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Assessment of ethanol extract of *Tinospora cordifolia*'s phytochemical, antioxidant, anti-inflammatory, and ion-chelating properties

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Abstract
Tinospora cordifolia is portrayed as "the one that prevents us from diseases" because it has been used by tribal people traditionally for treating jaundice, diabetes, chronic diarrhoea, asthma, cancer, dermatological diseases, and general debility. In the Indian System of Medicine, it is acknowledged as an important medicinal herb. The principal goal of this present communication was to highlight its biological activity in the ethanol extract of *Tinospora cordifolia* (EETC), such as its phytochemicals, anti-inflammatory, antioxidant, and ion-chelating nature. Initially, phytochemical analysis was performed for qualitative analysis of phytochemicals from the whole plant extract, which helped enhance the above biological activities. Due to its commercial importance, it has created interest in the field of research in recent decades because of its broad range of pharmacological properties.

Keywords: *Tinospora cordifolia*, DPPH, flavonoids, ascorbic acid, HRBC

Introduction
India is regarded as the world's botanical paradise and the global leader in the production of therapeutic herbs [1]. Medicinal plants have been used for their therapeutic benefits, which have been emerging since the start of human evolution. For thousands of years, our earth has served as a supply of medicinal materials. The traditional, folklore-based medical system is still essential to human health; about 80% of people on the planet rely mostly on herbal plants for primary health care [2]. The present study states the scientific evaluation of *T. cordifolia* (familiarly called Seenthil, Guduchi, Gurjo, or Giloy) to prove its medicinal efficacy; phytochemical analysis, anti-inflammatory, antioxidant, and ion chelating activities were analysed [3]. *T. cordifolia* is a versatile medicinal plant that has diverse varieties of bioactive compounds. Very few works have been conducted to analyse the biological activities and therapeutic applications of these plant compounds; hence, ample examination is needed to explore their medicinal implementation in conflict diseases [4]. The genus *Tinospora* belongs to a member of the family Menispermaceae and has been broadly explored by a large number of workers, and the reports of phytochemicals were marked with therapeutic activity. This family is a rich source of phytochemical compounds. The deciduous shrub *Tinospora cordifolia* climbs adjacent trees to reach a height of 1.0 metres (3.3 feet). It contains heart-shaped leaves, small chimes, and seeds that are usually hooked or uniform, and the stems of *Tinospora* are juicy and have elongated filiform fleshy aerial roots that rise from the branches. When a peeled fleshy stem is exposed, the bark appears greyish, thin, or creamy white. *T.* cordifolia has greenish flowers that are unisexual [5, 6]. Guduchi is the Sanskrit name of the plant, whereas Ayurveda literature quotes Guduchi as a constituent in several compound preparations, which have been useful in treating fever, general sickness, dyspepsia, and urinary infections. The bitter stomachic stem of T. cordifolia improves blood, induces bile secretion, alleviates thirst, stops vomiting, cures skin conditions, and heals jaundice; the extract form of the stem is useful for diabetes, vaginal discharge from a urethral, and enlarged spleen. The stems and roots of the plant are taken and mixed with other herbs for use as an antidote for scorpion snake bites and snake bites [7]. The present paper is to screen the biological activity of EETC. It evaluates the phytochemical analysis, antioxidant potential, and anti-inflammatory and ion-chelating activity.

Materials and Methods

Preparation of Extract

Tinospora cordifolia (Seenthil) whole plant sample is collected from roadside of thirumullaivoyal, Chennai and washed using normal tap water to eliminate dirt and shade dried. After drying the whole plant sample is ground as coarse powder. About 100 gm of coarse powder with 400ml of ethanol was extracted in a soxhlet extractor and filtered with muslin cloth.

Qualitative Study of Phytochemicals

Qualitative phytochemical analysis was utilized to examine the presence and absence of tannins, flavonoids, glycosides, phenols, saponins, quinones, coumarins, anthraquinones, phyobtannins, steroids, terpenoids, proteins, carbohydrates, and anthocyanins with EETC [8].

Antioxidant Assay

a) Assay for Hydrogen Peroxide (H_2O_2) Scavenging

The scavenging activity of hydrogen peroxide in EETC is produced *in vitro* with a peroxidase assay technique, and the amount of H_2O_2 lost during incubation of the scavenger is determined [17]. It is created by a dis-mutation reaction involving the superoxide anion, which results in the hydroxyl radical's emergence along with superoxide anions and metal ions [10]. The capacity of the EETC scavenging activity of hydrogen peroxide is analysed through UV spectrophotometry [9].

