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Strobilanthes heyneanus root extract as a potential source for antioxidant and antimicrobial activity

Vijayakumar Sundaram¹, Selvaraju Sadhasivam^{1*}, Sivaraj Chandrasekaran², Raaman Nanjian³ and Arjun Pandian⁴

Abstract

Background: *Strobilanthes heyneanus* (*Nilgiranthus heyneanus*) belongs to the family Acanthaceae that contains many species with potential for diverse medicinal uses. It is also called 'Karun kurinji' and is commonly found in the South-West regions of India. The species are commonly used in rheumatic complaints, sprain of the ankle, and hernia. The objectives of the study were to evaluate the antioxidant activity, phytochemical analysis, and antibacterial activities of the root extract of *S. heyneanus*.

Results: The radical scavenging and reduction assays such as DPPH• radical and OH• radical scavenging assays, as well as phosphomolybdenum reduction and Fe³⁺ reducing power assays, were determined for the root extract. The highest DPPH• radical scavenging activity was 88.23 ± 1.32 at 120 µg/mL concentration, and the calculated IC₅₀ was 38.52 µg/mL concentration. The highest OH• radical scavenging activity was 51.28 ± 1.06 at 120 µg/mL concentration, and the calculated IC₅₀ was 51.28 µg/mL concentration. The highest ABTS•⁺ radical scavenging activity was 91.28 ± 1.12 at 30 µg/mL concentration, and the calculated IC₅₀ was 33.92 µg/mL concentration. The highest phosphomolybdenum reduction was 87.43 ± 0.90 at 120 µg/mL concentration, and the calculated RC₅₀ was 24.74 µg/mL concentration. The highest Fe³⁺ reduction was 89.38 ± 0.98 at 120 µg/mL concentration, and the calculated RC₅₀ was 31.06 µg/mL concentration. The antibacterial activity of *S. heyneanus* showed the highest zone of inhibition of 24 mm for *Salmonella typhi* with 500 µg/mL concentration. The cytotoxicity limits of the root extracts were tested by MTT assay using human dermal fibroblast cell lines, reflecting > 90% cell viability at a concentration of 500 µg/mL.

Conclusions: The current study showed that the root extract of *S. heyneanus* has better antioxidant properties and potential anti-bacterial compounds. The phytochemical analysis of the root extract showed the presence of alkaloids, steroids, terpenoids, phenols, flavonoids, tannins, saponins, glycosides, and carbohydrates, which are responsible for the antibacterial root extract of *S. heyneanus* synergistically.

Keywords: *Strobilanthes heyneanus*, Phytochemicals, DPPH radical, MIC, MTT assay, Antioxidant, Antibacterial

Statement of novelty

The roots of *Strobilanthes heyneanus* are extremely used in the Ayurvedic drug preparation, one of the most efficient plant species available in India, and its pharmacological activity is not much revealed so far. The results

of antimicrobial activity revealed that the species has potential antibiotics against infections caused by the pathogens. Thus, our interest is to explore the pharmacological potential of bioactive compounds from the plant, *Strobilanthes heyneanus*, as a natural killer against disease to save people. This information on the species is useful for the discovery of the new drug for the pharmacological industry.

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Background

The family *Acanthaceae* contains several species with a potential diversity of ethnobotanical uses. One of the important species in this family is *Strobilanthes heyneanus* (*Nilgirianthus heyneanus*) mostly found in the southwest regions of India, commonly called Karun kurinji [1]. Ayurveda drugs prepared from this species are useful. They are commonly used in rheumatic complaints, sprain of the angle, and hernia. *S. heyneanus*, also called Karun Kurinji, is an undershrub, nearly a meter high, with a grooved stem, often covered with hairs. The leaves are oppositely arranged, unequal, ovate, and hairy and have a serrated margin. The flowers are blue urn-shape of 1–1.2 cm long, which occur in axillary spikes, and five sepals are combined at the base. The flower tube is swollen in the middle which is a white colour and has five rounded petals. In the flower, the stamens are 4, filaments are hairy at the base, and the capsule is 6–8 mm long, oblong, and 4-seeded. Karun kurinji is mostly found in the South-West of India and thought to be a type of Kurinji. Like Kurinji, it has a long flowering cycle and normally flowering from September to December [2]. *S. heyneanus* roots and leaves are used to treat leprosy, to control blood sugar, urinal problems, jaundice, inflammation, and excess menses [3]. The members of the genus *Strobilanthes* are used as an anti-diabetic, diuretic, laxative, and potent antimicrobial agents [4] (Fig. 1).



Fig. 1 Habitat of *Strobilanthes heyneanus* Nees. Taxonomic classification: kingdom, plant kingdom; division, angiosperms; class, Dicotyledoneae; subclass, Gamopetalae; family, Acanthaceae; genus, *Strobilanthes*; species, *heyneanus*

Methods

Plant collection and preparation of the extract

S. heyneanus plants were collected from Palchuram, Kottiyoor Road, Wayanad, Kerala, India. The specimen was deposited at the Centre for Repository of Medicinal Resources (C-RMR), Bangalore (Voucher No. FRLH 120018). The roots were separated, washed in distilled water, and dried for 2 days. The roots were powdered by using the mechanical blender and immersed in methanol for 3 days at room temperature. The clear supernatant above the plant powder residue was drained out through the filter paper and condensed by the rotary evaporator at 50 °C, which yields blood-red colour viscous extract [5, 6].

Phytochemical investigation

The root extract of *S. heyneanus* was tested for phytochemical analysis for different categories of secondary metabolites of phytochemicals using specific chemical reagents [7, 8].

