Research Article **CODEX: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

IN VITRO ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *GYNOCARDIA ODORATA* ROXB. LEAF

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ABSTRACT

The *in vitro* antioxidant activity *and* Preliminary Phytochemical investigation was carried out on hydroalcoholic extract *Gynocardia odorata roxb* leaves. It indicates the presence of alkaloids**,** carbohydrates, glycosides, flavonoids, phytosterols, phenols, tannins and lignins. The antioxidant activity was determined by *in-vitro* methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and Estimation of Total Phenol and Flavonol Contents. The highest radical scavenging was observed in the *hydroalcoholic extract Gynocardia odorata roxb* leaves with IC_{50} = 28.38 ± 1.02mg. The greater amount of phenolic compounds, flavonoids and flavonol content leads to more potent radical scavenging effect as shown by the *hydroalcoholic extract Gynocardia odorata roxb* leaves. The evaluation of total antioxidant capacity and IC_{50} value *of* hydroalcoholic extract *Gynocardia odorata roxb leaves* for DPPH activity showed significant antioxidant activity. The results of this research work are promising thus indicating the utilisation of the leaves of *Gynocardia odorata roxb* as a significant source of natural antioxidants.

KEY WORDS

Gynocardia odorata, Hydroalcohol, 2, 2-diphenyl-1-picrylhydrazyl and Antioxidant capacity.

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INTRODUCTION

Antioxidants get their name because they combat oxidation[1]. They are substances that protect other chemicals of the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation. During this reaction the antioxidant sacrifices itself by becoming

oxidized. However, antioxidant supply is not unlimited as one antioxidant molecule can only react with a single free radical[2]. Therefore, there is a constant need to replenish antioxidant resources, whether endogenously or through supplementation. a free radical is any atom with at least one unpaired electron in the outermost shell, and is capable of independent existence[3]. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom[4]. The *Gynocardia Odorata* Roxb. leaves are an old traditional medicament used in fever, the present study was designed to determine the antipyretic effect of hydroalcoholic extract. *Gynocardia Odorata* is a very large East Indian tree. The leaves are glossy, entire, and alternate. The flowers are yellow and sweet-scented. The fruit is round, ash - colored, and when mature, averages in weight from 10 to 20 pounds. The numerous seeds are imbedded in its pulp, and contain oil, which, according to Roxburgh, is mixed with fresh butter, and used by the natives as a remedy for cutaneous diseases. It is known as Chaulmoogra (Chaulmugra), and is said, when powdered, to have been used with advantage in scrofula, skin diseases, and rheumatism, the dose being about 6 grains. The seeds are grayish, irregularly ovoid, compressed, and angular and smooth, a little over an inch long, and have an oily taste and a peculiar, nauseous odor. It is a medicinal plant growing wildly throughout India and tropical countries of the world[4,5].

MATERIAL AND METHOD

The leaves were then shade dried and grinded and made a coarse powder and the coarse powder were used for further studies.

Preparation of Extract

Extraction was done according to standard procedures using analytical grade solvents. For hydroalcoholic extract 250 gm, powdered leaves was taken in a pouch of filter paper and kept inside the soxhlet thistle then it was extracted with petroleum ether for 48-72 hours for defatting after that it was extracted with 99.9 % ethanol 50ml + Water 50ml (hydroalcoholic extract) for 48-72 hours. The % yield of hydroalcoholic was found to be 10.3 %. Then Preliminary Phytochemical screening was performed. Furthermore, the dried hydroalcoholic extract were used for evaluation of antioxidant activities.

Preliminary Phytochemical Screening [6]

The extracts were subjected to preliminary phytochemical qualitative screening to evaluate the presence of various primary or secondary metabolites following standard procedures.

Identification of Constituents by Phytochemical Test

The extracts were subjected to qualitative tests for identification of phytoconstituents present in it viz. alkaloids, carbhohydrates, glycosides, phytosterols, fixed oils ans fats, phenolic compounds & tannins, proteins and free amino acids, gums & mucilages, flavanoids, lignins and saponins.