The Ruch *et al*. method [11] was used to analyse the extract's scavenging assay with hydrogen peroxide. H_2O_2 solution (2 mM) was added after 0.1 ml of EETC (25 to 400 µg/ml) was brought up to 0.4 ml using 50 mM phosphate buffer solution (pH 7.4), using ascorbic acid as the positive control. 230 nm is the measurement wavelength for the reaction mixture's absorbance. After it has been incubated for 10 minutes. The following equation is used to determine the EETC ability of H_2O_2 scavenging activity:

$$\text{Free radical scavenging activity \%} = \frac{\text{Abs(control)} - \text{Abs(sample)}}{\text{Abs(control)}} \times 100$$

b) DPPH Free Radical Scavenging Assay

An antioxidant that contributes hydrogen to diphenyl-picryl hydrazine (yellow) in a non-radical state was present when an alcoholic 1, 1-Diphenyl-2-picrylhydrazyl dark blue solution was reduced. The DPPH scavenging experiment was developed. In 100 ml of ethanol, 4 mg of DPPH is dissolved and left overnight for the production of DPPH radical. Using the technique described, it was verified that EETC could scavenge the activity of DPPH [12]. A mixture of a 3 ml solution containing 0.004% DPPH and 0.1 ml of EETC at different dosages was added. After vigorously stirring the mixture, it was left to settle at room temperature for about half an hour. This molecule had an absorbance of 517 nm [18]. The following formula was used to determine how much DPPH EETC could scavenge:

$$\text{Free radical scavenging activity \%} = \frac{\text{Abs(control)} - \text{Abs(sample)}}{\text{Abs(control)}} \times 100$$

c) Nitric oxide scavenging activity

A spectrophotometric measurement was made at 546 nm to assess the NO scavenging assay [13]. PBS with 5 mmol of sodium nitroprusside was combined with various EETC concentrations and allowed to incubate for 30 min at 25 °C. An equivalent volume of ethanol was used as a control. After 30 minutes, 1.5 mL of the incubation solution had been extracted and diluted with 1.5 mL of Griess solution. In the current investigation, EETC was used to decrease the nitrite that resulted from soaking sodium nitroprusside solution in phosphate buffer. This could be because the extract has antioxidant properties that prevent oxygen from reacting with nitric oxide, preventing the production of nitrite [19]. The ability of the EETC to scavenge nitric oxide was determined using an equation as follows:

$$\text{Scavenging activity \%} = \frac{\text{Abs(control)} - \text{Abs(sample)}}{\text{Abs(control)}} \times 100$$

In this case, (Abs = Absorbance)
Test was done in triplicate.

Anti-Inflammatory Effect

a) Denaturation of Albumin

The suppression of albumin denaturation technique was used to test the anti-inflammatory activity [14]. Inflammation has been linked to protein denaturation. The efficacy of EETC to prevent protein degradation was investigated as part of the inquiry into the workings of the reducing inflammation effect. It was successful in preventing albumin denaturation brought on by heat [21]. To 0.45 ml (5%) of BSA, various doses of EETC (1 to 5 ml) were added, and then 1N HCL was added in order to adjust pH 6.4. After 20 minutes of incubation and 4-5 minutes of boiling at 70 °C in a water bath, the solution was allowed to cool to room temperature, and then PBS was added to measure the absorbance. 2.5 ml of the buffering solution, 0.45 ml (5%) of bovine albumin fraction, and 2 ml of distilled water make up the reference mixture. Conventional anti-inflammatory medicine, aspirin, was utilised, and the absorbance levels were determined at 640 nm. The following equation was applied to convert the value of absorbance into the percentage inhibition:

$$\text{Percentage inhibition \%} = \frac{\text{Abs(control)} - \text{Abs(sample)}}{\text{Abs(control)}} \times 100.$$

b) Membrane Stabilization with HRBC suspension

After collecting anticoagulated human blood in a tube with a similar amount of Alsever's solution, it was centrifuged for ten minutes at a rate of 3,000 rpm. The obtained solution was washed three times with iso-saline solution until the supernatant became colorless. RBC layer was collected and diluted to make up to 10% v/v using iso-saline solution stored without disturbance at 4 °C.