Chemicals and reagents

DPPH, ABTS, and Folin–Ciocalteu phenol reagents were procured from SRL India. Quercetin, sodium carbonate (Na_2CO_3), absolute ethanol, Mueller-Hinton (MH) agar, and gallic acid were obtained from Himedia, India. The other analytical grade common chemicals were purchased from the local vendors (Chennai, India).

Total phenol estimation

The total phenolic content was determined by the Folin–Ciocalteu reagent method with small modifications [9]. One hundred microlitres of the root extract (1 mg/mL) of *S. heyneanus* was mixed with 900 μL of methanol and 1 mL of 1:10 diluted, Folin–Ciocalteu reagent. Next, 1 mL of 20% (w/v) Na_2CO_3 solution was added and shaken well. The reaction mixture was incubated in dark for 30 min at room temperature. At 765 nm, the absorbance was measured and the total phenolic content was calculated as gallic acid equivalent ($\mu\text{g}/\text{mg}$ of extract).

Total flavonoid estimation

The total flavonoid content of the root extract of *S. heyneanus* was estimated by the aluminium chloride reagent method with small modifications [10]. Five hundred microlitres of extract (1 mg/mL) was mixed with 500 μL of methanol, and to this, 0.5 mL of 5% (w/v) sodium nitrite solution was added. Next, 500 μL of aluminium chloride solution (10%, w/v) was added, followed by 50 μL of 1 M NaOH solution and shaken well. The reaction mixture was incubated for 30 min at room temperature, and the absorbance was measured at 510

nm. The flavonoid content was calculated as quercetin equivalent ($\mu\text{g}/\text{mg}$ of extract).

DPPH \bullet radical scavenging activity

The antioxidant activity of the root extract of *S. heyneanus* was measured by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay method [11]. One millilitre of the root extract with different concentrations (20–120 $\mu\text{g}/\text{mL}$) was mixed with 1 mL of 0.1 mM DPPH solution in methanol. One millilitre of DPPH solution mixed with 1 mL of methanol was used as the control. Then, the mixture was incubated in dark for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as the standard. The percentage of inhibition was calculated as:

$$\begin{aligned} \text{\% of DPPH}^\bullet \text{ radical inhibition} \\ = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100 \end{aligned}$$

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was analysed by the salicylic acid method with some minor modifications [12]. To 1 mL of various concentrations of the extract, 300 μL of FeSO_4 (8 mM) solution and 250 μL of H_2O_2 (20 mM) were added and shaken well. To start the reaction, 250 μL of salicylic acid in methanol (3 mM) was added, and then, the reaction mixture was incubated at 37 °C for 30 min. Then, 200 μL of distilled water was added, and the mixture was centrifuged at 5000 rpm for 5 min. At 510 nm, the absorbance of the supernatant was measured and the percentage of OH^\bullet radical scavenging activity was calculated as:

$$\text{\% of OH}^\bullet \text{ radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

ABTS \bullet^+ radical cation scavenging activity

The antioxidant ability of the root extract of *S. heyneanus* was evaluated by the ABTS \bullet^+ radical cation scavenging assay method with slight modifications [13]. ABTS \bullet^+ radical cation was obtained by 7 mM of ABTS reacted with 2.45 mM of potassium persulfate in 200 mM of PBS (pH 7.4) and incubated for 12–16 h in the dark before use. The ABTS \bullet^+ radical cation solution was diluted with PBS and set an absorbance of 0.70 ± 0.02 at 734 nm. Diluted ABTS \bullet^+ radical cation solution was prepared freshly every day for this assay. Different concentrations (5–30 $\mu\text{g}/\text{mL}$) of the root extract was mixed with 500 μL of diluted ABTS \bullet^+ radical cation solution, and after 5 min, the absorbance was measured at 734 nm. Ascorbic acid was used as the standard reference. The ABTS \bullet^+ radical cation scavenging activity was expressed as:

$$\text{\% of ABTS}^{\bullet+} \text{ radical cation inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Phosphomolybdenum reduction assay

The antioxidant capacity of the root extract of *S. heyneanus* was assessed by phosphomolybdenum reduction assay method [14]. One millilitre of various concentrations of the root extract (20–120 $\mu\text{g}/\text{mL}$) was mixed with 1 mL of reagent solution including ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulphuric acid (600 mM). The reaction mixture was incubated in a water bath at 95 °C for 90 min, and the absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as the standard. The percentage of Mo^{6+} reduction was calculated as:

$$\text{\% of Mo}^{6+} \text{ reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

Ferric (Fe^{3+}) reducing power assay

The assay for estimating the reducing power of the root extract of *S. heyneanus* was the potassium ferricyanide method with minor modifications [15]. One millilitre of the root extract of different concentrations (20–120 $\mu\text{g}/\text{mL}$) was mixed with 1 mL of potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (1% w/v) solution and 1 mL of phosphate buffer (0.2 M, pH 6.6) solution. Then, the mixture was incubated at 50 °C in a water bath for 20 min. Five hundred microlitres of trichloroacetic acid (10% w/v) was added to each mixture followed by 100 μL of freshly prepared FeCl_3 (0.1%, w/v) solution which was added and shaken well. The absorbance was measured at 700 nm. Ascorbic acid was used as the standard. The percentage of Fe^{3+} reduction was calculated as:

$$\text{\% of Fe}^{3+} \text{ reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

Antibacterial activity

Organisms

The Gram-positive organisms such as *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 98), as well as the Gram-negative organisms such as *Salmonella typhi* (MTCC 734) and *Klebsiella pneumonia* (MTCC 109), were chosen for the antibacterial activity study.

Standard

Tetracycline was used as the standard compound for all the bacterial cultures.

