ORIGINAL ARTICLE

Free radical scavenging activity of the plant extracts of *Synedrella nodiflora*

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Abstract

Introduction: *Synedrella nodiflora* (L.) Gaertn. (Asteraceae) is a native shrub of Ghana also found in the tropics and subtropics. The plant parts have been used in the treatment of rheumatism and mixtures used in the mitigation of stomach pain, interestingly, it is used in the cure of epilepsy and threatened abortion. Aim: This study focuses at investigating the probable mechanisms of free radical scavenging effects of the ethanolic and chloroform extract of the whole plant. Since the lipid cells are highly susceptible to peroxidase damage, our plant extract can be used to prevent the peroxidation of lipid cells. **Material and Methods:** With the help of thiobarbituric acid, the extract was screened for percentage inhibition. Similarly, the total reducing power assay was performed with respect to ferrous reduction and absorbance was studied. **Results and Discussion:** The extract with the increase in the concentration showed increase in the activity. Among all, the highest concentration of 200 µg/ml showed the greatest response and was relatively close to the standards used. In total reducing power and lipid peroxidation assay, ethanolic extracts of the plant seemed to show quite equal results.

Key words: Abortion, epilepsy, peroxidation, radical scavenging, thiobarbituric acid

INTRODUCTION

Synedrella nodiflora[1] (L.) Gaertn. (Asteraceae) is a shrub which was indigenous to tropical America grown as a weed. But now, it can be seen throughout the West African region. People of Ghana used to consume the leaves of the plant for its medicinal uses and this plant also forms a part of their fodder. According to Ghanaian traditional medicine, the decoction of the whole plant is believed to have anti-epileptic property. Moreover, leaves were used occasionally for the treatment of hiccups and abortion. In Nigeria, it is used to treat cardiac problems, inflammation and to stop bleeding. It is also reported with the potent anti-inflammatory activity and antioxidant property. Thus, the aim of the study is to evaluate the plant extract and provide an evidence for the claims around the globe. Implications in epilepsy and inflammation are in association with free radical liberation and lipid peroxidation. Thus, the role of free radical scavengers in the treatment of ailments caused by oxidative stress has been reported. The intend of this study of reporting the free radical scavenging and anti-lipid peroxidative properties of the ethanolic extract of the whole plant of *S. nodiflora* is successfully achieved.[2]

In this study, chloroform and ethanolic extract was taken and used for the studies. Two methods were chosen, namely, total reducing power assay and lipid peroxidation assay. Plant extract has showed up properties equal to that of standard used. Comparatively, the results for two different extracts were tabulated.[2]

MATERIALS AND METHODS

Collection and Processing of the Plant

The whole plant *S. nodiflora* was collected from Sengottai, Tirunelveli, Tamil Nadu, India, in the month of November 2016. Plant material was identified and authenticated by Mr. V. Chelladurai, Retired research officer botany, Central Council for Research in Ayurvedic Sciences, Government of India, Tirunelveli. The collected plant was free from diseases and also free from contamination of other plants. The

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collected plant was air-dried for few days and pulverized. The powder of the whole plant was used for extracting constituents subsequently using different solvent systems.

Preparation of Extract

Two kilograms of the powdered were cold macerated with 70% v/v of ethanol and chloroform for 48 h and 72 h, respectively. The hydroethanolic extract and chloroform extract were then concentrated to a syrupy mass under reduced pressure in Soxhlet apparatus, air-dried, and preserved in a silica desiccator. About 7% w/w yield was obtained after desiccation generally referred as the extract.[3]

Lipid Peroxidation Assay

Lipid peroxidation is an established mechanism of cellular injury in plants and animals, and oxidative stress in the cellular level can be indicated by lipid peroxidation.[4]

Principle

The lipids in the cell membranes are highly susceptible to peroxidase damage and are broken into number of small units to form malonyldialdehyde. This reacts with thiobarbituric acid (TBA) to form TBA reacting substance which has a pink color with absorption maximum at 532 nm.

Reagents Required

- KCl (0.15 M)
- Trichloroacetic acid (TCA) (15%)
- TBA (0.375%)
- Ferrous sulfate (25 µM)
- Ascorbic acid (100 µM)
- Preparation of extracts (10, 50, 100, 200, 400, 800, and 1000 µg/ml) in dimethyl sulfoxide (DMSO).

