



PHYTOCHEMICAL SCREENING, ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF *STROBILANTHES HEYNEANA* NEES (ACANTHACEAE)

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ABSTRACT

Objectives: The present study was carried out to evaluate antifungal and antioxidant activity of leaf extract of *Strobilanthes heyneana* Nees. (Acanthaceae). **Methods:** The shade dried, and powdered leaf material was extracted by maceration technique using methanol. Antifungal activity was determined by poisoned food technique against two seed-borne fungi. Antioxidant activity was evaluated by DPPH free radical scavenging and Ferric reducing assays. **Results:** Preliminary phytochemical analysis revealed the detection of alkaloids, flavonoids, tannins, saponins, sterols and triterpenoids. Extent of reduction of mycelial growth of *Aspergillus niger* and *Bipolaris* sp. by leaf extract was found to be >50%. Extract was shown to exhibit marked dose dependent radical scavenging and ferric reducing potential. **Conclusions:** Leaf extract of *S. heyneana* was promising in terms of antifungal and antioxidant potential. Further studies are to be undertaken to isolate active principles from the leaves and to investigate their biological activities.

KEY WORDS

Strobilanthes heyneana Nees., Maceration, Poisoned food technique, DPPH, Ferric reducing

INTRODUCTION

Plants have been extensively used since time immemorial for various purposes such as medicine, food, fodder, spices and construction tools. In various systems of medicine, plants have been used in certain formulations to treat several ailments or disorders. A majority of population, especially those living in remote places and having no access for modern medicines, rely on plant based traditional medicine for primary healthcare. It is well known that drugs such as vincristine, vinblastine, artemisinin, quinine, digoxin, reserpine and morphine are from plant origin. Herbal medicines are given more importance even in Western population owing to their negligible or no side effects. Advancement in bioanalytical techniques resulted in isolation and identification of several bioactive metabolites from higher plants. Crude extracts and

isolated components from plants exhibit a range of bioactivities including antioxidant and anticancer activity [1-12].

The genus *Strobilanthes* belongs to the family Acanthaceae and is one of the largest genera in the family. The genus comprises of several hundreds of species among which many are native to India. The genus encompasses certain species that bloom after several years [13,14,15]. Species of *Strobilanthes* have been used ethnobotanically for various purposes [13,16-21]. Several phytochemicals have been identified in members of *Strobilanthes* [22-28]. Species of *Strobilanthes* have been shown to exhibit various biological activities such as antiviral, antibacterial, antifungal, acetylcholine esterase inhibitory, anti-obesity, anti-inflammatory, anti-osteoarthritic, anticancer, antioxidant, antidiabetic, antinociceptive,

antihemolytic, antipyretic, hepatoprotective and antilipidemic activity [16,22,23,25,26,27,29-41].

Strobilanthes heyneana Nees. [*Nilgirianthus heyneanus* (Nees) Bremek], belonging to the family Acanthaceae, is an undershrub growing to a height of 1 meter. The plant is common in forests of Western Ghats of India. Stems are quadrangular and stout. Leaves are 15x7.5cm, unequal, broadly elliptic or ovate, base attenuate, acuminate at apex and serrate. Petiole is up to 7.5cm long. Flowers are in spikes. Flowering occurs annually during October to December. Calyx is 5-6mm long with lobes linear-oblong. Corolla is approximately 2cm in length, white flushed with pale lilac or purple in color and funnel shaped. Stamens are 4 in number. Fruit is a capsule; around 10mm in length, 4-seeded and seeds are pilose on the margins [14]. The plant is considered as alternative source for Sahachar i.e. *Barleria prionitis* [42]. The whole plant is used traditionally for treating gout, pruritus, rheumatoid arthritis and diseases of

nervous system [43]. The plant is shown to exhibit antihemolytic [38], antioxidant [38], and hypoglycemic [44] activity. In the present study, we evaluated antifungal and antioxidant potential of methanolic extract obtained from leaves of *S. heyneana*.