Mueller-Hinton agar

MH agar was procured from HiMedia (HIMEDIA-M173-500 G) to make up the medium for bacteria. The

media was calculated (38.0 g/L) depending upon the availability of strains and suspended in 150 mL of distilled water in a conical flask, stirred, boiled to dissolve, and then autoclaved at 15 lbs and 121 °C for 15 min. The pH range was between 7.0 and 7.5 [16]. The hot medium was dispensed in sterile petri plates which were kept in sterile laminar airflow chamber and allowed to solidify for 15 min.

Disc diffusion method

Antibacterial activity of root extract of *S. heyneanus* was carried out using the agar disc diffusion method. The MHA in the petri plates was inoculated by dispensing the inoculum using sterilised cotton swabs which are previously immersed in the inoculum containing test tube and spread evenly onto the solidified agar medium. Five sterile discs were taken, and the root extract was loaded onto the discs with different concentrations of 250 µg/mL, 375 µg/mL, and 500 µg/mL concentrations. The discs loaded with extract were placed onto an organism swabbed medium in the petri plates which were incubated for 24 h at 37 °C. The antibacterial activity was expressed by measuring the diameter zone of inhibition formed around the disc [17, 18]. Tetracycline (25 µg) was used as a positive control (standard).

Minimum inhibitory concentration

The antimicrobial activity of the root extract was determined by resazurin microtiter assay method [19]. The inoculum, equivalent to a 0.5 McFarland turbidity standard, was prepared from each bacterial isolate in MH broth. Sterile PBS (50 µL) was dispensed in each well of a sterile 96-well flat bottom plate. The root extract of various concentrations from 1000 µg/mL to 0.48 µg/mL was added by twofold serial dilutions. Tetracycline was used as the positive control in separate wells. Separate wells with negative control (culture with no plant extract) were also performed. To each well, 10 µL of resazurin indicator solution was added, followed by the addition of 30 µL of threefold strength MH broth. The plates were incubated at 37 °C in an incubator for 12–24 h for further growth if any was detected. The change in colour of the dye added to the wells from blue to pink indicated the growth of bacteria. The MIC was defined as the lowest concentration of each test compound or drug that prevented any colour change observed visually, i.e., the concentration that inhibited the microbial growth.

Cytotoxicity

MTT assay

Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, human dermal fibroblast (HDF)

cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then, the culture supernatant was removed and RPMI containing root extract of *S. heyneanus* of various concentrations of 10–500 µg/mL was added and incubated for 48 h. After treatment, the cells were incubated with MTT (10 µL, 5 mg/mL) at 37 °C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer [20]. Data are represented as the mean values for three independent experiments:

$$\text{Cell viability (\%)} = \left(\frac{\text{Mean OD}}{\text{Control OD}} \right) \times 100$$

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using Graph Pad Prism 8.0.1. Differences in the mean between observations were considered significant at level $p < 0.05$. All values acquired are cumulative of three replicates, and it was represented as a mean \pm standard deviation.

Results

Qualitative phytochemical analysis

The phytochemical analysis of the root extract of *S. heyneanus* showed the presence of compounds such as alkaloids, steroids, terpenoids, phenols, flavonoids, tannins, saponins, glycosides, and carbohydrates (Table 1).

Total phenols and flavonoids

Many phenolic acids and flavonoids possess antimicrobial, antiviral, and cytotoxic activity and have been reported to stimulate the secretion of bile acids and to prevent enzymatic reactions [21]. The total phenolic content of methanol root extract was 126.53 ± 2.29 µg/mg of GAE of extract, and the flavonoid content of methanol root extract was 32.79 ± 0.62 µg/mg of QE of extract (Table 2).

DPPH• radical scavenging activity

The DPPH radical is a stable free radical due to the delocalization of an odd electron over the molecule, thus preventing dimer formation. This radical is used in the DPPH radical scavenging assay to measure the ability of antioxidants to quench the DPPH free radical. The dark purple colour of DPPH will be changed to yellow when it is reduced to non-radical [22]. The reducing capacity increases by increasing the concentration of the extract, and the highest DPPH• radical scavenging activity was $88.23 \pm 1.32\%$ at 120 µg/mL concentration (Supplementary Information, Table S1). The methanol root extract of *S. heyneanus* has a reliable radical scavenging ability,

Table 1 Phytochemical analysis summary of root extract of *Strobilanthes heyneanus*

Phytochemicals	Test	Inference	Results
Alkaloids	Hager's test: To the extract, a saturated aqueous solution of picric acid was added and shaken well.	Formation of yellow precipitate	+
Steroids	Liebermann-Burchard's test: The extract was mixed with 1 mL of acetic anhydride and shaken well. To this, few drops of conc. H_2SO_4 were added along the sides of the test tube.	Appearance of dark violet colour	+
Terpenoids	Salkowski test: To the extract, chloroform was added and mixed well. Then, a few drops of conc. H_2SO_4 were added along the sides of the test tube.	Appearance of red ring	+
Phenols	$FeCl_3$ test: To the extract, a few drops of 5% $FeCl_3$ solution was added and shaken well.	Appearance of violet colour	+
Flavonoids	Alkaline reagent test: To the extract, a few drops of 2% NaOH solution was added and shaken well.	Appearance of yellow colour	+
Tannins	Lead acetate test: To the extract, a few drops of 5% $Pb(CH_3COO)_2$ solution was added and shaken well.	Appearance of white colour	+
Saponins	Foam test: To the extract, 3 mL of distilled water was added and shaken vigorously.	Appearance of foam	+
Glycosides	Legal's test: To the extract, few drops of pyridine and few drops of alkaline sodium nitroprusside solution was added and shaken well.	Appearance of blood red colour	+
Carbohydrates	Molisch test: To the extract, two drops of alcoholic α -naphthol solution was added and shaken well. To this, a few drops of conc. H_2SO_4 was added.	Appearance of violet ring	+

(+) = present; (–) = absent

and the IC_{50} was 38.52 μ g/mL concentration (Fig. 2). The IC_{50} was compared with the standard quercetin (IC_{50} = 3.11 μ g/mL concentration).

