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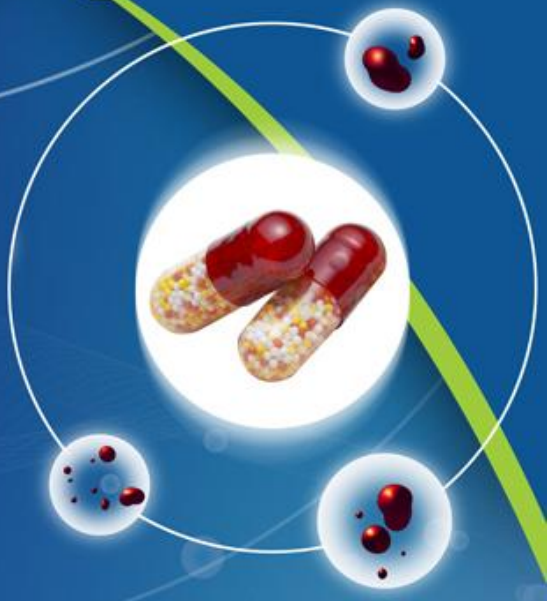

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Research Article

PHYTOCHEMICAL AND IN-VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *STROBILANTHES BARBATUS NEES* LEAVES

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ABSTRACT

Overproduction of oxidants in certain conditions may cause oxidative stress leading to oxidative damage to biomolecules and cells. Hence natural antioxidants are virtually important for human health. The ethanolic extract of Strobilanthes barbatus(EESB) leaves were evaluated for phytochemical tests and in-vitro antioxidant activity against DPPH(1,1-diphenyl-2-picrylhydrazyl)radical,superoxide radical and hydroxyl radical scavenging activity. The results of phytochemical tests revealed the presence of carbohydrates, alkaloids,phenolics, tannins,flavonoids, steroids and triterpenoids. The results of in-vitro antioxidant activity revealed that concentrations of the plant extracts required for 50% inhibition of DPPH radical scavenging effect (IC_{50}) were recorded as15µg/ml, Superoxide radical scavenging activity IC_{50} value of 250µg/ml and hydroxyl radical scavengingIC_{50} value of 525µg/ml as compared with standard drug ascorbic acid.

Keywords: *Antioxidants, DPPH, Hydroxyl radical, Superoxide, Strobilanthes barbatus*

INTRODUCTION

The current accepted modern medicine has gradually developed by the efforts of scientists. But ancient wisdom has been the basis of development of the modern medicine and therapies. Natural product research continues to explore a variety of lead structures which may be used as templates for the development of new drugs. It is pertinent that these discoveries are based on traditional medicines [1]. Herbal medicines are being used by about 80% of the worldpopulation primarily in the developing countries for primary health care [2]. The traditional medicine refers to a broad range of ancient natural health care practices as well as Ayurveda, Siddha and Unani.

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It is estimated that about 7500 plants are used in health traditions in mostly rural and tribal villages of India [3].Free radicals are highly reactive substances formed in the body as a result of metabolic processes. A free radical is a molecule with one or more unpaired electrons in its outer most orbital [4]. Many of these molecular species are oxygen and sometime nitrogen centered, oxygen free radicals and its non-radical products are associated withreactive oxygen species [5]. Free radicals are highly reactive, unstable molecules that reactrapidly with adjacent molecules via a variety of reactions including hydrogen abstraction (capturing), electron donation and electron sharing [6,7].

Free radicals can also react with DNA, proteins or lipids in the cell membrane and cause damage [8]. Flavonoidsare phytochemicals, known to possess antinflammatory, antioxidant, antiallergic,hepatoprotective, antithrombotic,

neuroprotective,anticarcinogenic activities[9]. Antioxidants are involved in the defense mechanism of the organism against the pathogenesis associated to the attack of free radicals. Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects [10]. The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases [11].

Strobilanthes barbatus is a gregarious shrub, 3-4 meter highbelonging to the family Acanthaceae.Literature survey of the *Strobilanthes* species revealed that the leaf of the plant is reported for the antioxidant activity[12],[13].Hence, the present study was undertaken to evaluate the phytochemical and antioxidant activity of ethanolic extract of *Strobilanthes barbatus* leaves.

