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Antioxidative Efficacy of Aqueous and Ethanolic Flower Extracts of *Senna auriculata*

Hebsibah Elsie B.[1]*, Gayathri G.A.[2], Pragathi N.[3], Nirmal Kumar Chinnakannu[4] and Mukesh Kumar Dharmalingam Jothinathan[5]

[1]PG &Research Department of Biochemistry D.K.M. College for Women (Autonomous), Vellore 632001, Tamil Nadu, India
[2]Department of Biochemistry, Arcot Sri Mahaalakshmi Women's College, Villapakkam, Ranipet, Tamil Nadu, India
[3]PG & Research Department of Foods & Nutrition, Marudhar Kesari Jain College for Women (Autonomous), Vaniyambadi 635751, Tirupattur District, Tamil Nadu, India
[4]PG & Research Department of Biochemistry, K.M.G. College of Arts & Science, Gudiyattam Vellore 632602, Tamil Nadu, India
[5]Centre for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India

**Corresponding Author*

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Abstract: From the origin of human on this globe, the usage of natural based ingredients for troublesome health diseases has been done. Especially, the Indian customary medicine is fully dependent on plants of medicinal features. In this investigation, *Senna auriculata* (*S. auriculata*) flower was chosen and extract was prepared using aqueous and ethanol solvent. The procured aqueous and ethanolic extracts of *Senna auriculata* flower was tested qualitatively to investigate the phyto-contents existing in it. The screening of phyto-content showed tannins, proteins, flavonoids, glycosides, steroids, phenols, alkaloids, saponins and carbohydrates. The quantified phyto-content in the aqueous extract was 45.21 mg/g total phenolic content (TPC), 29.04 mg/g total flavonoids content (TFC) and 16.38 mg/g (alkaloid), whereas in the ethanolic extract it was 59.22 mg/g (TPC), 36.02 mg/g (TFC) and 20.09 mg/g (alkaloid). The aqueous extract of *S. auriculata* showed IC_{50} of 9.10 µg/ml with 24.09 % of inhibition of DPPH radical, whereas the ethanolic extract provided IC_{50} of 12.76 µg/ml with 30.06% DPPH inhibition. The antibacterial assay showed the aqueous extract of *S. auriculata* flower had efficient inhibition zone for *Staphylococcus aureus* as 25 mm and by ethanolic extract of *S. auriculata* flower the inhibition zone given against *S. aureus* was 28 mm. Other bacterial strains also brought dose-mediated antibacterial role when screened with both the extracts. Precisely, the ethanolic extract provided superior antioxidant and antibacterial trait.

Keywords: *Senna auriculata*, *Staphylococcus* aureus, Phyto-content, Antioxidant, Antibacterial, DPPH

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Introduction

Medicines procured from the plants are the pivotal form of therapeutic aid in biomedicine sectors for managing numerous ill-health conditions. From the WHO (World Health Organization) data, it is known that major percentage (80%) of mankind relied on medicinal plants acquired drugs for promoting good health. Preparations of medicinal plants for therapeutic aid, solely based on the information from the traditionally known medicines. Not only in India, other countries also have been exploiting medicinal plants in their healing rituals. In the system of customary medicines (Siddha, Unani and Ayurveda), to combat chronic problems, poly-herbal formulations are employed (Prasathkumar *et al.*, 2021a).

In the usual cellular metabolism, free radicals are formed. Oxidative stress is the foremost agent in bringing reactive oxygen species (ROS) and its various forms such as singlet oxygen, NO radicals, hydrogen peroxide, hydroxyl radicals and superoxide anionic radicals. In addition to the endogenous agents, exogenous entities also tune ROS generation within the dwelling system such as pesticides and organic forms of chemicals, smokes, pollutants and ionizing radiations (Phaniendra *et al.*, 2015; Gonzalez-Palma *et al.*, 2016). ROS are the main culprit of chronic forms of health disorders like aging, rheumatoid arthritis (auto-immune disease), diabetes, neuro-degeneration and atherosclerosis, in addition to cancer. When the balance between the free forms of radical (ROS) and the antioxidant (inherent form) is disturbed, it brings the risk of aforementioned diseases (Saeed *et al.*, 2012). Plants phyto-contents are the natural neutralizers of free radicals. At present, natural forms of antioxidant extracted from plant origin are applied in health sections and extensively screened by the researchers owned to the safeness in contrary to the hazardous types of synthetically procured antioxidant (Noreen *et al.*, 2017). The diverse, bioactive and effective antioxidants in plants include tannins, vitamins, flavonoid, phenolics and alkaloids. In addition to antioxidant features they display antibacterial, antifungal and antiviral impact (Phong *et al.*, 2022).