MATERIALS AND METHODS

Collection, identification and extraction of plant material

The plant *S. heyneana* (Figure 1) was collected in the month of January 2018 at Haniya, Hosanagara taluk, Shivamogga district, Karnataka. The plant was identified by Dr. Vinayaka K.S, Principal, KFGC, Shikaripura. The leaves were separated, washed, dried under shade, powdered and extracted by maceration process using methanol [11]. The crude leaf extract was screened for the detection of phytochemicals by standard tests [45-48].



Figure 1: *Strobilanthes heyneana* Nees (Photograph by Prashith Kekuda)

Antifungal activity of leaf extract

Antifungal potential of leaf extract of *S. heyneana* was evaluated against two seed-borne fungi viz. *Aspergillus niger*, and *Bipolaris* sp. by poisoned food technique [11]. Extent of reduction in mycelial growth (%) of test fungi was calculated using the formula:

Antifungal activity (%) = $(D_c - D_t / D_c) \times 100$, where 'Dc' and 'Dt' denotes the colony diameter of test fungi in control and poisoned plates respectively.

Antioxidant activity of leaf extract

DPPH radical scavenging assay

The potential of various concentrations (12.5-200µg/ml of methanol) of leaf extract and ascorbic acid to

scavenge free radicals was evaluated by DPPH radical scavenging assay [11]. The extent of scavenging of DPPH radicals by leaf extract and ascorbic acid (reference antioxidant) was determined using the formula:

Scavenging of DPPH radicals (%) = $(A_0 - A_1 / A_0) \times 100$, where 'A₀' and 'A₁' refers to absorbance of DPPH control (extract replaced by methanol) and absorbance of DPPH in the presence of extract/ascorbic acid.

Ferric reducing assay

The reducing potential of various concentrations (12.5-200µg/ml of methanol) of leaf extract and ascorbic acid was evaluated by ferric reducing assay [11]. Absorbance of reaction mixture was measured at 700nm. An

increase in absorbance on increasing concentrations of extract and ascorbic acid indicates reducing power.

Statistical analysis

Experiments were done in triplicates and the results are presented as Mean \pm Standard deviation (S.D) of three trials.

RESULTS AND DISCUSSION

Phytochemicals in leaf extract of *S. heyneana*

Plants produce a myriad of metabolites and these metabolites are classified into primary and secondary metabolites. Metabolites such as alkaloids, polyphenolic compounds and terpenes are known to

exhibit a range of bioactivities including antimicrobial, antioxidant and anticancer activity and are shown to be responsible for therapeutic potential of plants. Hence, it is important to screen the plants for the presence of secondary metabolites [8,49-53]. In the present study, leaf extract of *S. heyneana* was screened for phytoconstituents by standard phytochemical analyses. Preliminary phytochemical analysis of leaf extract revealed the presence of all phytochemicals except glycosides (Table 1). Study of Fernandes and Sellappan [28] revealed the presence of alkaloids, saponins, terpenoids, flavonoids and phytosterols in the leaves of *S. heyneanus*. Krishnamoorthy et al. [38] identified phenolics, flavonoids, saponins, tannins and vitamin C.

Table 1: Phytochemicals detected in leaf extract

Phytochemical	Leaf extract
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Sterols	+
Glycosides	-
Triterpenoids	+
'+' Detected; '-' Not detected	

Antifungal activity of leaf extract of *S. heyneana*

Control of phytopathogenic fungi commonly employs the use of synthetic fungicides which is reported to have certain drawbacks including emergence of fungicide resistant strains, and adverse effects on humans. Higher plants seem to be potential alternatives for chemical agents as many studies revealed the inhibitory activity of crude solvent extracts and purified compounds from plants against a range of phytopathogenic fungi [11,54-61]. Poisoned food technique, one of the widely used antifungal assays, was used to evaluate antifungal activity of *S. heyneana* (1mg extract/ml of potato dextrose agar medium) against two seed-borne fungi. Poisoning of medium with the leaf extract caused a drastic reduction in the mycelial growth of test fungi

when compared to the growth of fungi in control plates. The leaf extract was effective in causing >50% reduction of mycelial growth of test fungi. The extent of inhibition of *A. niger* and *Bipolaris* sp. was found to be 60.43% and 61.31%, respectively (Figure 2; Table 2). In an earlier study, Honda and Tabata [62] isolated an antifungal compound tryptanthrin from *S. cusia* which has shown to exhibit activity against dermatophytes. Venkatachalapathi and Ravi [63] revealed the potential of petroleum ether and methanol extract of *S. ciliatus* to inhibit dermatophytic fungi. Mangang and Chhetry [32] showed the antifungal potential of *S. flaccidifolius* against *Rhizoctonia solani*, causal agent of root rot of French bean.