OH[•] radical scavenging activity

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and massive damage in proteins due to the reduction of disulphide bonds, specifically fibrinogen. In living organisms, hydroxyl radical is continuously formed in a reduction process of oxygen into the water, and it causes many diseases such as cancer, atherosclerosis, and neurological disorders and can be prevented by the antioxidant substances [23]. The highest OH[•] radical scavenging activity of root extract was 51.28 \pm 1.06% at 120 μ g/mL concentration (Supplementary Information, Table S1), and the IC_{50} was 117 μ g/mL concentration (Fig. 2). The IC_{50} was compared with the standard quercetin (IC_{50} = 4.21 μ g/mL concentration).

ABTS^{•+} radical cation scavenging activity

The antioxidant activity of the root extract was calculated by the decolorization of blue-green colour ABTS^{•+} radical cation, which was measured

spectrophotometrically at 734 nm. The root extract was added to the preformed ABTS^{•+} radical cation and can be reduced to ABTS in a dose-dependent manner [24]. The root extract showed the highest ABTS^{•+} radical cation scavenging activity of 91.28 \pm 1.12% at 60 μ g/mL concentration, and the IC_{50} was 33.92 μ g/mL concentration (Fig. 3; Supplementary Information Table S2). The IC_{50} was compared with the standard quercetin (IC_{50} = 4.21 μ g/mL concentration).

Phosphomolybdenum reduction activity

The reducing power of the root extract possesses various mechanisms such as the prevention of chain initiation, breakdown of peroxides, and inhibition of oxidation [25]. The highest phosphomolybdenum reduction of root extract was 87.43 \pm 0.90% at 120 μ g/mL concentration, and the RC_{50} was 24.74 μ g/mL concentration (Fig. 4; Table S3). The RC_{50} was compared with the standard quercetin (RC_{50} = 6.34 μ g/mL concentration).

Ferric (Fe^{3+}) reducing power assay

The root extract showed strong antioxidant activity due to the presence of phenolic compounds such as phenolic acids and flavonoids, which can donate electrons readily to the oxidising compounds [26]. The highest Fe^{3+} reduction was 89.38 \pm 0.98% at 120 μ g/mL concentration, and the RC_{50} was 31.06 μ g/mL concentration (Fig. 4; Supplementary Information Table S3). The RC_{50} was compared with the standard quercetin (RC_{50} = 7.72 μ g/mL concentration).

Table 2 Estimation of phenols and flavonoids from root extract of *S. heyneanus*

Phytochemicals	Amount (μ g/mg)
Phenols	126.53 \pm 2.29 GAE
Flavonoids	32.79 \pm 0.62 QE

GAE Gallic acid equivalent, QE Quercetin equivalent

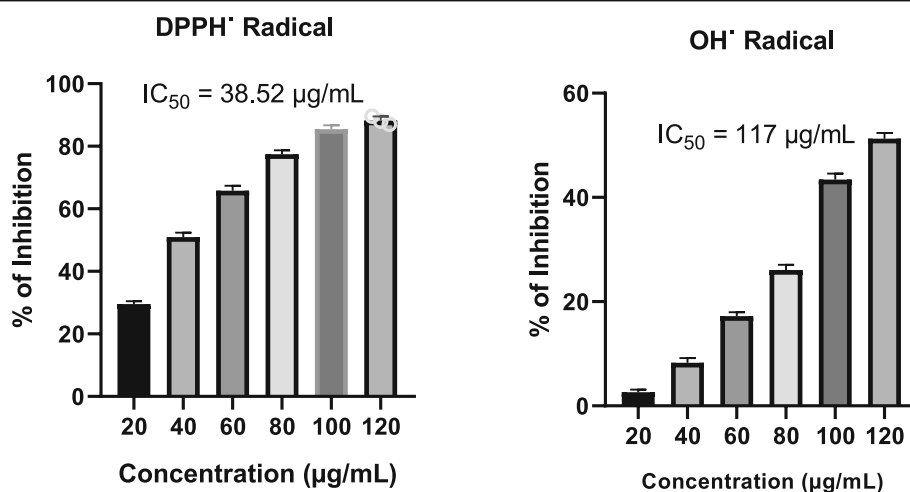


Fig. 2 DPPH[•] radical and OH[•] radical scavenging activities of the root extract of *S. heyneanus*. The results represent mean of triplicates along with standard deviation. Statistical significance—for DPPH[•], $t = -62.892$ and p value = $3.829\text{e-}07$; for OH[•], $t = -71.485$ and p value = $2.295\text{e-}07$

Antibacterial activity

Evaluation of the antibacterial activity of methanol root extract of *S. heyneanus* was determined by the disc diffusion method against the Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* as well as the Gram-negative bacteria such as *Salmonella typhi* and *Klebsiella pneumoniae*. The effectiveness of the root extract on bacterial strains was determined by calculating

the zone of inhibition (Fig. 5). The root extract showed the highest zone of inhibition of 24 mm against *Salmonella typhi* at 500 µg/mL concentration (Table 3).

Determination of MIC

Staphylococcus aureus and *Salmonella typhi* showed no change in colour of resazurin indicator at a concentration of 62.25 µg/mL (column 5), which was taken as the MIC (Table 4). Similarly, *Bacillus subtilis* and *Klebsiella pneumoniae* showed no change in colour of resazurin indicator at a concentration of 31.25 µg/mL (column 6), which was taken as the MIC for root extract of *S. heyneanus*. The complete colour change was observed for negative control, indicating the growth of the organism in all wells (Fig. 6) [27]. The presence of blue colour for the positive control in all wells indicates complete inhibition of growth of the organism (Tetracycline).