One milliliter containing 0.1 mM phosphate buffer solution with pH 7.4, 1 ml of EETC sample prepared in different concentrations, and half a milliliter of a ten percent HRBC suspension make up the reaction mixture (5 ml). In place of the test sample, 1.0 ml of saline was utilised as the control, and distilled water was utilised in place of hyposaline. After centrifuging the mixture for 20 minutes at 3000 rpm, the absorbed value of the haemoglobin amount in the remaining liquid was obtained using UV spectrophotometers at 540 nm [15].

$$\text{Percentage hemolysis \%} = \left[\frac{(\text{Abs}_0 - \text{Abs}_1)}{\text{Abs}_0}\right] \times 100$$

Ion Chelating Activity

One percent KCN with PBS is used to treat the substance with varying concentrations. After 20 minutes of incubation at 50

°C, 10 percent TCA had been added to the mixture to initiate the reaction, and it went through a centrifuge for ten minutes at 3000 rpm. A spectrophotometer has been employed to measure the absorption of a blue solution at 480 nm after adding 0.1% ferric chloride to 2.5 ml of supernatant. When chelating substances are present in EETC, ferrozine production is disrupted, and the violet colour's concentration diminishes as the chelating compound concentration rises [20]. Antioxidant peptides and proteins would thereby chelate transition metal ions, reducing the processes of oxidation and substantially lowering damages caused by oxidation. EETC digests capacity to chelate the transition metal ion Fe2+ calculated using the following formula; the inhibition percentage was determined:

$$\text{Percentage of metal chelating ability \%} = \left[\frac{(\text{Abs}_0 - \text{Abs}_1)}{\text{Abs}_0}\right] \times 100$$

Where Abs_0 represents the control's absorbance and Abs_1 represents the absorbance when sample or standard chemicals are present. The mean ± SD from three repetitions was used to express the results.

Results

Quantitative phytochemical analysis

The findings demonstrated that the EETC has components that are significant from a pharmacological standpoint and that it may be utilized to treat a variety of illnesses. The phytochemical analysis is summarized in Table1.

Table 1: Quantitative phytochemical analysis of EETC studied.

S. No	Tests	Ethanolic extract
1	Anthocyanin	-
2	Anthraquinones	-
3	Carbohydrate a) Benedict's test	+
	b) Fehling's test	+
	c) Molisch test	+
4	Coumarins	+
5	Flavonoids	+
6	Glycosides	-
7	Phenol	+
8	Phlobatannin	-
9	Protein a) Millon's test	+
	b) Ninhydrin test	+
10	Quinones	+
11	Saponins	-
12	Steroids	+
13	Tannins	-
14	Terpenoids	+

A sign with a (-) denotes the lack of a component in the corresponding screening test, while a sign with a (+) denotes its presence.

Activity of Antioxidants

Power to scavenge hydrogen peroxide radicals

The bar diagram (fig. 1) illustrates the % inhibition in H2O2 scavenging activity for various concentrations of EETC and ascorbic acid (vitamin C), and it indicates the relevance of *P < 0.005. Ascorbic acid, a conventional medication, has a maximal inhibition of 61.50% at a concentration of 125 µg/ml and an IC50 value of 45.61 +/- 0.670 µg/ml. It also exhibits inhibition of 71.43% at a concentration of 125 µg/ml and an IC50 value of 54.32 +/- 0.847 µg/ml.

