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Research Article

QUANTIFICATION OF PHYTOCHEMICAL CONSTITUENTS AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF *FICUS SEMICORDATA* LEAVES EXTRACTS

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ABSTRACT

Recently, natural plants have received much attention as sources of biological active substances including antioxidants. 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 *Ficus semicordata*. The quantification of the total phenolic and alkaloid contents were estimated by taking Gallic acid and atropine are as a standard, *In-vitro* antioxidant activity was evaluated for extracts by using different free radicals (Superoxide, Hydroxyl and DPPH). *Ficus semicordata* leaves ethanol extract have more phenolic and methanol extract have more 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 1280µg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. In the present study we found that the extracts of *Ficus semicordata* leaves showed good Antioxidant activity. Among the four extracts, the ethanol (70%) extract showed better activity than other extracts.

Keywords: *Ficus semicordata*, Leaves, Phenolic content, Alkaloid content, *In-vitro* Antioxidant activity.

INTRODUCTION

Antioxidants are the vital substances which possess the ability to protect the body from damage caused by the free radical induced oxidative stress [1]. There is an increasing interest in the study of antioxidant substances mainly due to the findings of the therapeutic effects of free radical scavengers on the organism. A great number of plants worldwide showed a strong antioxidant activity [2, 3] and a powerful scavenger activity against free radicals [4, 5]. The medicinal values of plants lie in their component phytochemical such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. In recent years, the research on medicinal plants was become more interest to produce certain bioactive molecules which were having biological activities including antioxidant activity.

Ficus semicordata is a small to medium sized tree, up to 15m tall with an irregular crown. Trunk is up to 2 m circumference, without aerial roots. Bark is grey, young twings are covered with white or pale brownish short hairs. Leaves are carried on 1-1.5 cm long stalks. Leaf blade is variable, mostly elliptic to oblong, lance shaped, 10-30 cm long, 5-10 cm broad, base highly unequal-sided with a 3.4-nerved rounded large lower lobe overlapping the stalk. *Ficus semicordata* is used as a fodder and edible. A bath made from the fruit and bark is a cure for leprosy. Latex is drunk to cure fever [6-8]. Raw fruits are eaten in diarrhea [8]. Young fruit juice is applied in forehead to relieve headache [10]. Young twigs are fed to cattle for facilitating the discharge of placenta [11].

In present study we have extracted dried leaves of *Ficus semicordata* in hexane, ethyl acetate, ethanol (70%v/v) and methanol. These extracts were checked out for their *In-vitro* antioxidant activity. The extracts were found to be potent antioxidant activity.

MATERIAL AND METHODS

Chemicals and Drugs

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

Preparation of extracts from *Ficus semicordata* leaves

The plant material used in present study was collected from Visakhapatnam, Andhra Pradesh and authenticated by the taxonomist Dr. Prayaga Murthy Pragada, Depart 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 hrs 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.,* [12]. 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 765nm.The intensity of the light absorption at that wave length 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 [13]. The plant extract (1mg/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 ± S.E.M.

In-vitro anti oxidant activity

For the assessment of free radicals scavenging activity, the 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 [14], 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 [15]. 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*., [16]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in color) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine.
Lower the absorbance higher the free radical scavenging activity [17].

RESULTS AND DISCUSSION

Quantification of Total phenolic and alkaloid contents

The quantified phenolic content of *Ficus semicordata* leaves extracts were ranging from 16.25±0.22 to 97.02±0.17 (mg/gm). The ethanol extract have more phenolic content 97.02±0.17 (mg/gm) than other extracts and alkaloid content was ranging from 18.91±0.16 to 45.68±0.55 (mg/gm). The methanolic extract has more alkaloid content 45.68±0.55 (mg/gm) than other extracts. Results of quantified phenolic and alkaloid contents were showed in Table-1.

In-vitro Antioxidant activity

Free radicals particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in the pathogenesis of several chronical and degenerative diseases such as inflammation, cardiovascular diseases, neurodegenerative diseases, cancer and aging related disorders. We have demonstrated the ethanol extract of *Ficus semicordata* leaves contained high level of total phenolic compounds and methanol extract have more level alkaloid content, these were capable of inhibiting, quenching free radicals to terminate the radical chain reaction, and acting as reducing agents. Furthermore, phenolic and alkaloid compounds present in the plant kingdom are mainly responsible for the antioxidant potential of plants. Accordingly in this study, a significant and linear relationship was found between the antioxidant activity and phenolic and alkaloid content, indicating that these compounds could be major contributors to antioxidant activity. The ethanol extract of *Ficus semicordata* leaves showed strong antioxidant activity by inhibiting DPPH radical scavenging activity when compared with other extracts and methanol extract showed strong antioxidant activity by inhibiting superoxide radical scavenging activity when compared with other extracts. In addition, the *Ficus semicordata* leaves found to contain a noticeable amount of total phenols and alkaloids which plays a major role in controlling antioxidants. Although the antioxidant activities found *In- Ficus semicordata* leaves *vitro* experiment were only indicative of the potential health benefit.

The Ethanol (70%v/v), methanolic, ethyl acetate and hexane extracts of *Ficus semicordata* leaves were found to possess concentration dependent scavenging activity on DPPH radicals and the results were given in table-2. The mean IC_{50} values for DPPH radical of alcoholic (ethanol 70%), methanolic, ethyl acetate and hexane extracts of *Ficus semicordata* leaves were found to be 112µg, 222µg, 266µg and 596µg respectively. The mean IC_{50} value of ascorbic acid was found to be 16µg. The results were given in fig-1.