MTT assay

MTT assay revealed that the root extract of *S. heyneanus* had no cytotoxic effect on HDF cells at the concentrations of 10 and 25 µg/mL (Fig. 7). But the root extract showed only mild toxicity resulting in cell viability of 97.76% at 50 µg/mL and 91.44% cells at 500 µg/mL (Supplementary Information Table S4). The root extract was tested for its cytotoxicity, considering that early screening of plant extract for toxicity might help in the evaluation of its biological and therapeutic value [28] (Fig. 8).

Discussion

Phytochemicals are naturally present in the plants, playing an essential role to defend themselves against various pathogenic microbes by showing antimicrobial activity. The secretion of these secondary metabolites is varying

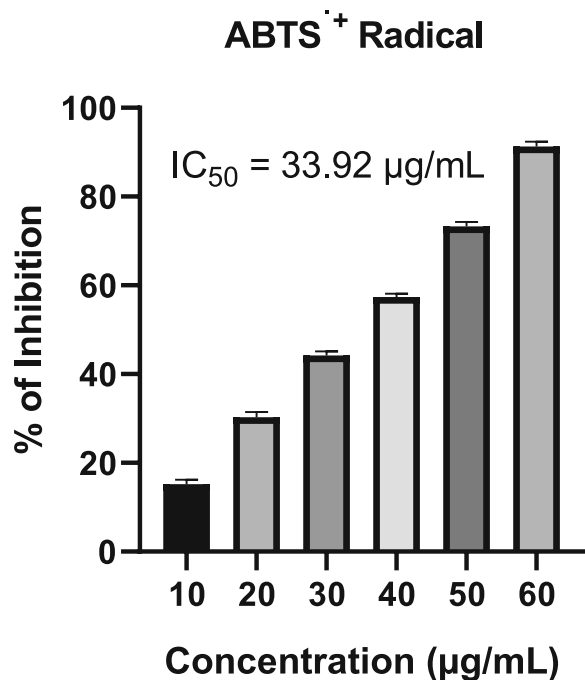


Fig. 3 ABTS^{•+} radical cation scavenging activity of root extract of *S. heyneanus*. Statistical significance, $t = -88.094$ and p value = $9.954\text{e-}08$

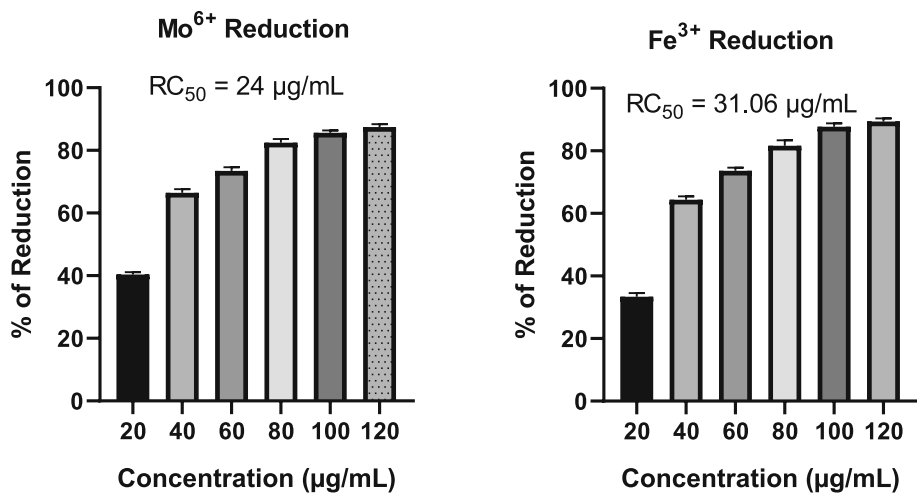


Fig. 4 Phosphomolybdenum reduction and Fe³⁺ reducing power activity of the root extract of *S. heyneanus*. Statistical significance—for phosphomolybdenum, $t = -70.397$ and p value = $2.44\text{e-}07$; for Fe³⁺, $t = -64.639$ and p value = $3.431\text{e-}07$

