Quantification of phytochemical constituents and *in vitro* antioxidant activity of *Synadium grantii*

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ABSTRACT

Introduction: In recent years, the search of biologically active compounds from plants has always been of great interest to scientists looking for new sources of useful drugs against infectious diseases. In this present study we investigated quantification of total phenolic, alkaloid content and *in vitro* antioxidant activity of ethanol (70%), methanol, ethyl acetate and hexane extracts of *Synadium grantii*. **Methods:** The quantification of the total phenolic and alkaloid contents were estimated by taking gallic acid and atropine are as a standards, *in vitro* antioxidant activity was evaluated for extracts by using different free radicals (superoxide, hydroxyl and DPPH). **Results:** *Synadium grantii* methanol extract have more phenolic and ethyl acetate alkaloid content than other extracts. The selected plant extracts were produced concentration dependent percentage inhibition of different free radicals and produced maximum activity at a concentration of 1280 µg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. **Conclusion:** In the present study, we found that the extracts of *Synadium grantii* showed good antioxidant activity. Among the four extracts, the ethyl acetate extract showed better activity than others.

Keywords: *Synadium grantii*, phenolic content, alkaloid content, *in vitro* antioxidant activity.

INTRODUCTION

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent biological activities, no side effects and economic viability. The biological activities of plants may be due to the presence of a diverse group of chemical compounds including steroids, glycosides, phenolics, glycosides, anthocyanins, flavonoids etc.[1-3] The search of biologically active compounds from plants has always been of great interest to scientists looking for new sources of useful drugs against infectious diseases. Many plant species have been investigated in the search for natural antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species. Several plant extracts have been shown to antioxidant activity.[4-6] Many studies have shown that natural antioxidants in medicinal and dietary plants are closely related to their biofunctionalities, such as the prevention or suppression of aging and many diseases associated with oxidative stress; cancer, cardiovascular diseases, rheumatoid arthritis, autoimmune diseases and AIDS. Thus, antioxidant capacity is widely used as a parameter to characterize food or medicinal plants. Phenolic compounds have antioxidant properties because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen and hydroxyl radicals.[7]

Synadium grantii is a succulent shrub with milky latex; leaves alternate, simple, and fleshy; flowers small and inconspicuous, in a small cup with a red rim of glands which belongs to the family Euphorbiaceae.

The objective of our research work was to investigate the total phenolic and alkaloid content and the antioxidant properties

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of the different fractions of the hydro alcoholic (70%) extract from *Synadium grantii* by using superoxide, hydroxyl, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals.

MATERIAL AND METHODS

Chemicals and Drugs

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic., Mumbai.

Preparation of extracts from synadium grantii

The plant material used in present study was collected from Visakhapatnam, Andhra Pradesh, and authenticated by taxonomist Dr. Prayaga Murthy Pragada, Dept. of Botany, Andhra University. Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. The powdered material was separately extracted in a Soxhlet apparatus for 6 h successively with hexane, ethyl acetate, hydro-alcoholic (ethanol 70% v/v) and methanol was concentrated to dryness under vacuum by using Rota-vapor.

Quantification of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteau reagent Singleton et al.[8] Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm (GAE).

Quantification of total alkaloid content

Total alkaloid content was determined by the Fazel et al., method.[9] The plant extract (1 mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean ± SEM (standard error of mean).

In vitro anti oxidant activity

For the assessment of free radicals scavenging activity, hexane, ethyl acetate, ethanol (70% v/v) and methanol extracts were dissolved in water and 5% dimethyl sulphoxide (DMSO), respectively.

Superoxide radical scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich method,[10] which depends on light-induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances.[11] Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the $Fe^{2+}/EDTA/H_2O_2$ system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS).

DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca et al.[12] The DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. The lower the absorbance, higher is the free radical scavenging activity.[13]

RESULTS

Quantification of total phenolic and alkaloid content

The quantified phenolic content of *Synadium grantii* extracts were ranging from 40.21 ± 0.10 to 9.10 ± 0.22 (mg/gm). The methanol extract have more phenolic content 40.21 ± 0.10 (mg/gm) than other extracts and alkaloid content was ranging from 34.27 ± 0.22 to 18.45 ± 0.56 (mg/gm). The ethyl acetate extract has more alkaloid content 34.27 ± 0.22 (mg/gm) than other extracts. Results of quantified phenolic and alkaloid content are showed in Table 1.