In today's world, the resistance emerging towards the life-threatening microbial species such as bacteria is the serious situation as the antibacterial medication (synthetic and semi-synthetic forms) have provided lack of killing impact on the bacteria. One of the causes for this is overexploitation of the antibacterial medicines, bringing resistant types of bacterial strains (Singh *et al.*, 2016). Moreover, in the developing nations, one of the death causes are infectious bacterial diseases. Even the antibacterial medications in addition brings negative impression on the human system such as hypersensitive reactions and immune system suppression. So, there is an immediate urge to design novel and potent antimicrobial medicaments by the pharmaceutical province (Stankovic *et al.*, 2016). The lower hazardous impact, budget friendly and easier availableness of the medicinal property plants have made them more preferable. The bioactive forms of secondary metabolites along with antioxidative properties creates defined physiological and pharmacological efficacy on the human system (Atef *et al.,* 2019).

Senna auriculata belongs to family Fabaceae/ Leguminosae. It is also known as *Cassia auriculata* (Juan-Bafaturuge *et al.*, 2011). It is entirely branched form of shrub, holding alternative leaf pattern. The bark is darkish brown and the flowers are bright yellowish. They are usually grown in the dry parts of India. The glucosides existing in this plant is an ultimate remedy for corneal (conjunctivitis), gonorrhea and urinary tract infections (UTI) (Muthu *et al.*, 2017). In choornam form as Avarai Panchaga, the sections of this plants ranging from stem, bark, roots, flowers and fruits (unripen) are applied in the Indian healing system of traditional medicine. It is majorly used as curative for diabetes mellitus and auto-immune disease (rheumatism). Scientific testing had proven the antioxidant, antibacterial, antifungal, anti-inflammatory and analgesic

potency of *S. auriculata* (Prasathkumar *et al.,* 2021b). In this investigation, *Senna auriculata* flower was chosen and extract was prepared using aqueous and ethanol solvent. The procured aqueous and ethanolic extracts of *Senna auriculata* flower was tested qualitatively to investigate the phyto-contents, antioxidative and antibacterial efficacy.

Materials and Methods

Collection and preparation of flower of S. auriculata:

The flower of *S. auriculata* were freshly collected. The damaged and infected flowers were removed and the remaining flowers were thoroughly washed multiple times in the flowing tape water to discard the adhered residues and soil particles. Following the washing the flowers were rinsed five times by applying double distilled water. After rinsing, the flowers were placed uniformly on a sterile dry plastic tray and made to dry for 2 weeks. Once the flower was entirely free from moisture, the dried content was blended into fine powder by applying a kitchen blender. The acquired powder was sieved and stored in an air-resistant jar for various investigations.

Preparation of aqueous extract of S. auriculata flower:

30 g of powered form of *S. auriculata* flower was mingled with 250 ml of double distilled water in a 500 ml of sterile glass beaker and retained on a hot pre-set magnetic stirrer for 30 min at 60 ℃. Post heating, the content was left undisturbed for an hour. Once the solution was cooled, it was filtered twice applying Whatman No.1 filter paper. The clear golden yellowish flower extract procured was collected and transferred into a glass container (50 ml) with screw capped and stocked in the refrigerator operated at 4 ℃ for further research needs.

Preparation of ethanolic extract of S. auriculata flower:

To prepare the ethanolic flower extract of *S. auriculata*, exactly, 30 mg of *S. auriculata* flower powder was taken in a 250 ml of sterile beaker and added 100 ml of 90% ethanol. The mixture was blended well and let to react for a week. After a week, the solution was filtered and the ethanolic flower extract of *S. auriculata* was acquired. The filtrate was again filtered with the aid of Whatman No. 1 filter paper. The clear flower extract was refrigerated to prevent any contamination and further usage in the experiment.

