition was found to be increased with enhancing the concentration of HDAE. The inhibition was 19.31% (12.5 µg/ml) and 51.72% (200 µg/ml) for HDAE, whereas the values were 48.40% (12.5 µg/ml) and 93.85% (200 µg/ml) for AS respectively. IC₅₀ value of HDAE found to be 107.2 µg/ml and IC₅₀ value of AS found to be 13.75 µg/ml.

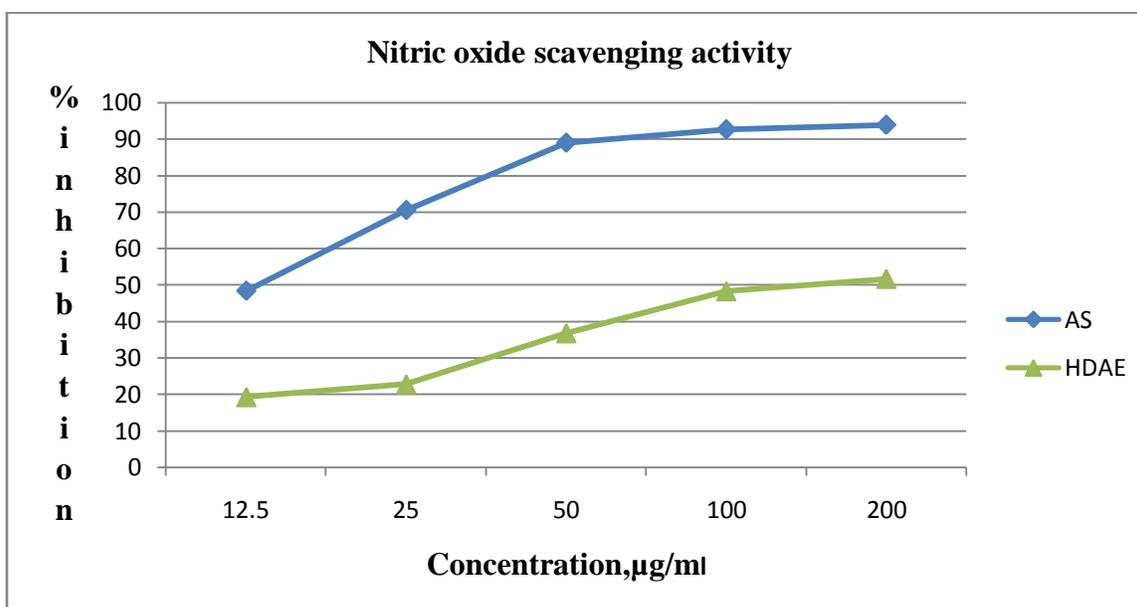


Figure 5: Nitric oxide scavenging activity of AS and HDAE

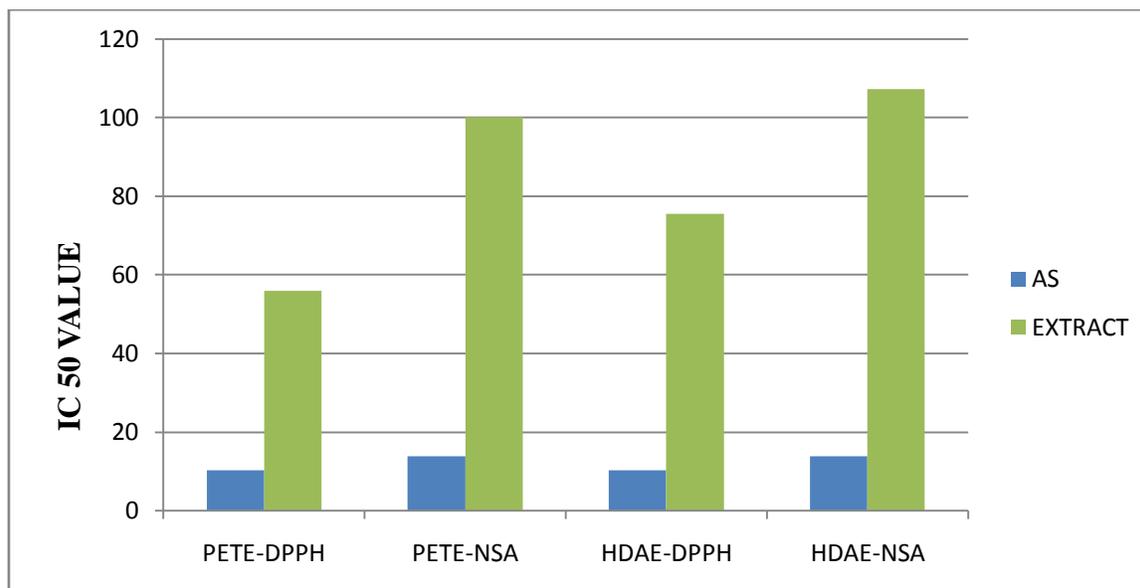


Figure 6: Comparison of Antioxidant Activities of Two Extracts

From the above results IC₅₀ value shows that petroleum ether extract is having good antioxidant activity when compared with that of hydro alcoholic extract.

CONCLUSION

This study reports the phytochemical analysis and antioxidant activities of the petroleum-ether leaf extracts and hydro alcoholic leaf extracts for the first time from *Strobilanthes barbatus*. The plant shows better, antioxidant activity by means of two methods such as DPPH method and nitric Oxide Scavenging method. From the comparative study, petroleum-ether extracts found to be having good antioxidant activity than hydro alcoholic extract. Future studies could include the isolation, characterization studies and various other biological activity studies in this plant using various solvents. Many pharmacological activities are yet to be proven for this plant.

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