MATERIALS AND METHODS

Collection and identification of plant materials

The leaves of *strobilanthes barbatus*were collected from Kurupumthara, Kottayam District, Kerala, India, in the month of April 2014. The plant material was identified and authenticated by Dr.A.G. Pandurangan,scientist F &Head PS &ES Division, Trivandrum,Kerala, India.

Preparation of extract

The leaves of *Strobilanthesbarbatus* was washed thoroughly with distilled water and shade dried at room temperature. The dried leaves were powdered and extracted by using Soxhlet apparatus with alcohol [14],[15].

Phytochemical screening [16],[17]

Phytochemical screenings were carried out using standard procedures.

Detection of carbohydrates

Small quantity of the crude extract was dissolved in distilled water and filtered. The filtrate was subjected to

Molisch's test: To the filtrate few drops of alcoholic α-napthol was added and 2ml of sulphuric acid was added slowly through the sides of the test tube. Purple colored ring was formed at junction of the two layers indicates presence of carbohydrates.

Fehling's test: Small portion of the extract was treated with Fehling's solution I and II and then heated on water bath. Brick red colored precipitate formed indicates presence of carbohydrates

Barfoed's test: Small portion of the extract was treated with barfoed's reagent. Red precipitate formed indicates presence of carbohydrates.

Detection of proteins and amino acids

Small quantity of the crude extract was dissolved in few ml of water and was subjected to million's, biuret and ninhydrin test.

Million's test: The extract was treated with million's reagent. Red precipitate produced shows the presence of proteins and free amino acids.

Biuret test: To the extract equal volume of 5 %w/v NaOH and four drops of 1 %w/v $CuSO_4$ solution were added. Pink or purple colour formed indicates the presence of proteins.

Ninhydrin test: The extract was treated with ninhydrin reagent. Purple colour produced indicates the presence of proteins.

Xanthoprotein test: (For protein containing tyrosine or tryptophan): Mixed 3ml extract with 1 ml concentrated H_2SO_4, observed for white precipitate.

Detection of phenolic compounds and tannins

The extract were diluted with distilled water and filtered. The filtrates were treated with following reagent.

Ferric chloride test: The filtrate was treated with 5% of ferric chloride solution.

Black precipitate found indicating the presence of tannins and phenolic compounds.

Test with Lead acetate Solution: Few ml of filtrate were treated with lead acetate solution and observed for white precipitate.

Gelatin test: To the extract, add 1ml of 1% solution of gelatin. White precipitate was seen, which indicates presence of tannin in plant.

Test for alkaloids

Small amount of extract was stirred with a few ml of dilute HCl and filtered. The filtrate was tested with various alkaloidal reagents such as Mayer's, Dragendroff's, Wagner's and Hager's reagent.

Mayer's test: To the small amount of filtrate add few drops of Mayer's reagent. A white colour precipitate was formed, indicates the presence of alkaloids.

Dragendroff's test (potassium bismuth iodide): To the small amount of filtrate add few drops of Dragendroff's reagent. An orange red colour precipitate was formed, indicating the presence of alkaloids.

Wagner's test: To the small amount of filtrate add few drops of Wagner's reagent. A brown colour precipitate was formed, indicating the presence of alkaloids.

Hager's test (picric acid):To the small amount of filtrate add few drops of Hager's reagent. A yellow crystalline precipitate was formed, indicating the presence of alkaloids.

Test for glycosides

A small amount of the extract was subjected to the following tests

Legal's test: To the extract 1ml pyridine few drops of sodium nitroprusside solution was added and then made alkaline with sodium hydroxide solution. Pink colour was obtained.

Balget's test: To a solution of extract sodium picrate solution was added. Yellowish orange colour was obtained showing the presence of glycosides.

Borntrager's test: Extract was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Pink colour was observed in ammoniacal layer, confirms the presence of glycosides.

Modified borntrager's test: The extracts were boiled with few ml of dilute HCl and 5ml of ferric chloride solution. The contents are cooled and shaken with organic solvent. Organic layer was separated and to this equal volume of ammoniacal solution was added. The ammoniacal layer showed pink colour, which shows the presence of anthraquinone glycoside.

Test for flavonoids

The extract was dissolved in ethanol and then subjected to the following tests.

Ferric chloride test: To a small quantity of extract few drops of neutral ferric chloride was added. Blackish red colour was observed, showing the presence of flavanoids.

Shinoida's test: To the alcoholic solution a small piece of magnesium ribbon was added along with concentrated HCl. Magenta colour was formed, showing the presence of flavanoids.