Table 2: Colony diameter of test fungi in control and poisoned plates

Test fungi	Colony diameter in cm	
	Control	Leaf extract
<i>A. niger</i>	5.56 \pm 0.05	2.20 \pm 0.00
<i>Bipolaris</i> sp.	4.73 \pm 0.05	1.83 \pm 0.05

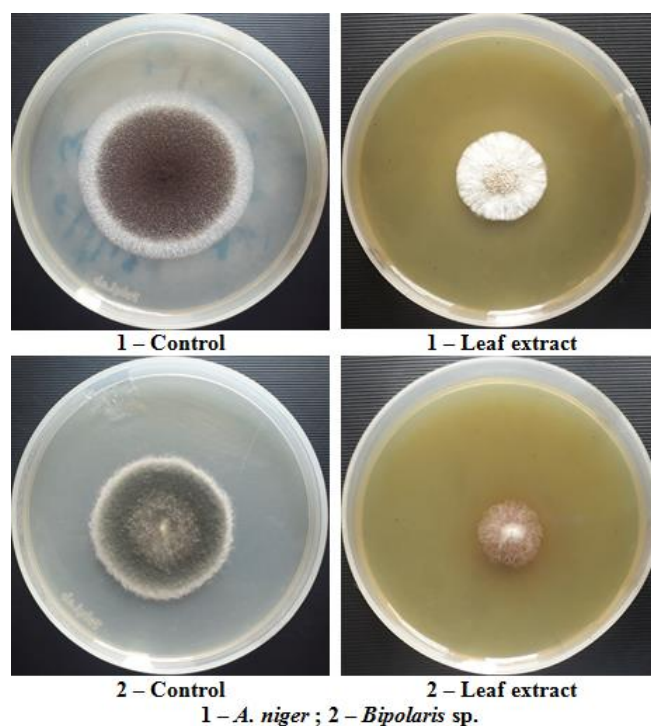


Figure 2: Growth of test fungi in control and poisoned plates

DPPH radical scavenging activity of leaf extract of *S. heyneana*

The method of scavenging of stable, organic, nitrogen centred DPPH radicals is widely used to evaluate antiradical activity of extracts and purified metabolites of higher plants [11,64-70]. Figure 3 shows the scavenging potential of leaf extract and ascorbic acid against DPPH free radicals. Both leaf extract and ascorbic acid scavenged radicals dose dependently with >50% scavenging activity at 12.5µg/ml and higher. A

scavenging activity of >90% was observed at concentration 100µg/ml of both extract and ascorbic acid. Scavenging activity of ascorbic acid was higher than that of leaf extract. In an earlier study, the acetone, ethanol and water extracts of leaves of *S. heyneana* were shown to scavenge DPPH radicals with IC₅₀ values viz. 342.6, 247.8 and 121.0µg/ml, respectively [38]. It is shown that *S. ciliatus* [71], *S. barbatus* [72], *S. crispus* [73], *S. asperimus* [35] and *S. sessilis* [17] exhibit DPPH radical scavenging potential.