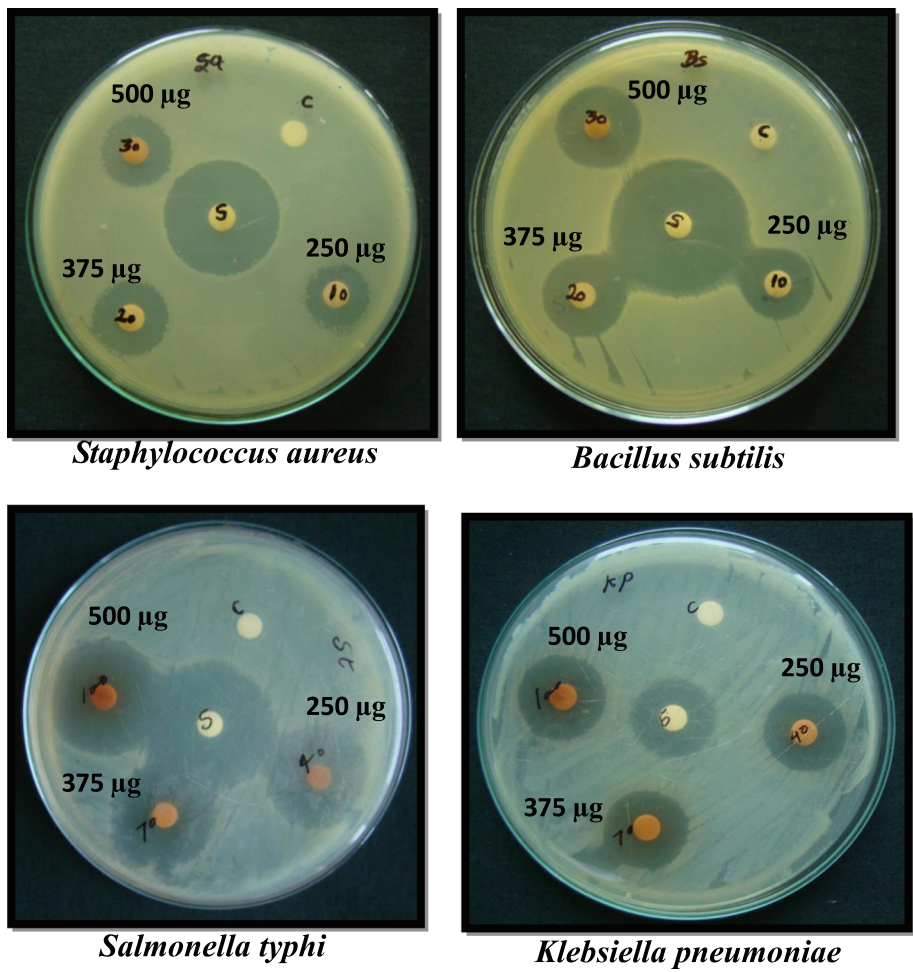


Fig. 5 Antibacterial activity of root extract of *S. heyneanus* against four different bacterial species by disc diffusion tests. S, standard; C, negative control

Table 3 Antibacterial activity of root extract of *S. heyneanus* against selected bacterial strains. NA, no activity

Organism	Zone of inhibition (mm)				Negative control
	250 µg/mL	375 µg/mL	500 µg/mL	Standard (Tetracycline)	
<i>Staphylococcus aureus</i>	12	15	18	25	NA
<i>Bacillus subtilis</i>	14	16	20	30	NA
<i>Salmonella typhi</i>	17	19	24	26	NA
<i>Klebsiella pneumoniae</i>	15	17	22	18	NA

from plant to plant; while some plants produce more, others produce them only in minimal quantities. Some phytochemicals can be harmful and some can be quite helpful for the plants' survival. Phytochemicals in fruits, vegetables, and leaves may reduce the risk of diseases due to the presence of polyphenols, and terpenoids act as antioxidants and antimicrobial and anti-inflammatory agents.

The phytochemical analysis of the root extract showed the presence of terpenoids, phenolic compounds, flavonoids, and tannins [29, 30] which are responsible for antioxidant and antibacterial activity. Antioxidants are substances that may protect cells from ROS (reactive oxygen species) and free radicals which cause damage to cells. The phenolic acids and flavonoids, as effective antioxidants, play an important role in the control of different oxidative human diseases such as cancer inflammation, tissue damage, and DNA injury [31–33]. Antioxidant substances from natural products are good free radical scavengers leading to reduced risk of cancer development and protecting cells against the harmful effects of ROS on macromolecules, such as proteins, lipids, and DNA [34, 35]. Flavonoids and phenolic compounds as potential antioxidants may assist in health promotion by preventing oxidative damage responsible for many diseases [36]. Phenolic compounds increase the antioxidant enzyme activity and indirectly affecting the concentration of harmful oxygen radicals in the living cells and showed anti-inflammatory and anticancer activity [37]. Flavonoids eliminate pathological changes on capillaries and are used for the treatment of hypertension, diabetes, and atherosclerosis.

The radical scavenging ability of the root extract of *S. heyneanus* was carried out by DPPH• (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay method. In this

method, the purple coloured DPPH (1,1-diphenyl-2-picrylhydrazyl) radical solution turned to yellow coloured 1,1-diphenyl-2-picrylhydrazine, after the reduction of DPPH radical by the root extract due to the transfer of H-atom from the extract [38]. The hydroxyl radical is a highly reactive free radical formed in the biological systems and has been capable of damaging proteins in living cells. The hydroxyl radicals can have the capacity to join nucleotides in DNA and can cause strand breakage, which leads to mutagenesis cytotoxicity and carcinogenesis [39]. The Fenton reaction generates hydroxyl radicals that degrade DNA deoxyribose and Fe²⁺ ions as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of radical products. H₂O₂ is a non-free radical, and it can inactivate enzymes by the oxidation of thiol groups. It can cross the cell membrane quickly and slowly oxidise the various cell compounds and generate dangerous OH• radicals by the catalytic reaction using the metal ions Fe²⁺ and Cu²⁺, and this may be the triggering point of all its toxic effects.

ABTS^{•+} radical cation is produced by the reaction between ABTS and potassium persulfate, which is a stable blue-green chromophore, with an absorption at 734 nm. The antioxidants present in the root extract reacts with ABTS^{•+} radical cation and reduces it to ABTS depending on the concentration and reactivity of the antioxidant. Antioxidants reduce the blue-green colour to colourless of the solution which is proportional to the antioxidants present in the root extract [40].

The reducing capacity of *S. heyneanus* root extract was determined by the phosphomolybdenum reduction assay method. The root extract reduces Mo (VI) to Mo (V) during the reaction and the blue or green phosphate/Mo (V) complex developed at acidic pH, which has absorption at 695 nm. The reducing power of Fe³⁺ to Fe²⁺ by the root extract of *S. heyneanus* was measured by the potassium ferricyanide reduction assay method, and the reduction capacity was found to be concentration-dependent. Fe³⁺ reducing power activity showed the electron-donating ability of an antioxidant and subsequent formation of the Ferrous-ferric complex [41].