Lead acetate solution: To a small quantity of extract a few drops of 10% lead acetate solution was added. Yellow precipitate was produced, shows presence of flavanoids.

Detection of saponins

The extracts were diluted, with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. A layer of foam was formed, indicating the presence of saponins.

Detection of coumarins

To a small quantity of extract were dissolved in alcohol and exposed to UV light, shows green fluorescence.

To small quantity of extract were dissolved in alcohol and add ferric chloride solution, shows green color, indicating the presence of coumarins.

Test for Steroids and Triterpenoids

Salkowski Test: To the solution of extract in chloroform, a few drops of sulphuric acid were added and the mixture was shaken and allowed

to stand for some time. Red colour was produced in the chloroform layer, which indicates the presence of steroids.

Libbermann-Burchard Test: Small portion of extract was dissolved in chloroform. To this 1 ml acetic anhydride and then 2 ml concentrated sulphuric acid was added through the sides of the test tube. A reddish violet colour at the junction of two liquids indicates the presence of steroid and triterpenoids.

DETERMINATION OF ANTIOXIDANT ACTIVITY

DPPHRadical scavenging activity

The radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-picryl hydrazyl radical were determined by UV spectrophotometry at 517 nm. Different volumes (1.25-20µl) of plant extracts were made up to 40µl 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. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{control}-\text{test}}{\text{control}} \times 100$$

Super oxide free radical scavenging activity [19],[20]

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical.Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in riboflavin-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan product. Different concentrations of ethanolic extract of *Strobilanthes barbatus* leaf (125-2000µg/ml), 0.05 ml of Riboflavin solution (0.12 mM), 0.2 ml of EDTA solution [0.1M], and 0.1 ml NBT (Nitro-blue tetrazolium) solution [1.5mM] were mixed in test tube and reaction mixture was diluted up to 2.64ml with phosphate buffer [0.067M]. The absorbance of solution was measured at 560nm using DMSO as blank afterillumination for 5 min and difference in OD was determined after 30 minutes incubation in fluorescent light. Absorbance was measured after illumination for 30 min. at 560 nm on UV visible spectrophotometer.

*Hydroxyl radical scavenging activity [*21],[22]

This assay is based on the qualification of the degradation product of 2 deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} - ascorbate- EDTA $–H_2O_2$ system (The Fenton reaction). The reaction mixture contained in the final volume of 1 ml.2 deoxy 2 ribose (2.8mM) KH_2PO_4—KOH buffer (20 mM pH 7.4), FeCl3 (100µm), EDTA (100µm), H_2O_2 (1.0mM), ascorbic acid (100µm) and various concentrations (0-200µg/ml)of the ethanolic extract of *Strobilanthes barbatus* leaves. After incubation for 1hour at 37°C, 0.5 ml of the reaction mixture was added to 1ml of 2.8% TCA, then 1ml aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532nm against an appropriate blank solution.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The qualitative phytochemical investigation of ethanolic extracts of leaves of*Strobilanthes barbatus* was carried out to check the presence of various phytoconstituents in extract. It is observed from the phytochemical study that flavonoids,terpenoids,tannins,steroids,alkaloids, carbohydrate,phytosterols were present in the extract.

TableI: Preliminary phytochemical investigation of EESB

SL.NO	PRELIMINARY TEST	EESB
1	**Carbohydrate**	
	Fehling's test	+
	Mohlish's test	+
	Barfoed's test	+
2	**Protein and amino acid**	
	Biuret test	-
	Millon's test	-
	Xanthoprotein test	-
	Ninhydrin test	-
3	**Alkaloids**	
	Mayer's test	+
	Wagner's test	+
	Dragendorff's Test	+
	Hager's Test	+
4	**Glycoside**	
	Bontrager's test	-
	Balget Test	-
	Legal's test	-
	Modified Borntrager's Test	-
5	**Phenolic and Tannins**	
	Lead acetate test	+
	Gelatin test	+
	Ferric chloride test	+
6	**Saponins**	
	Foam test	-
7	**Flavanoids**	
	Shinoda test	+
	Ferric chloride test	+
	Lead acetate test	+
8	**Steroidsand triterpenoids**	
	Salkowisski test	+
	Libermann- buchard test	+
9	**Coumarins**	-

IN-VITRO ANTIOXIDANT ACTIVITY
DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl)