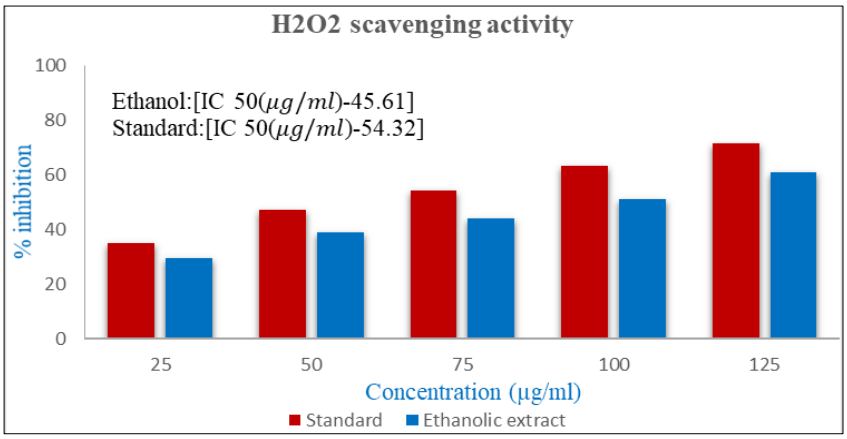

Fig 1: % scavenging activity of H2O2 in EETC

Radical scavenging action of DPPH

The bar diagram (Fig. 2) illustrates the overall percentage inhibition in DPPH scavenging efficiency with respect to various concentrations of EETC and ascorbic acid, and it indicates the relevance of *P < 0.005. It shows maximum inhibition of 62.31 % at concentration 125 µg / ml and IC50 value is 43.16 +/- 0.972 µg / ml with ascorbic acid as a standard drug shows inhibition of 87.31% at concentration 125 µg /ml and IC50 value is 49.63 +/- 0.689 µg / ml.

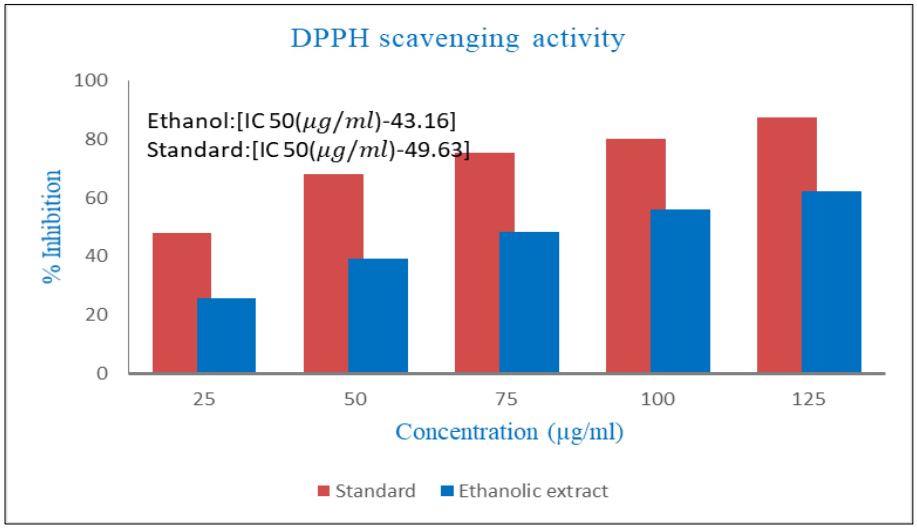

Fig 2: % inhibition in DPPH scavenging activity in EETC

Activity of nitric oxide scavenging

The graph (Fig. 3) illustrates the percentage inhibition of NO scavenging capacity for varying doses of EETC and ascorbic acid; it shows the significance of *P < 0.005. It shows maximum inhibition of 35.42 % at concentration 125 µg / ml and IC50 value is 56.71 +/- 2.367 µg / ml with ascorbic acid as a standard drug shows inhibition of 87.66 % at concentration 125 µg / ml and IC50 value is 60.93 +/- 1.343 µg / ml.

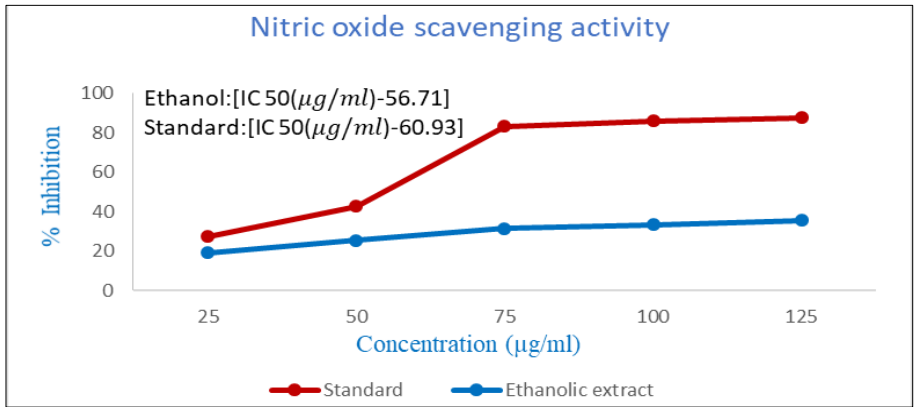

Fig 3: % inhibition in NO scavenging activity in EETC.