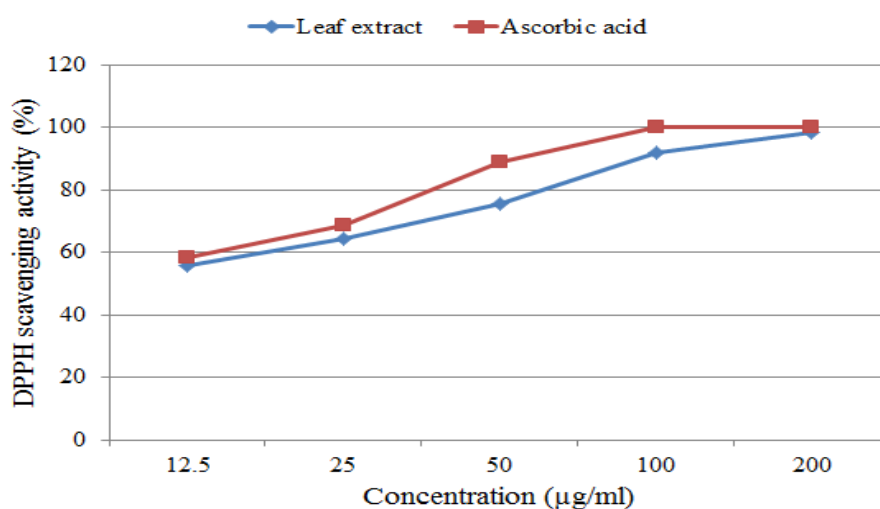


Figure 3: DPPH radical activity of leaf extract and ascorbic acid

Ferric reducing activity of leaf extract of *S. heyneana*

The reducing ability is due to the presence of reductones in the samples and the reducing potential is considered as a significant indicator of antioxidant activity. Substances that have reducing ability will react with potassium ferricyanide ($K_3Fe^{3+}(CN)_6$) to form potassium ferrocyanide ($K_4Fe^{2+}(CN)_6$) which on reacting with ferric chloride forms a ferric-ferrous complex (Perl's Prussian blue) having an absorption maximum at 700nm. The capability of crude extracts of to exhibit ferric reducing activity is widely followed to evaluate antioxidant activity of plants [11,64-70,74,75,76]. In the present study, the reducing property of leaf extract was

determined by measuring the absorbance due to the formation of Perl's Prussian blue complex. The result of ferric reducing activity of leaf extract and ascorbic acid is shown in Figure 4. It was observed that, on increasing the concentration of extract, an increase in the absorbance was observed. The reducing potential of extract was lesser than that of ascorbic acid. The study of Krishnamoorthy et al. [38] revealed ferric reducing ability of aqueous, acetone and ethanol extract of *S. heyneana* leaves. Other species of *Strobilanthes* such as *S. sessilis* [11], *S. crispus* [29] were found to exhibit ferric reducing efficacy.

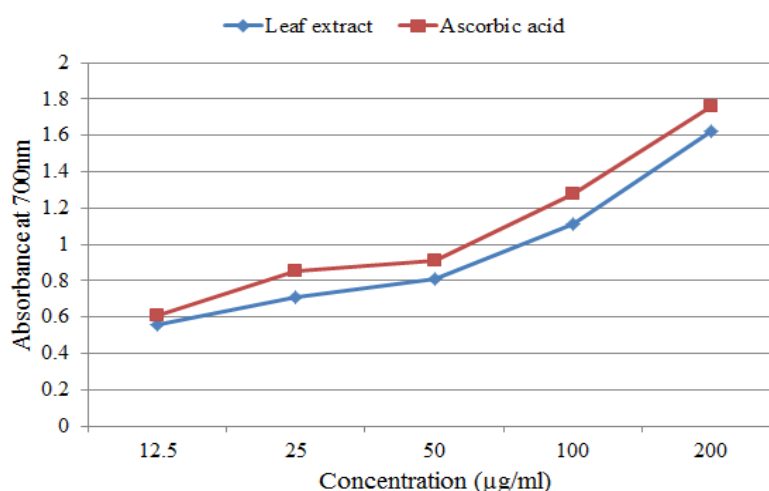


Figure 4: Ferric reducing activity of leaf extract and ascorbic acid

CONCLUSIONS

The leaf extract of *S. heyneana* showed promising antifungal and antioxidant activity in this study. The observed activities could be related to the presence of secondary metabolites that have been detected in the leaf extract. Isolation of bioactive principles from leaf extract and their bioactivity demonstrations are to be conducted in future studies.

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None

CONFLICTS OF INTEREST

None declared

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