Table II: DPPH radical scavenging activity of ascorbic acid and extracts of *Strobilanthes barbatus* leaves

Concentrations (µg/ml)	% free radical scavenging activity	
	Ascorbic acid	EESB
12.5	50.10	42.58
25	83.75	61.23
50	86.62	68.92
100	88.53	70.12
200	90.28	75.28

IC_{50} VALUE OF ASCORBIC ACID:12.5 µg/ml IC_{50} VALUE OF EESB: 15 µg/ml

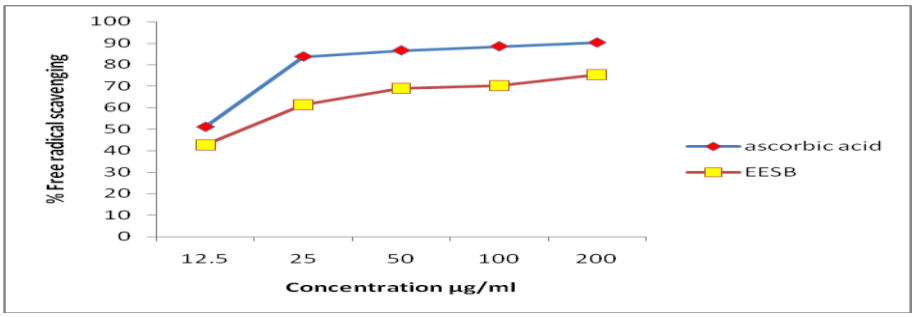

Figure 1: DPPH radical scavenging activity of ascorbic acid and extracts of *Strobilanthes barbatus* leaves

Super oxide free radical scavenging activity

Table III: Super oxide radical scavenging activity of ascorbic acid and extracts of *Strobilanthes barbatus* leaves

Concentration (µg/ml)	% free radical scavenging activity	
	ASCORBIC ACID	EESB
125	41.41	41.81
250	54.54	50.00
500	63.63	62.27
1000	72.72	65.45
2000	81.81	70.45

IC_{50} VALUE OF ASCORBIC ACID 175µg/ml IC_{50} VALUE OF EESB 250µg/ml

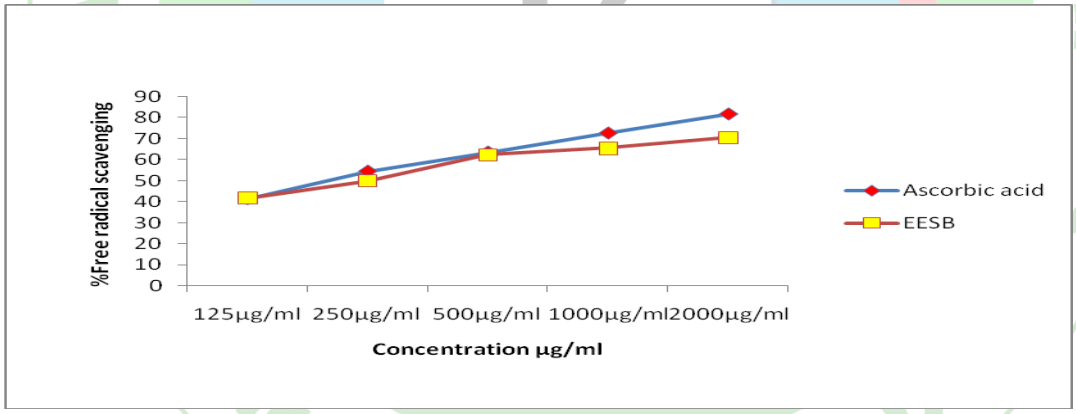

Figure 2: Superoxide radical scavenging activity of ascorbic acid and extracts of*Strobilanthes barbatus* leaves

Hydroxyl radical scavenging activity

Table IV:Hydroxyl radical scavenging activity of ascorbic acid and extracts of *Strobilanthes barbatus* leaves

Concentration (µg/ml)	% free radical scavenging activity	
	ASCORBIC ACID	EESB
125	36.85	33
250	44.96	41.90
500	51.88	46.34
1000	58.98	52.98
2000	69.77	57.12