Anti-Inflammation

Albumin denaturation: The graph (Fig. 4) depicting the percentage inhibition of anti-inflammatory action at varying doses of EETC and standard (aspirin) indicates a significant *P < 0.005. When aspirin is used as a regular medication, it exhibits a maximum inhibition of 60.29% at a concentration of 125 µg/ml and an IC50 value of 44.32 +/- 1.986 µg/ml. At the same concentration, aspirin exhibits 63.3% inhibition and an IC50 value of 53.56 +/- 2.245 µg/ml.

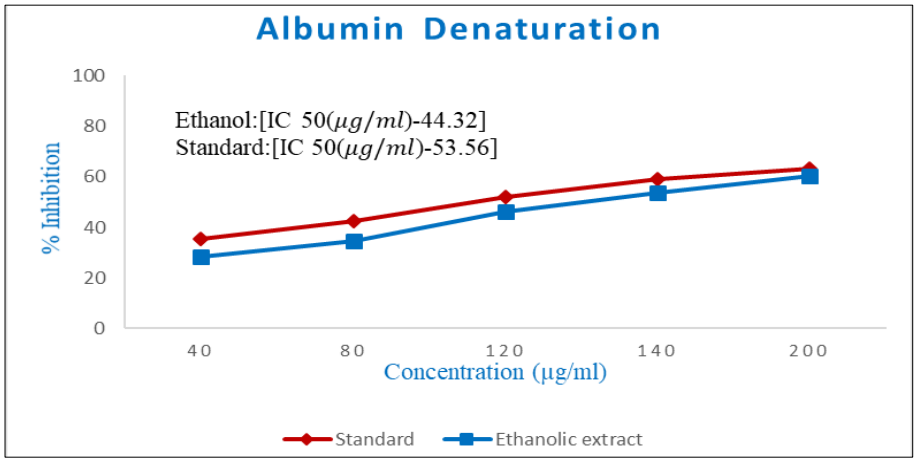

Fig 4: % inhibition of Anti-inflammatory activity in EETC.

Activity of the HRBC Membrane Stabilisation: Figure 5 shows a bar diagram that plots the percentage reduction in HRBC membrane stabilisation activity between EETC and standard; it shows the significance of *P < 0.005. It shows maximum inhibition of 59.78% in 125 µg / ml concentration and IC50 value is 43.76 +/- 2.689 µg / ml with Aspirin as a standard drug shows inhibition of 62.12% at concentration 125 µg / ml and IC50 value is 49.54 +/- 2.453 µg / ml.

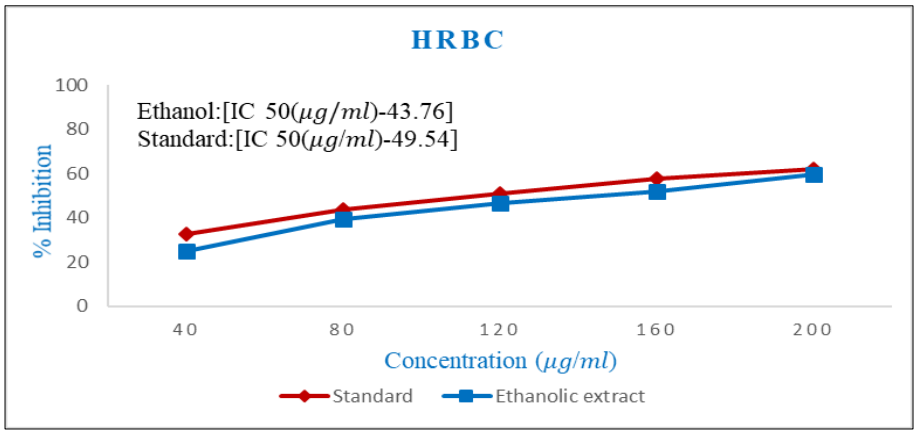

Fig 5: % inhibition in HRBC membrane stabilization activity in EETC.

