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Effect of antiradical and anti-inflammatory properties of terpenoid rich fraction from the leaves of *Sphaeranthus Indicus*

S M Kirubhakaran[1*], S Vadivelan[2], T Sarojini[3], S Mathukumar[4]

[1] Reader, Department of Sattam Sarntha Maruthuvamum Nanju Maruthuvamum, Sairam Siddha Medical College and Research Centre, Sai Leo Nagar, West Tambaram, Chennai, Tamil Nadu, India

[2] Lecturer, Department of Maruthuvam, Sairam Siddha Medical College and Research Centre, Sai Leo Nagar, West Tambaram, Chennai, Tamil Nadu, India

[3] Lecturer, Department of Udal Thathuvam, Sairam Siddha Medical College and Research Centre, Sai Leo Nagar, West Tambaram, Chennai, Tamil Nadu, India

[4] Professor, Department of Kuzhanthai Maruthuvam, Sairam Siddha Medical College and Research Centre, Sai Leo Nagar, West Tambaram, Chennai, Tamil Nadu, India

Abstract

To evaluate the antioxidant and anti-inflammatory activities of terpenoid rich fraction from the leaves of *Sphaeranthus indicus*. In this framework, the *in vitro* antioxidant activity was demonstrated by ABTS radicals, lipid peroxidation, nitric oxide scavenging activities and anti-inflammatory activity was evaluated first by studying inhibiting the denaturation of albumin and inhibition of lipoxygenase activities. Total phenolic and flavonoid content were found respectively (63.21 ± 1.78) mg gallic acid equivalent/g, and (52.34 ± 0.89) mg quercetin equivalent/g. The terpenoid rich fraction displayed significant scavenging activity of some radicals such as ABTS EC_{50} at. Terpenoid rich fraction rich fraction from the leaves of *S. indicus* showed *in vitro* anti-inflammatory activity by inhibiting albumin denaturation and inhibition of lipoxygenase. Our results show that terpenoid rich fraction from the leaves of *S. indicus* has good antioxidant activity and interesting anti-inflammatory properties. Terpenoid rich fraction from the leaves of *S. indicus* extract can be used to prevent oxidative and inflammatory processes.

Keywords: antioxidant, anti-inflammatory, *Sphaeranthus indicus*

Introduction

Plant based medicines are used for the handling of various ailments from ancient times and it is not an overestimation to say that the use of the plant based drugs is as old as mankind. Plant based medicines are synthesized from the therapeutic experience of generation of practicing physicians of ancient system of medicine for more than hundreds of years (Abbas *et al*., 2014) [1]. Nowadays, researcher shows a great interest in those medicinal agents that are derived from plants because the currently available drugs are either have certain side effects or are highly expensive. Nature has blessed us with enormous wealth of herbal plants which are widely distributed all over the world as a source of therapeutic agents for the prevention and cure of various diseases (Kim *et al*., 2013) [6]. According to WHO, world's 80% population uses herbal medicines for their primary health care needs. Herbal medicines will act as parcels of human society to combat disease from the dawn of civilization. The medicinally important parts of these herbal plants are chemical constituents that produce a desired physiological action on the body (Biren *et al*., 2006) [2].

Since ancient time India uses herbal medicines in the officially alternative systems of health such as Sidha, Ayurveda, Homeopathy and Naturopathy. In India, there are more than 2500 plants species which are currently used as herbal medicines. For than 3000 years, the herbal medicines are used either directly as folk medication or indirectly in the preparation of recent pharmaceuticals. Thus, from the knowledge of traditional plants, one might be able to discover new effective and cheaper drugs. In this review article, we have tried to cover all the ayurvedic strategies that are followed for the treatment of RA without any possible side effects. The future treatment of RA should provide more effective relief.

Materials and Methods

Plant Material

Sphaeranthus indicus was obtained from Herbal garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamilnadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-297/2021. The flowers were sufficiently air-dried in 5 days at the ambient room temperature, while the flower was cut into smaller pieces and air-dried in 7 days.

Phytochemical Screening

The aqueous extract of leaves of *Sphaeranthus indicus* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973) [4].