Qualitative profiling of phytochemicals of aqueous extract and ethanolic extract of S. auriculata (*Jenila Jose Jancy et al., 2020*; *Dhrubajyothi Sarkar et al.,* 2020; Ezhilarasan *et al., 2021*; *Sanjeed et al., 2023*; *Subbulakshmi Packirisamy et al., 2023*):

To assure the phytochemicals present in the aqueous extract and ethanolic extract of *S. auriculata*, a standard qualitative examination protocol was executed out.

(A) *Screening of proteins*:

(a) Million's test: Exactly, in a test tube, 0.5 ml of extract of *S. auriculata* flower was poured, along with Million's reagent (0.5 ml). Immediate development of whitish precipitate that turns to reddish precipitate on moderate heating defines protein presence.

(b) Ninhydrin test: Crude extract of *S. auriculata* flower (1 ml) was treated with ninhydrin solution (0.2 %). Violet colouration manifest for amino acids as well as proteins.

(B) *Screening of carbohydrates*:

(a) Fehling's test: Precisely, equal quantities of Fehling A (0.5 ml) and Fehling B (0.5 ml), were blended with *S. auriculata* flower extract (1 ml). Manifestation of brick-reddish precipitate signifies reducing sugars.

(b) Benedict's test: 1 ml of Benedict's reagent was heated and boiled with 1 ml of crude *S. auriculata* flower extract. Reddish-brown colouration indicates presence of carbohydrates.

(c) Molisch's test: To the Molisch's reagent (1.5 ml), *S. auriculata* flower extract (1 ml) was added and mingled gently by shaking the tube contents. Approximately, 1 ml of concentrated sulphuric

acid (H_2SO_4) was taken in a dropper and added along the side of the tube. An immediate formation of violet coloured ring at the junction of two solutions, signifies carbohydrate in the tested extract.

(C) *Screening of phenols and tannins*:

Exactly, 1 ml of *S. auriculata* flower extract was amalgamated with 2% ferric chloride (1 ml). Instant visibility of blackish or bluish-green colouration displays phenols and tannins.

(D) *Screening of flavonoids*:

(a) Lead acetate test: To three drops of lead acetate, added 0.5 ml of *S. auriculata* flower extract. Immediate precipitate development with yellowish colouration justifies flavonoids.

(b) Sulphuric acid test: Orangish colouration formation on adding two drops of sulphuric acid to the *S. auriculata* flower extract authenticates flavonoids in the extract.

(E) *Screening of saponins*:

A tenacious soapy bubble appearance in the test tube, that retains for more than 5 min on strongly shaking the crude extract of *S. auriculata* flower (1 ml) with 5 ml of double distilled water, depicts the traces of saponins.

(F) *Screening of glycosides*:

(a) Liebermann-Burchard test: 1.5 ml of chloroform was brought in contact with the 1 ml of crude extract of *S. auriculata* flower, in addition to acetic acid (2 ml). The tube content was retained in the ice for 5 min. Then poured two drops of sulphuric acid to the reacting contents in the tubes. A shift in the colouration to bluish-green from violet, validate glycosides.

(b) Salkowaski's test: Crude extract of *S. auriculata* flower was reacted with chloroform along with 2 ml of dilute sulphuric acid and moderately shaken. Genesis of reddish-brown steroidal ring validates glycosides.

(G) *Screening of steroids*:

Chloroform (1 ml) and crude *S. auriculata* flower extract was reacted with concentrated sulphuric acid (1 ml). The lower section of tube content showing red colouration manifest steroid's presence.

(H) *Screening of alkaloids*:

(a) Mayer's test: To 1 ml of the crude *S. auriculata* flower extract Mayer's reagent (1 ml) was applied. Yellowish colouration determines the persistence of alkaloids.

(b) Wagner's test: A combination of Wagner's reagent (1 ml) and *S. auriculata* flower extract (1 ml) when provides brownish to reddish precipitation, it ascribes the alkaloid's existence in the extract.