IC_{50} VALUE OF ASCORBIC ACID 425µg/ml IC_{50} VALUE OF EESB 525µg/ml

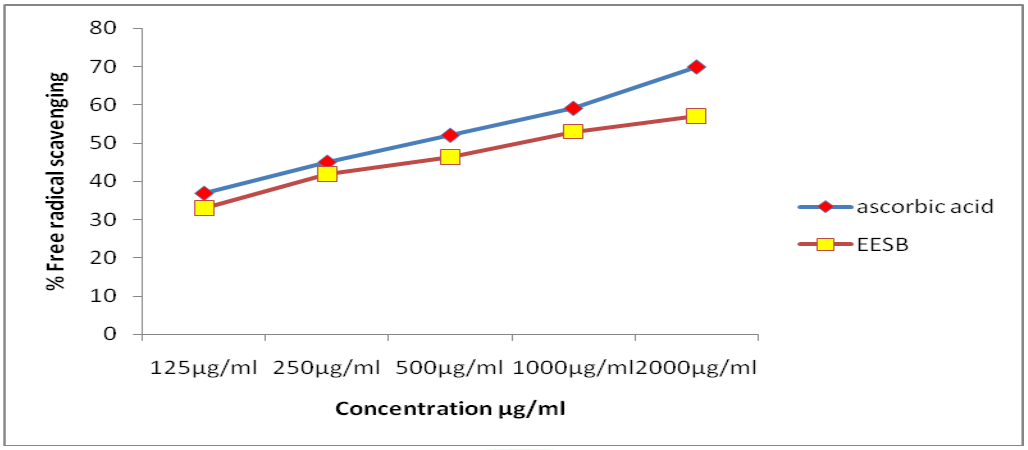

Figure 4: Hydroxyl radical scavenging activity of ascorbic acid and extracts of *Strobilanthes barbatus* leaves

DISCUSSION

The plant leaf *Strobilanthes barbatus* was subjected to extraction using ethanol. Ethanolic extraction was carried out using soxhlet extraction method.The extract were subjected to preliminary phytochemical screening. Phytochemical screening showed the presence of active components like alkaloids, carbohydrates, proteins, flavonoids, phenolics, tannins, steroids and triterpenoids (Table I).

Free radicals involved in the process of lipid peroxidation are considered to play a cardinal role in numerous chronic pathologies such as cancer and cardiovascular diseases among others and are implicated in the ageing process

Ethanolic extracts have shown dose dependent increase in reducing power of DPPH comparable to that of standard ascorbic acid. The DPPH scavenging activity of the ethanolic extract of *Strobilanthes barbatus* leaves at different concentrations (12.5-200 µg/ml) were compared with ascorbic acid at varying concentrations (12.5- 200µg/ml).Table II and graph:1 illustrates a significant decrease in the DPPH radical due to the scavenging ability of extracts and ascorbic acid.

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in riboflavin-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan product. The Superoxide radical scavenging activity of the ethanolic extract of Strobilanthes barbatus leaf at different concentrations (125-2000µg/ml) was compared with Ascorbic acid at varying concentrations (125-2000µg/ml).Table III and graph:2 illustrates a significant decrease in the superoxide radical due to the scavenging ability of extracts and ascorbic acid. The ethanolic extracts showed maximum activity of 70.45% at 2000 µg/ml respectively, whereas ascorbic acid at the same concentration exhibited 81.81% inhibition. The IC_{50} values were found to be 175 µg/ml, 250 µg/ml for ascorbic acid and ethanolic extract respectively.

This assay is based on the qualification of the degradation product of 2- deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} - ascorbate- EDTA – H_2O_2 system (The Fenton reaction).The EESB and ascorbic acid at different concentrations (125-2000µg/ml). The scavenging of hydroxyl by the extracts was increased in dose dependent manner. Table IV andgraph:3 illustrates a significant decrease in the hydroxyl radical due to the scavenging ability of extracts and ascorbic acid. The ethanolic extracts showed maximum activity of 57.12% at 125 µg /ml, whereas ascorbic acid at the same concentration exhibited 69.77% inhibition. The IC_{50} values were found to be 425µg/ml and 525 µg/ml for ethanolic extract and ascorbic acid respectively.

CONCLUSION

This study reports the phytochemical analysis and antioxidant activities of the ethanolic extracts of *Strobilanthes barbatus* for the first time. The ethanolic extract of *Strobilanthes barbatus* shows better antioxidant activity by means of three methods such as DPPH method,Superoxide scavengingand Hydroxyl scavenging method due to their percentage inhibition in free radical scavenging ability.

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