Extraction of Terpenoid

The terpenoids of *Sphaeranthus indicus* was partitioned using the method described by Kuo *et al*. [3]. The crude extract was reconstituted in water and partitioned using chloroform, butanol and ethyl acetate. The organic layer was collected and dried in a drying oven at 50°C. The remaining aqueous layer was further extracted with the same and fresh solvent in twice. The organic layer was combined and dried to determine the yield of organic fraction. The remaining aqueous layer was also collected and dried in the oven. Both dried aqueous and organic phases were refrigerated at -20°C before further analysis.

ABTS (2, 2'-Azino-Bis-3-Ethyl Benzthiazoline-6-Sulphonic Acid) Radical Scavenging Assay

ABTS radical scavenging activity of terpenoid rich fraction from the leaves of *S. indicus* was followed by Re *et al*. (1999) [10]. ABTS radical was newly prepared by addition 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to produce an absorbance of 0.70 at 734 nm and the same was used for the antioxidant activity.

The final solution of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid. Correspondingly, in the experiment group, 1 ml reaction mixture encompassed 950 µl of ABTS solution and 50 µl of different concentration of each extracts. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

ABTS Scavenging Effect (%) = $[(A_0- A_1/A_0) \times 100]$ Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of extract.

Inhibition of Lipid Peroxidation Activity

Lipid peroxidation induced by Fe^{2+}ascarbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al*. (1979) [8]. The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06 mM); and different concentrations of terpenoid rich fraction from the leaves of *S. indicus* in a final volume of 0.5 ml. The experimental mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The final volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV–Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard.

Inhibition of lipid peroxidation (%) by the each extracts was calculated according to 1-(E/C) × 100

Where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample.

Nitric Oxide Radical Scavenging Activity

Nitric oxide scavenging ability of terpenoid rich fraction from the leaves of *S. indicus* was measured according to the method described by Olabinri *et al*. (2010) [9]. 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of extracts and incubated at room temperature for 150 min. After treated period, 0.2 ml of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N-(1-Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the experimental sample was read at 546 nm against blank. All readings were taken in triplicate and ascorbic acid was used as standard. The percentage of inhibition was calculated by following equation:

% Nitric oxide radical scavenging capacity = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of control and A_1 was the absorbance of flavonoid rich fraction.

Inhibition of Albumin Denaturation

A solution of 0.2% (w/v) of egg albumin was prepared in a PBS (pH 6.4). A volume of 50 µL of the extract or standard at different concentrations was added to 5 mL of this stock solution.

The test tubes were heated at 72 °C for 5 min and then cooled. The absorbance of these solutions was determined at 660 nm (Karthik *et al*., 2013) [5].

Lipoxygenase Inhibition Assay

Determination of LOX activity by spectrophotometric assay followed by Kemal *et al*., (1987). Inhibition experiments were run by measuring the loss of soybean 15-LOX activity (5µg) with 0.2µM linoleic acid (Sigma) as the substrate prepared in solubilized state in 0.2M borate buffer (pH 9.0). Inhibition studies in presence of various concentrations of extracts (25, 50, 75, 100 µg/mL) and reference compound *viz*., quercetin was recorded at 234 nm using UV-Vis spectrophotometer (Deep Vision, DU 730). The inhibitory effect of the terpenoid rich fraction from the leaves of *S. indicus* was also expressed as percentage of enzyme activity inhibition. EC_{50} indicating the concentration required to inhibit 50 % LOX activity was also calculated. Values of hydroperoxide content and lipoxygenase activity were calculated from equation,

Specific activity (LOX) =$\Delta A. V/\varepsilon.l.c$

Result and Discussion

Phytochemical Screening

The phytochemical screening of the *Sphaeranthus indicus* leaves was studied presently showed the presence of alkaloids, flavonoids, phenol, terpenoids, glycosides and saponin, and absence of glycosides and Saponin (Table -1).

Table 1: Phytochemical screenings of *Sphaeranthus indicus*

Sl. No.	Phytochemical Constituents	Observation	*Sphaeranthus indicus*
1	Alkaloids		
	-Dragendorff's test	Orange / red precipitate	+
	-Mayers test	Creampie ppt	+
2.	Flavonoids		
	-Alkalai Reagent	Intense yellow colour	+
	-Lead aceate test	Precipitate formed	+
3.	Glycosides		
	-Keller-Killiani test	Pink colour (Ammonia layers)	-
4.	Tannin		
	-$FeCl_3$ test	Blue-black colour	+
5.	Saponins		
	-Frothing test	Foam	-
6.	Terpenoids		
	-Salkowski test	Reddish brown colour ring formed in interface	+
7.	Polyphenols		
	-Ferrozine test	Reddish blue	+
8.	Anthocyanin		
	-Ammonia test	Pink color in ammonia layer	+