Quantification of S. auriculata phyto-content:

(a) TPC (Total phenolic content):

To identify the total phenolic content in the aqueous and ethanolic extracts of *S. auriculata* flower, spectrophotometric assay was done. 1 ml of the Folin-Ciocalteau reagent (F-C) reagent, a commercially available form was made into dilution (50%) and to that added 2 ml of freshly prepared sodium carbonate solution (20%). The tube contents were mixed well and placed in a pre-heated water bath at 90℃ for 1 min. After that cooling of the tubes were done and at 650 nm, spectrophotometrically the optical density was noted. The values were provided as GAE/g of extract (Jain *et al*., 2012).

(b) TFC (Total flavonoid content):

Aluminium chloride protocol was used to check the quantity of flavonoid existing in the aqueous and ethanolic flower extracts of *S. auriculata*. For this quantification, 1 mlof each extract was diluted with distilled water (4 ml). To this added, sodium nitrite (5%, 0.3 ml). Then, 10% of aluminium chloride (0.3 ml) was poured into the reaction content, in addition to 2 ml of sodium hydroxide (1 M) and diluted with distilled water to get a 10 ml volume. Quercetin served as reference. The absorbance of the screened extracts and quercetin, a commercial flavonoid was monitored through spectrophotometric scanning at 510 nm. From the

plotted graph, the TPC was found out. The quantity of flavonoid was mentioned as QE/g of extract (Shubhangi *et al.*, 2023).

(c) Total alkaloid content:

To quantify the alkaloids, precisely, 3 g of each extract of *S. auriculata* flower was placed in a beaker (250 ml) and reacted with 10% solution of acetic acid (200 ml) prepared in ethanol. The whole mixture was maintained to react for 4 h and then the filtrate was obtained which was concentrated to one-quarter of the acquired volume. Then to this added saturated solution of freshly made ammonium hydroxide. Again, filtered and the residue left was taken dried and weighed (Sheela Rani *et al*., 2013).

Antioxidant efficacy by DPPH assay (*Tulsidas et al., 2021*; *Chew Khe Jiea et al., 2022*; *Berihu Tekluu et al., 2023*):

The efficacy with which both the extracts of *S. auriculata* flower has impact on free form of hazardous radical is tested. For this assay, 1 ml of DPPH methanolic solution (0.3 mM) was exposed to 2.0 ml of various ranges (50 µg/ml, 100 µg/ml, 150 µg/ml and 250 µg/ml) of extracts and incubated for 30 min in a dark condition. After incubation, at 517 nm absorbance was noted. A blank solution (methanol) was also used. As negative control, mixture of DPPH solution and methanol was used. Vitamin C/Ascorbic acid was used as positive reference. Applying the following formula the IC_{50} value was calculated:

% of decolouration of DPPH radical = [$Absorbance_{517}$ (Control) – $Absorbance_{517}$ (Sample)/ $Absorbance_{517}$ (Sample) x 100

Where, $Absorbance_{517}$ (Control)= Control absorbance; $Absorbance_{517}$ (Sample)= Sample absorbance

Antibacterial assay (*Raveesha and Ashalatha*, 2018; Sugashini *Settu et al., 2019*):

The efficacy of both the aqueous and ethanolic extract of *S. auriculata* flower to inhibit the growth of pathogenic strains of bacteria was checked through a commonly adopted standard agar-well diffusion methods as already reported in the studies with moderate alterations. Under a sterile environment, Mueller-Hilton (MH) agar media was made in a sterile 250 ml conical flask and then autoclaved. Three sterile petri-plates were taken and the autoclaved MH agar media was gently poured into all the petri-plates in an equal volume. The MH agar media was retained for solidification. Once the media was solidified, each petri-plate was evenly swabbed with freshly formed five pathogenic bacterial strains namely, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Bacillus subtilis.* Then applying a sterile cork-borer even sized wells were made in the petri-plate media. Various ranges of both the extracts of *S. auriculata* (100 µg/ml, 200 µg/ml and 300 µg/ml) were poured into the wells using the micropipette. All petri-plates loaded with the extracts were retained in an incubator maintained at 37°C for a day. Post-incubation, the formed inhibitory zone was measured in mm (millimetres). Here, a standard antibiotic (Chloramphenicol) was utilized as comparative compound in this assay.

Results and Discussion

Qualitative profiling of S. auriculata phyto-content:

The testing of phyto-content in the *S. auriculata* flower aqueous and ethanolic extract displayed the bioactive ingredients which are illustrated in Table 1 and Figures 1 and 2.