In the present study, the Ethanol (70%v/v), methanolic, ethyl acetate and hexane extracts of *Ficus semicordata* leaves 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 of *Ficus semicordata* leaves were found to be 305µg, 144µg, 213µg and 590µg respectively. The mean IC_{50} value of ascorbic acid was found to be 59.3µg. The results were given in fig-1.

The Ethanol (70%v/v), methanolic, ethyl acetate and hexane extracts of *Ficus semicordata* leaves were found to possess concentration dependent scavenging activity on hydroxyl radicals and the results were given table-4. The mean IC_{50} values for hydroxyl radical of ethanol (70%), methanolic, ethyl acetate and hexane extracts of *Ficus semicordata* leaves were found to be 265µg, 229µg, 211µg and 752µg respectively. The mean IC_{50} value of ascorbic acid was found to be 66µg. The results were given in fig-1.

Table 1: Total phenolic and alkaloid content (mg/gm) of *Ficus semicordata* leaves extracts

Name of the extract	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)
Hexane	16.25±0.25	18.91±0.16
Ethyl acetate	34.65±0.11	27.22±0.35
Methanol	45.68±0.55	32.64±0.22
Ethanol (70%v/v)	97.02±0.17	45.66±0.55

Table 2: Concentration dependent percent inhibition of DPPH radical by different extracts of *Ficus semicordata* leaves 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. of *Ficus semicordata*	19.53±0.5	32.76±1.1	45.66±0.9	55.92±0.6	63.42±0.6	72.83±0.8	83.86±0.9
Methanol ext. of *Ficus semicordata*	17.54±0.62	28.46±0.76	37.53±0.91	47.62±0.35	54.52±0.41	61.36±0.31	70.52±0.22
Ethyl acetate ext. of *Ficus semicordata*	15.42±0.77	26.52±0.14	35.53±0.91	44.42±0.32	52.86±0.58	60.28±0.13	68.46±0.39
Hex. ext. of *Ficus semicordata*	8.23±0.3	15.36±0.4	23.71±0.6	35.12±0.6	43.72±0.2	51.23±0.5	60.36±0.4
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	--

Table 3: Concentration dependent percent inhibition of Superoxide radical by different extracts of *Ficus semicordata* leaves 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. of *Ficus semicordata*	14.96±0.2	28.68±0.8	34.80±0.3	41.05±0.2	51.02±0.5	55.45±0.3	66.41±0.6
Methanol ext. of *Ficus semicordata*	22.45±0.19	31.52±0.46	43.69±0.22	51.62±0.71	60.58±0.54	70.35±0.89	79.65±0.43
Ethyl acetate ext. of *Ficus semicordata*	19.98±0.41	26.45±0.63	38.12±0.14	47.45±0.71	55.96±0.46	62.55±0.26	71.44±0.51
Hex.ext. of *Ficus semicordata*	12.26±0.3	20.33±0.5	28.50±0.3	36. 5±0.5	44.26±0.5	51.33±0.3	60.23±0.4
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 *Ficus semicordata* leaves 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. *Ficus semicordata*	16.74±0.58	25.01±0.39	33.79±0.38	41.47±0.84	54.91±0.90	67.02±0.45	78.67±0.34
Methanol ext. *Ficus semicordata*	15.76±0.63	24.35±0.52	33.49±0.46	44.99±0.32	56.96±0.19	65.99±0.43	74.36±0.43
Ethyl acetate ext. of *Ficus semicordata*	17.44±0.72	28.63±0.93	35.65±0.33	46.72±0.21	58.66±0.51	67.56±0.22	78.81±0.32
Hex.ext. *Ficus semicordata*	6.32±0.55	15.66±0.31	23.41±0.62	31.22±0.72	40.19±0.42	47.31±0.63	58.23±0.41
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

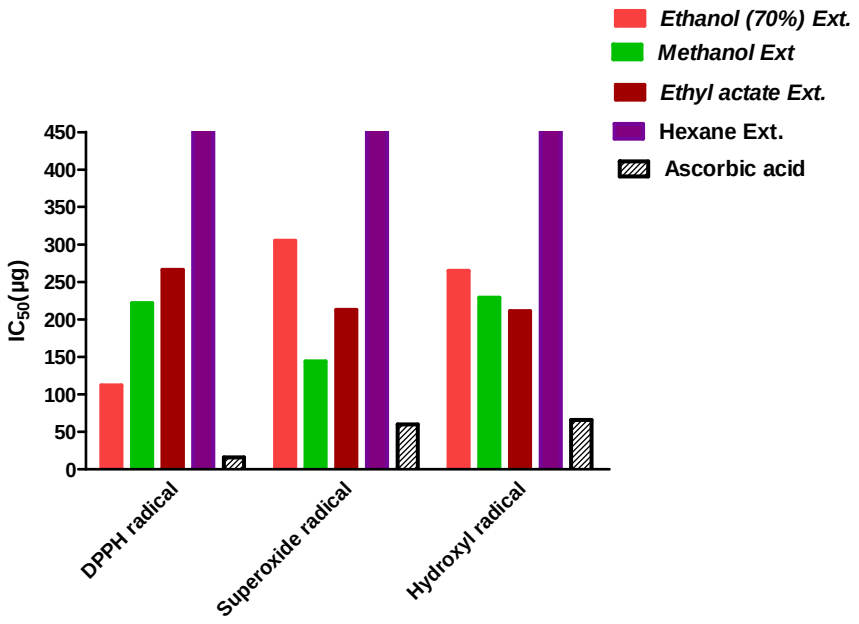

Fig. 1: *In vitro* 50% inhibition concentration (IC_{50}) of alcoholic extracts of *Ficus semicordata* leaves on DPPH, Superoxide and Hydroxyl free radicals.

CONCLUSION

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

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