Test of Alkaloids

A small portion of the solvent free petroleum ether, hexane, alcohol and aqueous extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloidal reagents such as,

a. Mayer's reagent - Cream precipitate
b. Dragendroff's reagent - Orange brown ppt
c. Hager's reagent - Yellow ppt
d. Wagner's reagent - Reddish brown ppt

Test for Carbohydrates & Glycosides

The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

a. Molisch's Test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha napthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube.

b. Fehling's Test

The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained shows the presence of carbohydrates.

Another portion of the extracts was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legals,

Borntrager's test to detect the presence of different glycosides.

c. Legal's Test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Purple colour in ammoniacal layer was observed.

Test for Phytosterol (Libermann Burchard Test)

One gram of the extract was dissolved in few drops of dry acetic acid, 3 ml of acetic anhydrade was added followed by few drops of conc sulphuric acid. Appearance of bluish green colour showed the presene of phytosterol.

Test for Fixed oils and Fats

A small quantity of the various extracts was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for Tannins and Phenolic Compounds[7]

Small quantities of extracts were dissolved separately in water and tested for the presence of phenolic compounds and tannins with

i. Dilute Ferric chloride solution 5% - Violet colour

ii.1% solution of gelatin containing 10% NaCl - White precipitate

iii. 10% Lead acetate solution- White precipitate.

Test for Proteins and Free Amino Acids

Small quantities of extracts were dissolved separately in a few ml of water and treated with:

i. Million's reagent - Appearance of red colour shows the presence of proteins and free amino acids.

ii. Ninhydrin reagent - Appearance of purple colour shows the presence of proteins and free amino acids.

iii. Biuret test - Equal volume of 5% solution of sodium hydroxide and 1% solution of copper sulphate were added. Appearance of pink colour shows the presence of proteins and free amino acids.

Test for Gums and Mucilages

About 10ml of extract were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for Flavonoids

With aqueous sodium hydroxide solution, blue to violet colour (Antho cyanins), yellow colour (Flavones), yellow to orange (Flavonones).

With concentrated sulphuric acid, yellowish orange colour (anthocyanins), yellow to orange colour (Flavones), orange to crimson (Flavonones).

Shinoda's Test

The various extracts were dissolved separately in alcohol, to this a piece of magnesium followed by conc. hydrochoric acid drop wise were added & heated. Appearance of magenta colour shows the presence of flavonoids.

Test for lignin[8]

With alcoholic solution, phloroglucinol and conc. hydrochloric acid, appearance of red colour shows the presence of lignin. The results of chemical tests of whole plant powder and extracts were shown in Table No.1.

Acute toxicity study[9]

Acute oral toxicity studies were performed according to OECD no. 423 guidelines. Three rat and mice of either sex were selected for the study. The animals were fasted overnight for food with free access for water prior to test extract *Gynocardia odorata roxb*. The hydroalcoholic leaves extracts were administration orally up to dose 2000 mg/kg. Individual animal was observed after dosing at least once during first 30 min., periodically during 24 hrs, with a special attention given during the first 4 hrs and daily thereafter, for a 7 days .

Statistical analysis

The significance of difference among the control group and various treated groups were analyzed by means of one-way ANOVA followed by Dunnett's multiple comparison tests. The experimental results are represented as ± SEM (standard error mean)[10].

Experimental Animals

***In Vitro* Antioxidant Activities**

To study the comparative antioxidant activity of the successive and hydroalcoholic extracts of *Gynocardia odorata roxb* in vitro antioxidant studies were carried out. The extracts having better antioxidant property. The scavenging activities of the extracts against different radicals were carried out according to the procedures described below. The absorbance was measured against a blank solution that contained the extract or standard, without the reagent. A control was performed without adding extract or standard. The final concentration of the extract or standard was 1000 to 0.45 µg/ml. The results of the *in vitro* antioxidant scavenging activities were expressed in terms of IC_{50}, which is the concentration of the sample required to scavenge 50% of free radicals.

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured using the modified methods. About 2.8 ml of test solution or standard ascorbic acid (in methanol), at different concentrations and 0.2 ml of DPPH (100 µM in methanol) were mixed and incubated at 37°C for 30 min. The absorbance of the resulting solution was measured at 517 nm using spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with the control (not treated with extract) using the following formula [11, 12].