+ Positive activity; - Negative activity

Free Radical-Scavenging Ability Using ABTS Assay by Terpenoid Rich Fraction from the Leaves of *Sphaeranthus Indicus*

The radical scavenging ability was measured by ABTS assay as per given in table 4. The inhibition percentage of the ABTS radical activity was assessed on average and high free radical-scavenging values were found in terpenoid rich fraction from the leaves of *S. indicus.* In ABTS assay, inhibition percentage was high terpenoid rich fraction from the leaves of *S. indicus* 76.34% with EC_{50} value 63.21µl/ml. The pure ascorbic acid was lower activity (71.32% with EC_{50} value 66.34) (Table-2). Nevertheless, in present study, it is showed that these activities were mainly due to anthocyanin and flavonoids compounds. It is known that vitamin C (ascorbic acid) and carotenoids are chief source of discrepancy of antioxidant/ antiradical activities in plant materials.

Table 2: Free radical-scavenging ability using ABTS assay of terpenoid rich fraction from the leaves of *S. indicus*

Different concentration of extract	Percentage of ABTS radical activity	
	Terpenoid rich fraction from the leaves of *S. indicus*	Ascorbic acid (+ve control)
25 µl/ml	16.35±1.56	15.44±1.64
50 µl/ml	34.75±2.67	31.68±2.36
75 µl/ml	56.31±1.89	55.34±1.78
100 µl/ml	76.34±2.78	71.32±0.89
EC_{50} value	63.21	66.34

[a] Results are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean+SD of three experiments

Inhibition of Lipid Peroxidation Activity of Terpenoid Rich Fraction from the Leaves of *Sphaeranthus Indicus*

The terpenoid rich fraction from the leaves of *S. indicus* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in terpenoid rich fraction from the leaves of *S. indicus* (73.32%) and lowest inhibition percentage of ascorbic acid was found in 68.32% (Table-3). As it is identified that lipid peroxidation is the net result of any free radical attack on membrane and other lipid components present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). In the present study, egg yolk was used as substrate for free radical mediated lipid peroxidation, which is a non-enzymatic method. Normally, the mechanism of phenolic compounds for antioxidant activity includes neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals.

Table 3: Inhibition of lipid peroxidation activity of terpenoid rich fraction from the leaves of *S. indicus*

Different concentration of extract	Percentage of lipid peroxidation	
	Terpenoid rich fraction from the leaves of *S. indicus*	Standard Vitamin-C
25 µl/ml	20.31±1.46	17.34±1.79
50 µl/ml	33.62±1.78	30.32±2.48
75 µl/ml	51.23±2.56	47.36±2.36
100 µl/ml	73.32±1.45	68.32±1.45
EC_{50} value	61.23	64.31

[a] Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

Nitric Oxide Radical Scavenging

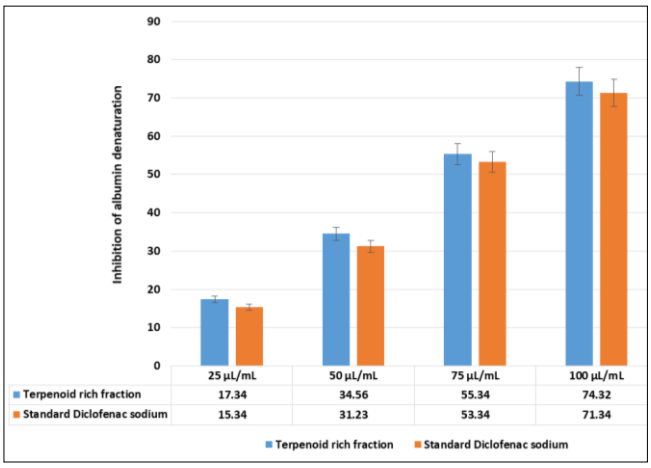
Terpenoid rich fraction from the leaves of *S. indicus* indicated a strong nitric oxide scavenging ability which was equivalent to the standards ascorbic acid. The EC_{50} value (61.23) of terpenoid rich fraction from the leaves of *S. indicus* was less than ascorbic acid (63.32). Percentage of nitric oxide radical scavenging activity Terpenoid rich fraction from the leaves of *S. indicus* and standards were presented in Table-4. In the present outcome, nitrite was formed by incubation of sodium nitroprusside in standard phosphate saline buffer at 25°C was reduced by polyphenol rich fraction. Imperative scavenging activity may be due to the antioxidant property of polyphenol, compounds present in *C. occidentalis* flower, which contest with oxygen to respond with nitric oxide, prominent to less production of nitric oxide. Blando *et al.* (2019) evaluated the antioxidant activity of polyphenolic extracts from cladodes of *Opuntia ficus-indica*. In particular, *O. ficus-indica* cladodes extracts exhibited *in vitro* strong free radical scavenging activity and increased antioxidant activity in human erythrocytes