Table 1 Total phenolic and alkaloid content (mg/gm) of *S. grantii* extracts

Name of the extract	Total phenolic content (mg/gm)	Total alkaloid content (mg/gm)
Hexane	9.10 ± 0.25	18.45 ± 0.56
Ethyl acetate	35.62 ± 0.55	34.27 ± 0.22
Methanol	40.21 ± 0.10	26.68 ± 0.29
Ethanol (70% v/v)	33.15 ± 0.25	29.31 ± 0.16

Table 2 Concentration dependent percent inhibition of DPPH radical by different extracts of *Synadenium grantii* and ascorbic acid in *in vitro* studies

Extracts	Percentage inhibition of DPPH radical						
	Quantity of extracts/ascorbic acid in micrograms (µg)						
	20	40	80	160	320	640	1280
Alc. ext. *Synadenium grantii*	13.52 ± 0.3	20.51 ± 0.9	30.15 ± 0.6	40.00 ± 0.4	46.97 ± 0.2	56.16 ± 0.6	66.10 ± 0.8
MeOH. ext. *Synadenium grantii*	16.25 ± 0.65	24.45 ± 0.26	32.89 ± 0.45	43.74 ± 0.52	52.16 ± 0.54	60.66 ± 0.41	69.89 ± 0.49
EA. ext. *Synadenium grantii*	16.81 ± 0.29	26.99 ± 0.65	34.32 ± 0.53	46.56 ± 0.24	54.27 ± 0.23	62.23 ± 0.62	72.23 ± 0.23
Hex. ext. *Synadenium grantii*	9.12 ± 0.3	17.75 ± 0.4	25.51 ± 0.2	36.06 ± 0.6	44.37 ± 0.4	52.46 ± 0.7	62.44 ± 0.6
Ascorbic acid	48 ± 0.5	88.08 ± 1.0	90.68 ± 0.3	93.63 ± 0.5	94.21 ± 0.3	94.74 ± 1.1	–

In vitro antioxidant activity

The ethanol (70% v/v), methanolic, ethyl acetate and hexane extracts of *S. grantii* were found to possess concentration dependent scavenging activity on DPPH radicals and the results are given in Table 2. The mean IC_{50} values for DPPH radical of alcoholic (ethanol 70%), methanolic, ethyl acetate and hexane extracts of *S. grantii* were found to be 424 µg, 284 µg, 211 µg and 531 µg, respectively. The mean IC_{50} value of ascorbic acid was found to be 16 µg. The results are given in Figure 1.

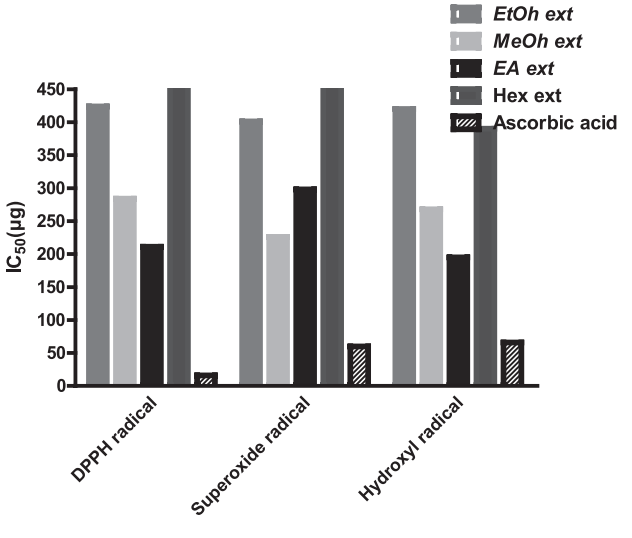

Figure 1. *In vitro* 50% inhibition concentration (IC_{50}) of different extracts of *Synadenium grantii* on DPPH, superoxide and hydroxyl free radicals.

In the present study, the ethanol (70% v/v), methanolic, ethyl acetate and hexane extracts of *S. grantii* were found to possess concentration-dependent scavenging activity on superoxide generated by photoreduction of riboflavin and the results are given in Table 3. The mean IC_{50} values for superoxide radical of Ethanol (70% v/v), methanolic, ethyl acetate and hexane extracts *S. grantii* were found to be 401 µg, 226 µg, 298 µg and 742 µg, respectively. The mean IC_{50} value of ascorbic acid was found to be 59.3 µg. The results are given in Figure 1.