Ion Chelating Activity

A graph (Fig. 6) illustrates the EETC protein chelating activity, which shows the significance of *P < 0.005. It shows maximum inhibition of 56.12 % at concentration 125 µg / ml and IC50 value is 50.81 +/-1.098 µg / ml with ascorbic acid as a standard drug shows inhibition of 97.63 % at concentration 125 µg / ml and IC50 value is 60.67 +/- 1.783 µg / ml.

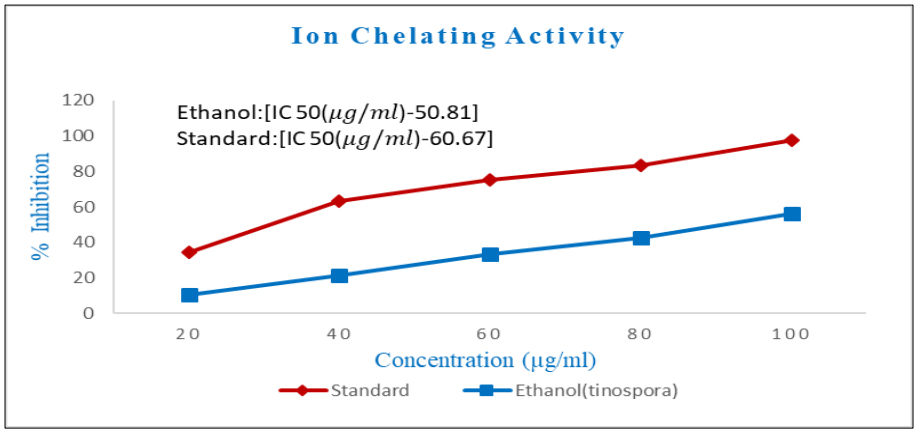

Fig 6: % inhibition of ion chelating activity in EETC.

Discussions

Tinospora cordifolia is the subject of research on phytochemicals, which have the potential to prevent damage from free radicals and yield a wide range of bioactive substances. Supplementing the diet with molecules of antioxidants derived from plants is one potential method of enhancing an individual's resistance to disease [22]. In addition to identifying the components of the EETC, phytochemical screening aids in the search for bioactive substances that are employed in the production of pharmaceuticals. Flavonoids, which are hydroxylated phenolic substances and are present in EETC, have been demonstrated to have anti-tumor properties through a free-radical quenching process (OH, ROO) [23]. Antioxidants (BHT, BHA, propyl gallate, and tetrabutyl hydroquinone) can be classified as natural or synthetic. The carcinogenicity and toxicity of synthetic antioxidants produce problems, including liver damage, which is why many experts are concerned about utilising them. Thus, safer antioxidants derived from natural sources, such as plants, have been developed and are being used traditionally to treat a variety of ailments [24]. The human body contains hydrogen peroxide, which is absorbed through the skin, eyes, or by inhaling vapour or mist. It breaks down into O2 and H2O, releasing radicals containing hydroxyl groups (OH-) and starting the process of lipid peroxidation, which repairs DNA [25]. Nitric oxide is also an essential chemical transmitter that is produced by neurons, mesophages, and endothelial cells. It is involved in the control of numerous physiological functions. Significant cytotoxic consequences caused by excessive levels of nitric oxide (NO) have been linked to AIDS, cancer, Alzheimer's, and arthritis, among other conditions [26]. Furthermore, the anti-inflammatory action of albumin denaturation is investigated using aspirin and other medications as useful benchmarks to compare with the anti-inflammatory properties characteristic of EETC. RBC lysis is measured by HRBC, and in order to control the inflammatory response, lysosomal components of stimulated neutrophils, such as bacteria and proteases, must be stabilized. Failure to do so might lead to increased tissue damage and inflammation due to extracellular releases [27].

Conclusion

Tinospora cordifolia is an extraordinary source of various components with complex chemical structures. Relatively few

studies have been performed, and full-scale research is needed to maximise their therapeutic use for disease control. We have demonstrated that the ethanolic extract of *Tinospora cordifolia* suggests a huge amount of biological potential. We firmly believe that the comprehensive information presented in this paper regarding the chemical makeup of the plant extract will provide ample proof about this plant's activity for use in different medications. It's an amazing drug that could be an excellent remedy and is frequently employed in ayurvedic medicine for treating various ailments.

Conflict of interest

No conflicts of interest regarding this investigation**.**

Acknowledgments

Nil.

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