Quantification of S. auriculata phyto-content in the flower extracts:

The bioactive secondary form of phyto-contents was quantified by executing the standard assays and the phyto-content quantity is depicted in Table 2.

DPPH assay:

The aqueous and the ethanolic flower extract of *S. auriculata* were noted for their free radical neutralizing trait through inhibiting the impact of DPPH which was checked through the percentage inhibition. When tested from 50 µg/ml to 100 µg/ml concentration, the inhibition (%) appeared was 6.23%, 10.11%, 14.87%, 19.73% and 24.09%

Table 1: Depicting the phyto-content screened in both the extracts of *S. auriculata* flower

Phyto-content	Aqueous extract of *S. auriculata*	Ethanolic extract of *S.auriculata*
Protein	+	+
Carbohydrate	+	+
Phenols and tannins	+	+
Flavonoids	+	+
Saponins	+	+
Glycosides	+	+
Steroids	+	+
Alkaloids	+	+

(+): Presence

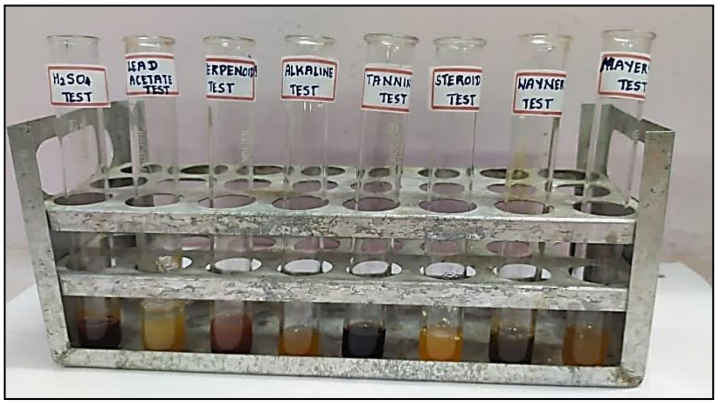

Fig. 1: Phyto-content visualized in the *S. auriculata* flower aqueous extract.

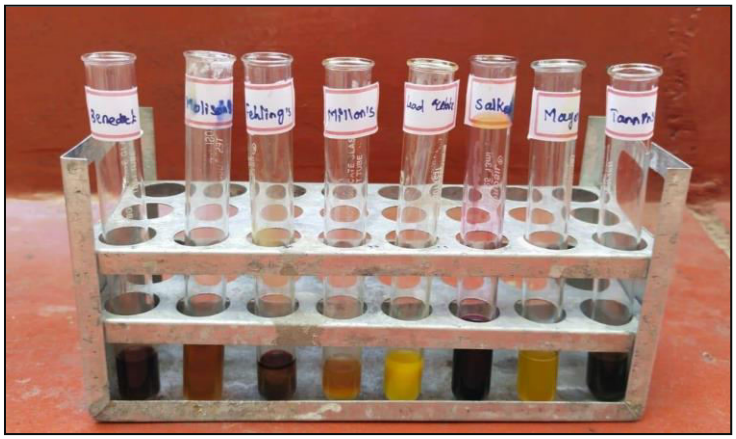

Fig. 2: Phyto-content visualized in the *S. auriculata* flower ethanolic extract.

Table 2: Providing the quantity of phyto-content found in the *S. auriculata* flower aqueous and ethanolic extract

Phyto-content	Quantity mg/g	
	Aqueous extract	Ethanolic extract
TPC	45.21	59.22
TFC	29.04	36..02
Alkaloids	16.88	20.99

Table 3: DPPH quenched by the aqueous and ethanolic flower extract of *S. auriculata* presented as % inhibition

Concentration (µg/ml)	Inhibition of radical DPPH in %		
	Aqueous extract	Ethanolic extract	Ascorbic acid/Vitamin C
50	6.23 ± 0.44	9.11 ± 0.50	12.09 ± 0.62
100	10.11 ± 0.23	15.02 ± 0.76	19.52 ± 0.33
150	14.87 ± 0.57	20.22 ± 0.99	23.44 ± 0.53
200	19.73 ± 0.77	28.09 ± 075	31.45 ± 0.68
250	24.09 ± 0.51	30.06 ± 0.45	39. 08 ± 0.48