The ethanol (70% v/v), methanolic, ethyl acetate and hexane extracts of *S. grantii* were found to possess concentration dependent scavenging activity on hydroxyl radicals and the results are given in Table 4. The mean IC_{50} values for hydroxyl radical of ethanol (70%), methanolic, ethyl acetate and hexane extracts *S. grantii* were found to be 420 µg, 268 µg, 195 µg and 390 µg, respectively. The mean IC_{50} value of ascorbic acid was found to be 66 µg. The results are given in Figure 1.

DISCUSSION

The phytochemical analysis conducted on *S. grantii* extracts revealed the presence of carbohydrates, alkaloids, steroids, terpenoids, phenols, glycosides etc. Thus the present study shows the significant antioxidant potential of *S. grantii* in all *in vitro* assays. Further, *S. grantii* was found to possess significant amount of total phenolic and alkaloid content. Antioxidant property of many phenolic and alkaloid compounds has been reported in other

Table 3 Concentration dependent percent inhibition of superoxide radical by different extracts of *Synadenium grantii* and ascorbic acid in *in vitro* studies

Extracts	Percentage inhibition of superoxide radical						
	Quantity of extracts/ascorbic acid in micrograms (µg)						
	20	40	80	160	320	640	1280
Alc. ext. *Synadenium grantii*	9.63 ± 0.4	14.26 ± 0.6	21.14 ± 0.5	34.24 ± 0.7	48.16 ± 0.5	56.67 ± 0.8	60.79 ± 0.9
MeOH. ext. *Synadenium grantii*	20.69 ± 0.63	29.54 ± 0.47	37.36 ± 0.26	45.66 ± 0.39	56.49 ± 0.66	64.69 ± 0.43	72.46 ± 0.63
EA. ext. *Synadenium grantii*	18.51 ± 0.35	26.45 ± 0.72	34.74 ± 0.24	41.48 ± 0.73	51.62 ± 0.73	59.36 ± 0.73	66.82 ± 0.73
Hex. ext. *Synadenium grantii*	8.12 ± 0.5	16.55 ± 0.3	24.46 ± 0.8	31.88 ± 0.4	39.44 ± 0.2	48.15 ± 0.4	56.26 ± 0.6
Ascorbic acid	28.15 ± 0.5	43.19 ± 1.1	56.87 ± 0.2	74.46 ± 0.7	80.72 ± 0.4	84.41 ± 1.5	–

Table 4 Concentration dependent percent inhibition of hydroxyl radical by different extracts of *Synadenium grantii* and ascorbic acid in *in vitro* studies

Extracts	Percentage inhibition of hydroxyl radical						
	Quantity of extracts/ascorbic acid in micrograms (µg)						
	20	40	80	160	320	640	1280
Alc. ext. *Synadenium grantii*	24.07 ± 1.1	35.89 ± 0.37	43.02 ± 0.30	59.45 ± 0.34	67.65 ± 0.29	73.03 ± 0.39	84.34 ± 0.55
MeOH. ext. *Synadenium grantii*	16.22 ± 0.61	24.32 ± 0.43	34.34 ± 0.46	43.54 ± 0.3822	52.61 ± 0.36	62.15 ± 0.38	70.35 ± 0.18
EA. ext. *Synadenium grantii*	17.99 ± 0.35	26.32 ± 0.77	35.71 ± 0.84	47.33 ± 0.38	59.79 ± 0.32	69.61 ± 0.43	77.44 ± 0.31
Hex. ext. *Synadenium grantii*	7.84 ± 0.73	15.41 ± 0.41	24.51 ± 0.43	39.72 ± 0.55	48.22 ± 0.71	56.07 ± 0.17	64.51 ± 0.58
Ascorbic acid	24.32 ± 0.4	35.12 ± 0.6	55.61 ± 1.0	65.31 ± 0.6	76.25 ± 0.4	82.11 ± 1.0	91.22 ± 1.3

investigations.[14,15] Plants with antioxidant activities have been reported to possess free radical scavenging activity. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism.

The antioxidant activity of *S. grantii* in the present study may be attributed to its phenolic and alkaloid content. Antioxidant property is widely used as a parameter for medicinal bioactive components. The antioxidant property of *S. grantii*, revealed in the present investigation may be important in future research works to study the underlying mechanism of its various medicinal properties. Further research can also explore the particular antioxidant principle(s) from the *S. grantii* extracts which can be one of the potent lead molecule(s) from the arsenal of natural products.

CONCLUSION

The data clearly indicated that the extracts ethanol (70%), hexane, ethyl acetate and methanol of *S. grantii* showed good antioxidant activity. Among all the extracts, ethyl acetate extract showed better activity.

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