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In-Vitro Assessment Of Antioxidant Activity, Total Phenolic And Flavonoid Content For Various Extracts Of *Gardenia Resinifera Roth*

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ABSTRACT

Gardenia resinifera (Rubiaceae) stem possess traditional uses such as Anti-spasmodic, Expectorant, Anti-microbial, Anti-helmintic, Anti-epileptic, Anti-convulsant properties due to presence of complex phytochemicals. In the present study we evaluated Phenolic, Flavonoid and Antioxidant activity of Hexane, Ethylacetate, methanol and Aq.methanolic extracts of stems of ***Gardinea resinifera*** by application of Folin Cio-calteu assay method, Aluminium Chloride assay method and DPPH radical scavenging assay, respectively The results indicated high amount of phenolic and flavonoid content in Aq.Methanoic extract. The Aq.methanoic extract was found to display highest TPC (46.60 mg GAE/g) and TFC(158.06mg QE/g, 55.63mg CE/g) , interestingly the better anti-oxidant activity was also found in Aq.methanolic extract with the percentage inhibition rate 44.59% at 50µg/ mL. The results suggested that *G.resinifera* has the potential to be source of alternative medicine due to its reportedly good Anti-Oxidant activity.

Keywords:

Gardinea resinifera, Total Phenolic content, Total flavonoid content, Anti-oxidant, DPPH radical scavenging activity.

1. INTRODUCTION

Gardenia resinifera is native to both tropical and subtropical regions of Asia, Africa[1]etc., it is a flowering plants in the coffee family, Rubiaceae. Gardenia resinifera are evergreen shrubs and small trees growing to 1–15 m (3.3–49.2 ft) tall. Flowering is from mid-spring - mid-summer. Gardenia is commonly used to treat infections, particularly bladder infections, abscesses, jaundice & blood in the urine, sputum, or stool. It is also used to treat anxiety or insomnia. It is also helpful in menopausal imbalance reflected in insomnia and depression, nervous tension, dizziness, and headache. This plant is rich in diverse chemical constituents like methyl 7-keto-octadec-cis-ll-enoic acid[2], dikamaliartanes A – F[3], Gardenin A, B, D, E, 5-Desmethylnobiletin, Xanthomicrol and Acerosin[4]. Some special pharmacological activities reported on *Gardenia resinifera* were antispasmodic, expectorant, anti-microbial and anti-

helmintic[5], Anti-epileptic, anti-pyretic[6] and anti- convulsant activities[7], antiproliferative activity against lung[8], breast, colon , hepatic and leukemia cell lines.

MATERIALS AND METHODS

Plant collection:

Gardenia resinifera (Stems) were collected from CSIR-CIMAP Research Centre, Mallapur, Boduppal, Telangana. The plant *G.resinifera* was taxonomically authenticated and identidied by Dr. A.Sabitha Rani, Assistant Professor, Department of Botany, Osmania University, college for women, koti, Hyderabad. A voucher specimen (CIMAP-2019/G.R) was stored at CIMAP Research Centre, Hyderabad. Shade dried, milled to a coarse powder by Cutter mill.

Extraction:

The stem powder (100 gms each) extracted using various solvents like hexane, ethyl acetate, methanol by ultrasonication for 30 mins (30 min x 4 times) and 30% aq. Methanol by overnight maceration, filtered and concentrated under reduced pressure by rota-evaporator. These extracts were used further for *in-vitro* antioxidant, total Phenolic and Flavonoid content estimation studies.

In-Vitro Anti-Oxidant activity

The anti-oxidant activity of the *G.resinifera* extracts was determined using 1, 1-diphenyl-1-picrylhydrazyl radical (DPPH) method.[9] Briefly, a freshly prepared DPPH solution in methanol (0.3mM) was added to 2.5 ml of plant extract solution of varying concentrations (50, 20, 15, 10 and 5 µg/ml) to start the radical antioxidant reaction and Corresponding blank sample were prepared and Ascorbic acid (1-100 µg/ml) was used as reference standard. The absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer against the corresponding blank solution and the results were shown in (Table 1 Figure 1). The inhibition % was calculated using the following formula.

$$\textbf{Percentage of Inhibition (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100$$

Estimation of Total Phenolic Content

The total phenolics of *G.resinefera* extracts were determined using the Folin and Ciocalteu reagent.[10] The mixture of the sample solution (1ml), 1ml of Folin-ciocalteu's reagents, 7% Na_2CO_3 (1ml) and distilled water (9ml) of varying concentrations (50, 20, 15, 10 and 5 µg/ml) and Gallic acid standards were also prepared accordingly and was vortexed and incubated for 90 min in dark at room temperature. The quantification of phenolic compounds was performed spectrophotometrically by measuring the absorbance in UV-VIS spectrophotometer at 750nm against blank which contain a mixture of 1ml methanol water, 1ml of Folin-ciocalteu's reagents and 1ml of 7% Na_2CO_3.