Table 4: Antibacterial assay presenting the inhibition zone by the aqueous flower extract of *S. auriculata*

Microorganism	Zone of inhibition (in mm diameter)			
	Concentration in µg/ml			Positive control (Chloramphenicol) 10 µg/ml
	100	200	300	
Pseudomonas aeruginosa	9	11	21	24
Escherichia coli	13	16	18	20
Bacillus subtilis	16	18	20	22
Klebsiella pneumonia	19	20	22	24
Staphylococcus aureus	21	21	25	28

Table 5: Antibacterial assay presenting the inhibition zone by the ethanolic flower extract of *S. auriculata*

Microorganism	Zone of inhibition (in mm diameter)			
	Concentration in µg/ml			Positive control (Chloramphenicol) 10 µg/ml
	100	200	300	
Pseudomonas aeruginosa	19	22	25	19
Escherichia coli	18	19	20	24
Bacillus subtilis	20	21	22	18
Klebsiella pneumonia	21	23	25	24
Staphylococcus aureus	23	24	28	26

for the aqueous flower extract of *S. auriculata* (Table 3). The ethanolic extract of flower of *S. auriculata* provided 9.11%, 15.02%, 20.22%, 28.09% and 30.06% of inhibition. Both the extracts of flowers had concentration-based activity, with the upsurge of extract dose, the radical nullifying role also increased. Here, the IC_{50} for aqueous extract was 9.10 µg/ml and for the ethanolic extract it was 12.76 µg/ml. In comparison, ascorbic acid gave inhibition as 12.09%, 19.52%, 23.44%, 31.45% and 39.08% with IC_{50} of 7.32 µg/ml (Table 3). Our results were in relation with the already experimented and provided literature data (Sriram Prasath *et al*., 2019). DPPH is a radical, which exist in a free form, on mingling with antioxidative trait components, the purple colour of DPPH gets faded away (Sriram Prasath *et al*., 2019., Xiao *et al*.,

2020) atoms to the electron deficient free radicals and removes their harmful impact through redox action (Speisky *et al.*, 2022).

Antibacterial activity of aqueous and ethanolic flower extract of S. auriculata:

The agar-well diffusion assay, a conventional protocol followed to notice the antibacterial impact of ethanolic extract of flower of *S. auriculata.* A concentration-based increase in the size of the zone was seen. The aqueous extract of flower of *S. auriculata* gave inhibition width as 25 mm for *Staphylococcus aureus*, 22 mm for *Klebsiella pneumoniae*, 21 mm for *Pseudomonas aeruginosa*, 20 mm for *Bacillus subtilis* and 18 mm for *Escherichia coli*. At 300 µg/ml, the more inhibition zone width was shown by *Staphylococcus aureus* (28 mm), followed by *Pseudomonas aeruginosa* (25 mm) and *Klebsiella pneumonia* (25 mm) and *Bacillus subtilis* (22 mm) and least width of inhibition by *Escherichia coli* (20 mm) (Tables 4, 5). Flavonoids creates irreparable damage to the cellular membrane of bacteria, hinders the functioning of synthase enzymes which are the contributor of nucleic acid formation, respiratory chain mechanism in the bacterial cells and cellular envelope creation. Usually, the interactivity of flavonoids with the phospholipid of cellular membrane causes leakage in the cellular content and lysis of the cell (Yuan *et al.*, 2021). Intercalation with the bacterial genome (DNA), halting the transcription mechanism of m-RNA and cell wall depolarization are the pivotal mechanism executed by the alkaloids to kill the virulent pathogenic bacteria (Shamsudin *et al*., 2022).

Conclusion

In this study we performed screening of the phyto-contents, antioxidant activity and anti-bacterial activity of aqueous and ethanolic flower extract of *S. auriculata* which gave noteworthy results. In both the screened assays, the ethanolic extract had superior functioning comparative to the aqueous extract of *S. auriculata* flower. This revealed the efficacy of this plant to be applied in the pharmacological domain with further deep investigations for providing strong antibiotics in a natural form with antioxidative traits for managing health-issues.

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