Table 4 Minimal inhibitory concentration of root extract of *S. heyneanus*

S. no	Organism	MIC (µg/mL)
1	<i>Staphylococcus aureus</i>	62.25
2	<i>Bacillus subtilis</i>	31.25
3	<i>Salmonella typhi</i>	62.25
4	<i>Klebsiella pneumoniae</i>	31.25

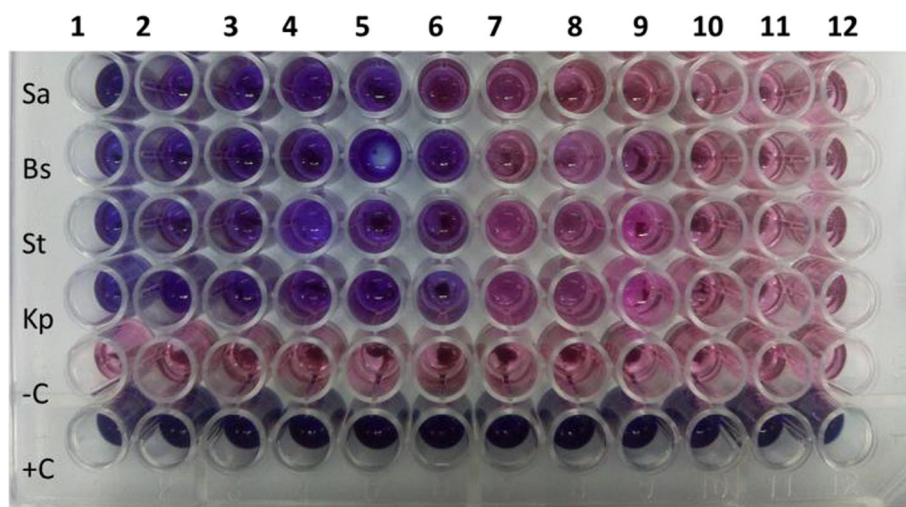


Fig. 6 MIC of root extract of *S. heyneanus*. Sa, *Staphylococcus aureus*; Bs, *Bacillus subtilis*; St, *Salmonella typhi*; Kp, *Klebsiella pneumoniae*; -C, negative control; +C, positive control

The mechanism of action of the plant extracts revealed that plant polyphenols and terpenoids play important roles against bacteria, fungi, and viruses. Antibacterial activity in *S. heyneanus* has not been much reported yet, and the inhibition was reported mainly due to the presence of polyphenols [42]. The mechanism of bacterial inhibition by polyphenols is complex and they can act by chelating iron, which is important for the survival of many bacteria [43]. Antibacterial compounds in the root

extract of *S. heyneanus* work together and producing an effect on microbial membranes, adhesins, enzymes, and cell envelope transport proteins. Polyphenols interact with proteins and/or phospholipids of the pathogens and increasing membrane permeability, modifying ion transport processes, and damaging cell membranes [44, 45]. Plant extracts would help cells to survive under conditions that progressively break intracellular machinery (free radical scavenging activities). On the other hand, the most efficient modern drugs kill cells, without discriminating between normal and cancer cells (free radicals attack). A single healthy cell mutation can provoke its transformation into a malignant phenotype. The MTT assay of the root extract of *S. heyneanus* on dermal fibroblasts cells showed the highest cell viability even at 500 µg/mL concentration [46].

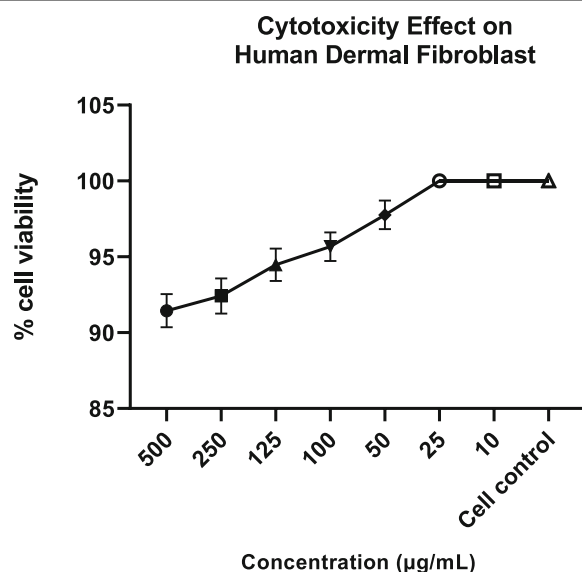


Fig. 7 Cytotoxicity effect of root extracts of *S. heyneanus* on human dermal fibroblast cells. Cell control represents sample without the addition of any extract. Statistical significance— $t = 13.065$ and p value = 0.000169

Conclusion

Several natural products are derived from plants and have been used traditionally to treat many diseases such as wound healing, injuries, and pain. Current synthetic medicine and combinatorial chemistry linked with the new technological tools such as proteomics, genomics, and metabolomics covered the broader use of natural products. Currently, most natural products are produced and industrialised as pharmacologically active agents with potential antioxidant, anti-angiogenic, anti-inflammatory, anti-infective, and anti-carcinogenic properties. Natural products have been engaged as the lead compounds to obtain extremely active pharmacological semi-synthetic derivatives for healing use with increased efficiency. The knowledge and practice from ancient times will significantly move to produce potential drugs