Table 4: Nitric oxide radical scavenging assay of terpenoid rich fraction from the leaves of *S. indicus*

Different concentration of extract	Percentage of Nitric oxide radical scavenging activity	
	Terpenoid rich fraction from the leaves of *S. indicus*	Standard Vitamin-C
25 µl/ml	18.34±1.78	15.34±1.78
50 µl/ml	37.64±2.67	31.23±2.34
75 µl/ml	56.34±1.89	53.34±1.63
100 µl/ml	72.34±2.45	69.34±1.48
EC_{50} value	61.23	63.32

[a] Results are expressed as percentage of Nitric oxide radical activity with respect to control. Each value represents the mean+SD of three experiments.

Inhibition of Albumin Denaturation



Graph 1: Inhibition of albumin denaturation activity of terpenoid rich fraction from the leaves of *S. Indicus*

Protein denaturation is involved in inflammation and plant extracts showing inhibition of denaturation are often tested for anti-inflammatory activity.

For inhibiting thermally induced denaturation of albumin, the extract showed an astonishingly effect at different concentrations as shown in Graph-1. During the investigation of the activity of the plant extract on albumin denaturation we observed that terpenoid rich fraction from the leaves of *S. indicus* showed a greater protection comparatively to data observed for *Erythrina indica* (65.21 ± 1.77%) at 100 µg/mL (Chen *et al*., 2016). According to the fact that proteins denaturation is the cause of inflammation and rheumatoid arthritis, the protection of albumin denaturation confirms and contributes to anti-inflammatory activity of terpenoid rich fraction from the leaves of *S. indicus*.

Lipoxygenase Inhibition Activity of Terpenoid Rich Fraction from the Leaves of *S. Indicus*

The inhibition of LOX using linoleic acid as substrate was determined for the anti-inflammatory activity of terpenoid rich fraction from the leaves of *S. indicus.* The flavonoid rich fraction at 100µl/ml concentration exhibited more inhibition than the other concentration. The inhibition percentage was above 65.34% at 100 µl/ml (Table-4). The standard diclofenac sodium was showed 62.34% inhibition at 100 µl/mL.

The terpenoid rich fraction was showed higher inhibition activity than positive control. Plant derived products may also contain phytochemicals that act as enzyme inhibitors (Chen *et al*., 2016) [6].

These compounds have the ability to bind to enzymes and inhibit their activity. These products are further converted into others that play a key role in inflammatory processes. Hence, terpenoid rich fraction from the leaves of *S. indicus* which are able to inhibit that enzyme can be considered as antioxidants and possessing anti-inflammatory properties.

Table 5: Inhibition activity of Lipoxygenase by terpenoid rich fraction from the leaves of *S. indicus*

Terpenoid rich fraction from the leaves of *S. indicus*	Inhibition percentage of LOX	Diclofenac sodium (+ve control)
25 µl/ml	17.36±2.78	15.34±0.89
50 µl/ml	28.34±1.23	25.34±1.23
75 µl/ml	42.34±2.34	37.32±1.45
100 µl/ml	65.34±1.45	62.34±2.34
EC_{50} Value	66.23	69.34

Results are expressed as percentage inhibited Lipoxygenase with respect to control. Each value represents the mean+SD of five experiments

Conclusion

A positive direct correlation between antioxidant activities and the total phenolic and flavonoid content of the plant extracts was found. The plant extracts with high phenolic and flavonoid content also exhibited significant anti-inflammatory activity with good cell viability. The selected herbs could be a rich source of antioxidants and free radical scavenging compounds. The levels of Terpenoid compounds were correlated with the antioxidant and anti-inflammatory activities of the herb extracts.

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