Procedure

Liver was quickly excised after decapitation, washed several times with ice-cold saline solution (0.15 M KCl, pH 7.4), and homogenized in the same saline solution. About 10% liver homogenate was prepared and test solution contained homogenate with a protein concentration of 500 µg/ml. The lipid peroxidation was initiated by addition of 25 µM ferrous sulfate, 100 µM ascorbate, and 10 µM potassium hydrogen phosphate and the homogenates were incubated at 37°C for 30 min with different concentrations of extracts. A 1 ml of 15% TCA and 0.375% TBA were added to all the tubes, placed in a boiling water bath for 30 min, centrifuged and the supernatant was measured at 532 nm.

Percentage inhibition = OD control − OD test/OD control * 100.

Total Reducing Power

Principle

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. Antioxidant compound forms a colored complex with potassium ferricyanide, TCA, and ferric chloride, which is measured at 700 nm.[5,6]

Reagents required

1. Phosphate buffer (pH 6.6, 0.2 M).
2. Potassium ferricyanide (1%).
3. TCA (10%).
4. Ascorbic acid (1 mg/1 ml).
5. Preparation of extracts (10, 50, 100, 200, 400, 800, and 1000 µg/ml) in DMSO.

Procedure

For the measurement of reductive ability, we investigated the Fe^{+3}–Fe^{+2} transformation in the presence of the various extracts of *S. nodiflora* at different doses using the method (Oyaizu method). Definite amount of the extract and standard drugs, 2.5 ml phosphate buffer pH 6.6, and 2.5 ml 1% potassium ferricyanide[7] were incubated at 50°C for 20 min, 2.5 ml of 10% TCA were added to the mixture and centrifuged for 10 min at 3000 r.p.m. After centrifugation, 2.5 ml of the

Table 1: Lipid peroxidation assay

Concentration (µg)	Percentage inhibition		
	Chloroform extract	Ethanolic extract	Curcumin
5	3.77±0.73	7.14±0.51	22.37
10	14.82±0.62	22.63±0.90	40.48
20	13.88±0.68	52.02±0.83	54.44
50	29.51±0.42	63.20±0.85	81.53
100	49.59±0.53	74.12±0.52	86.11
200	66.84±0.63	78.70±0.20	89.89

Table 2: Reducing power assay

Concentration (µg)	Absorbance (nm)		
	Chloroform	Ethanol	Ascorbic acid
5	0.338	0.362	0.427
10	0.341	0.387	0.481
20	0.346	0.399	0.532
50	0.349	0.411	0.792
100	0.356	0.446	1.317
200	0.381	0.487	1.838
400	0.435	0.551	1.999
800	0.605	0.703	1.999
1600	0.775	1.072	1.999

supernatant were diluted with 2.5 ml of water and shaken with 0.5 ml freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. The control solution was prepared as above, but contained water instead of samples. An increase in absorbance indicated higher reductive ability.

RESULTS AND DISCUSSION

Data Analysis

As shown in Table 1 for in lipid peroxidation activity, the percentage inhibition was found to be 66.84 % (chloroform), 78.70% (ethanol), and 89.89% (curcumin). All the extracts showed positive response. The chloroform and ethanol extracts showed dose-dependent response. This effect may be due to the presence of steroids, alkaloids, and flavonoids present in various extracts.

Data analysis

In total reducing power assay, the absorbance value of chloroform extract was found to be 0.775 nm and for ethanolic extract its 1.072 nm as shown in Table 2. The standard ascorbic acid has shown the results around 1.999 nm. Relatively, the ethanol extract of the plant has shown better results when compared to the standard. The chloroform and ethanol extracts showed an increase in response with increase in dose. This property may be due to the presence of several phytoconstituents such as steroids, alkaloids, and flavonoids present in various extracts.

The intended study clearly demonstrates the free radical scavenging and anti-lipid peroxidative effects of the ethanolic and chloroform extracts of the plant *S. nodiflora* quantitatively based on the scientific data obtained from the above reports. On chemical screening of the extracts of the plant, phytochemical constituents of *S. nodiflora* showed the presence of steroids, reducing sugar, alkaloids, and phenolic, flavonoids compounds which could be the reason behind the plant's antioxidant properties.[8] Apart from these phytoconstituents, quinoline alkaloids, beta-carboline, and tannins like catechins are present which are potent free radical destroyers. The presence of polyphenolic compounds is electron donating and contributes significantly by changing oxidation pattern by inhibiting or delaying oxidation of biomolecules.