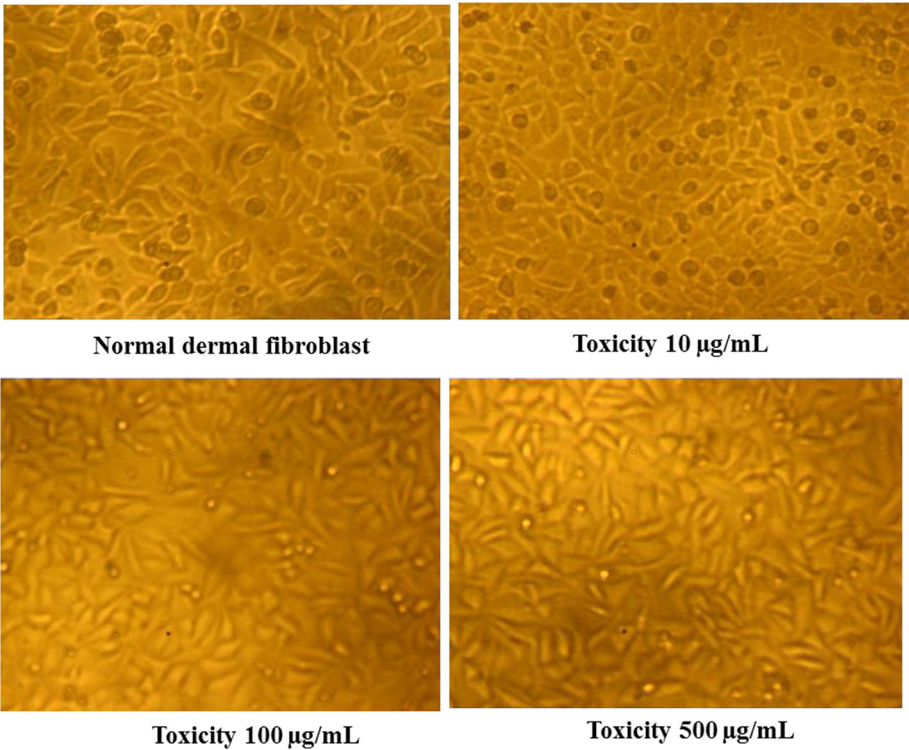


Fig 8 Microscopic image of cytotoxicity tests of human dermal fibroblast with the root extracts of *S. heyneanus*

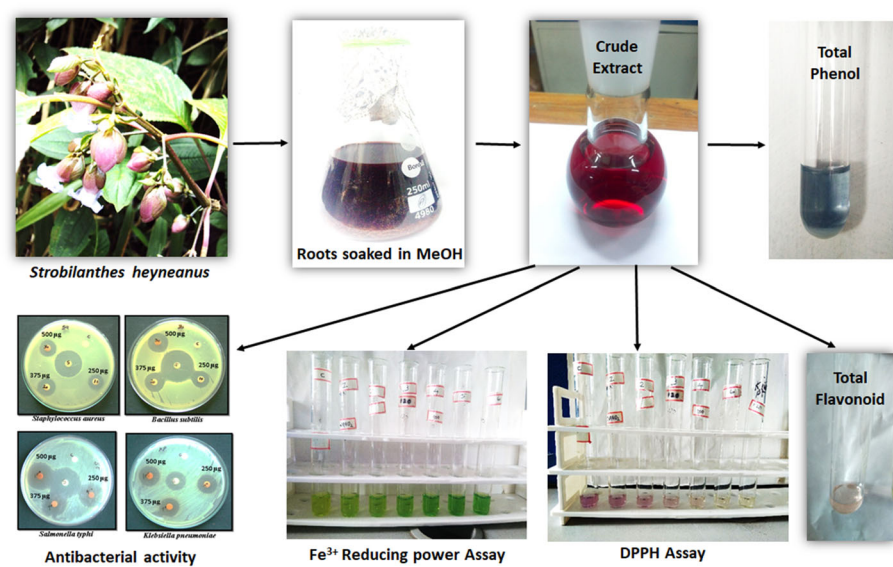


Fig. 9 ToC figure: pictorial representation of antioxidant and antibacterial activities of *S. heyneanus*

for therapeutic strategy. There is considerable evidence that plant extracts, essential oils, and purified compounds have been used as a preventative agent or to treat various diseases. Hence, clinical trials of such natural products are essential for the safety and efficacy to offer therapeutic benefits before coming into the market. It should be helpful to use either alone or in combination with conventional therapies to reduce the overall burden of diseases worldwide. The present study showed that the root extract of *S. heyneanus* has good antioxidant properties and potential antibacterial agents. Further studies are necessary to isolate active compounds responsible for antibacterial activity in order to overcome the demand for natural antibiotics for human welfare (Fig. 9).

Abbreviations

ABTS^{•+}: (2,2'-and-bis (3-ethylbenzothiazoline-6-sulfonic acid); QE: Quercetin; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; OH: Hydroxyl radical; FC: Folin-ciocalteu; GAE: Gallic acid equivalent; Na₂CO₃: Sodium carbonate; PBS: Phosphate buffer saline; MTCC: The microbial type culture collection, and gene bank. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) invitrogen, USA. Acridine orange was obtained from Sigma, USA.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-021-00242-2>.

Additional file 1: Supplementary Table S1 DPPH[•] radical and OH radical scavenging activities of the root extract of *S. heyneanus*. **Supplementary Table S2** ABTS^{•+} radical cation scavenging activity of root extract of *S. heyneanus*. **Supplementary Table S3** Phosphomolybdenum reduction and Fe³⁺ reducing power activity of root extract of *S. heyneanus*. **Supplementary Table S4** Cytotoxicity effect of root extract of *S. heyneanus* on Human dermal fibroblast

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Plant authentication

The plant material was authenticated by Dr. S. Noorunnisa Begum. The herbarium specimen was deposited at the Centre for Repository of Medicinal Resources (C-RMR), FRLH herbarium (voucher number is FRLH 120018), School of Conservation of Natural Resources, Transdisciplinary University, Bangalore.

Authors' contributions

VS evaluated and interpreted the experimental data of the antioxidant, phytochemical screening, and antimicrobial activity, contributed to the characterization and bioassay, and inscribed the major part of the manuscript. SS contributed to the writing of the manuscript. SC was accompanying the supervision of the experiment. RN contributed to the guiding and configuring of the manuscript. AP contributed to the arranging of the MTCC organisms. All authors read and accepted the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

No competing interest to declare.

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