% DPPH radical-scavenging = [(Ac − At)/Ac] ×100

Where, Ac and At are absorbances at 517 nm of the control and the test sample.

Estimation of Total Phenol and total flavonol Content

In a test tube, 200 µl of the extract (1 mg/ml to 0.1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu reagent and 800 µl of sodium carbonate. After shaking, it was kept for 2 h for reaction. The absorbance was measured at 750 nm. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 10-50 µg/ml. Using the standard curve, the total phenol content of the extract was determined and expressed as gallic acid equivalent in mg/g of the extract. A known volume (0.5 ml) of the extract was mixed with 1.5 ml methanol, and 0.1 ml of 1 M potassium acetate. After 5 min, 0.1 ml of 10% aluminium chloride was added and finally the mixture is diluted with 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm in spectrophotometer. The total flavonol content was expressed as rutin equivalent in mg/g of the extract [13, 14].

RESULTS AND DISCUSSION

Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free radical scavenging, lipid peroxidation and chelating of metal ions. There was no change in normal behavioral pattern of animals and no sign and symptoms of toxicity were observed during the observations which was done continuously for the first two hours and then observed up to twenty four hours for mortality[15]. The extracts were safe up to a maximum dose of 2000 mg/ kg body weight. The % yield of hydroalcoholic extract was found to be 10.3%. This study showed the presence of phytoconsituents such as alkaloids**, carbohydrates, glycosides, flavonoids, phytosterols, phenols, tannins, lignins** were observed[16]. The DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. Thus antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 2, 2- diphenyl-1-hydrazine is formed and as a result of which the absorbance (at 517 nm) of the solution is decreased. Hence the more potent antioxidant, more decrease in absorbance is seen and consequently the IC50 value will be minimum. The free radical scavenging activity of different solvent extracts of grape seed powders were determined by the DPPH method[17, 18]. The highest DPPH scavenging activities were shown by hydroalcoholic extract of *Gynocardia odorata roxb.* This result

indicates that the amount of DPPH scavenging activity appeared to depend on the phenolic concentration of the extracts of *Gynocardia odorata roxb* was show in Table No.2.

Table No.1: The preliminary phytochemical screening of *hydroalcoholic extract Gynocardia odorata roxb* leaves

S.No	Phytoconstituents	Alcoholic extract
1	Alkaloids	(+)
2	Carbohydrates	(+)
3	Glycosides	(+)
4	Flavonoids	(+)
5	Phytosterols	(+)
6	Fixed oils and Fats	(-)
7	Saponins	(-)
8	Phenolic and Tannins	(+)
9	Lignins	(+)
10	Proteins, Amino Acids	(-)
11	Gums and Mucilage	(-)

Table No.2: Antioxidant Activity of of *hydroalcoholic extract Gynocardia odorata roxb* leaves by DPPH Method

S.No	Extracts/ standards	IC_{50} values (µg/ml)*
		Gynocardia odorata roxb
1	Hydro-alcoholic extract	28.38 ± 1.02***
2	Ascorbic acid	2.70 ± 0.05*
3	Rutin	2.91 ± 0.1**

Values are expressed as mean ± S.E.M (*n* = 6); ***P≤0.001, **P≤0.01,*P≤0.05

Table No.3: Total Phenol and Flavonol Contents of Various Extracts of *E. Jambolana* Leaves

S.No	Extracts	Total phenol content (mg/g)*	Total flavonol content (%mg/g)*
1	Hydro-alcoholic extract	83.16 ± 0.14	39.36 ± 0.12

***Average of three determinations**

CONCLUSION

It can be concluded that the extracts of *Gynocardia odorata roxb* possess antioxidant activity. The plants may be considered as a source of natural antioxidants for medicinal use. However, the components responsible for the antioxidant activity are currently unclear. Therefore, further investigation is needed to isolate and identify the constituents present in the leaves extracts. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

ACKNOWLEDGEMENT

The authors are sincerely thanks to Bharathi College of Pharmacy, Mandya, Karnataka, India for providing the facilities to complete this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Jagan Mohan C. *et al*. *In vitro* antioxidant activity of hydroalcoholic extract of *gynocardia odorata* roxb. Leaf, *International Journal of Research in Pharmaceutical and Nano Sciences,* 2(3), 2013, 351-357.