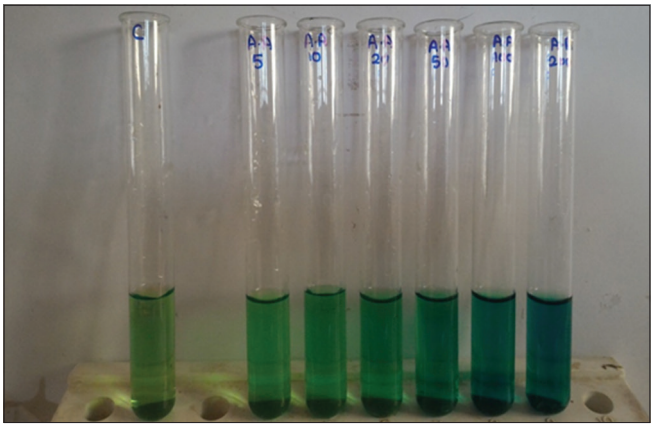

Figure 1: Testing samples of ascorbic acid

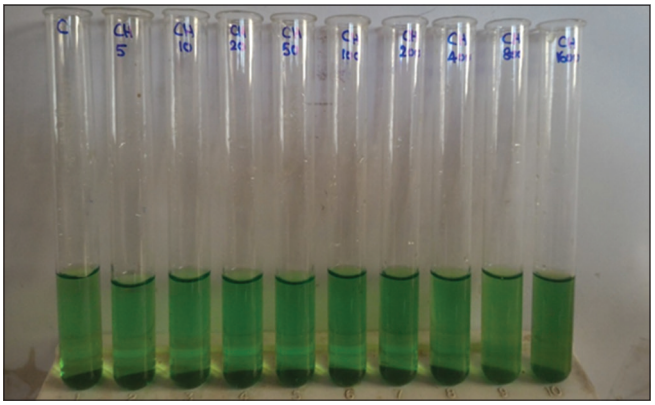

Figure 2: Testing samples of chloroform extract of *Synedrella nodiflora*

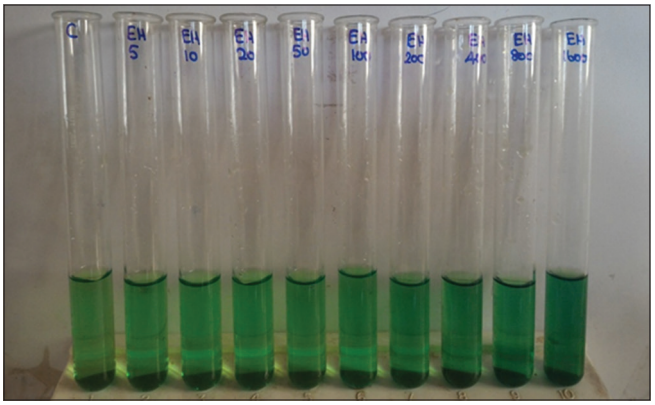

Figure 3: Testing samples of ethanolic extract of *Synedrella nodiflora*

Reducing power of the extract increased as concentration increased as depicted in the [Figures 1-3]. This measurement, as described by Oyaizu method, describes the Fe3+ to Fe2+ transformation in the presence of the extract. Iron is very commonly used as a catalyst in oxidation reaction due to its property of easily donating and accepting electrons. Due to pro-oxidative effects that iron has on cells and tissues, the release of iron from the breakdown of RBCs can be harming the cellular membrane. Other authors have observed that the process of reduction has a direct correlation between antioxidant activity and reducing power of certain plant extract. Reported activities of reductions include reacting with peroxide precursors and inhibiting peroxide formation. There are various levels at which antioxidants exert their activities among which prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging are pronounced.

Inhibition of lipid peroxidase by the extracts of *S. nodiflora* was reported.

CONCLUSION

The *in vitro* studies on the whole plant extracts of *S. nodiflora* showed the presence of significant free radical scavenging activities. The ethanol extract shows more antioxidant activities. All these activities may be due to the presence of terpenoids, steroids, alkaloids, flavonoids, and tannins. Our future aim is to isolate the chemical constituents responsible for the above activities and also to carry out the *in vivo* investigation.

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