The total phenol content was expressed as Gallic acid equivalents (mg of GAE/g sample) through the calibration curve of Gallic acid. (Table-3)

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Blank preparation: 9 ml distilled water, 1 mL Folin-Ciocalteu reagent was added and 10 mL of 7% Na_2Co_3 solution was added to 25 mL volumetric flask. The final volume was made upto (25 mL) with distilled water and shaked, and incubated for 90 min in dark condition at RT. The phenolic content expressed in mg Gallic acid equivalents (GEA)/100 g fresh weight. All samples were analysed in duplicates. Total phenolic content was calculated by

$$\textbf{Total Phenolic Content} = \text{Concentration} \times \frac{\text{Vol. of the Sample}}{\text{Weight of the Sample}}$$

Estimation of Total Flavonoid content

The aluminum chloride colorimetric method[11] was used for the determination of the total flavonoid content of extract of *G.resinifera* stem. For total flavonoid determination, Quercetin and Catechin was used to make the standard calibration curve.

$$\textbf{Total Flavonoid content} = \text{Concentration} \times \frac{\text{Vol. of the Sample}}{\text{Weight of the Sample}}$$

a. Colorimetric assay (Quercetin)

The total flavonoids contents of different crude extracts were estimated by using $AlCl_3$ colorimetric assay method. Different extracts and standard solution of Quercetin (100, 200, 300, 400 and 500 mg/lit) was added to 10 mL volumetric flask, containing 4 ml of distilled deionized water and sodium nitrate (0.3ml) then mixed together. All the test tubes were kept in the dark place for about 6 min. Then $AlCl_3$ 10% (0.3ml) was added into the test tube and wait for 5 min in the dark for complete reaction. Finally, 5% NaOH (2 mL) and total volume was made up to 10 ml with distilled deionized water. The absorbance was measured of all samples at a wavelength 510 nm using UV spectrophotometer. Quercetin was used as standard for the calibration curve. . Flavonoid content expressed as mg Quercetin equivalents (QE) /100 g fresh mass (Table 5).

b. Colorimetric assay (Catechin)

The flavonoids content was determined by aluminium trichloride method using catechin as standard. An aliquot of extracts and standard solution of Catechin (100, 200, 300, 400 and 500 mg/lit) was added separately to 10 mL volumetric flask, containing 4 ml of distilled deionized water. To the flask add 0.3ml of a 5% $NaNO_3$ solution. The mixture was allowed to stand for 5 min, then 0.3ml of $AlCl_3$ (10%) was added and incubated for 6 min, followed by the addition of 2ml of NaOH (1M). The final volume of the solution was adjusted to 10ml with distilled water. After 15 min of incubation the mixture turned to pink and the absorbance

was measured at 510 nm Flavonoid content expressed as mg Catechin equivalents (CE) /100 g fresh mass (Table 5).

***Blank preparation*:** To the 10 mL capacity volumetric flask, water (10 mL), 5% sodium nitrate and 10% $AlCl_3$(0.3 mL each) were added one after the other with a gap of 5mins time interval.1 M sodium hydroxide (2 mL) was added after 5 mins and final volume was made up to 10 mL with deionised water.

The darker the colour complex, higher is the complexation with aluminum and indicates the presence of more number of flavonoid principles

RESULTS AND DISCUSSION

In-Vitro Antioxidant Activity (DPPH radical scavenging activity)

The leaf (hexane, ethyl acetate, methanol and aq.methanol) extracts of *G.resinefera* determined for *in-vitro* antioxidant assay by DPPH method showed dose dependent inhibition of DPPH radicals. Percentage scavenging of DPPH radical examined at different concentrations (by leaf hexane, ethyl acetate, methanol and aq.methanol extracts) was depicted in (Table-1, Fig-1).

S. No	Concentration	Ascorbic Acid	% Inhibition by Leaf Extracts		
			Ethyl acetate	Methanol	Aq. Methanol
1	25	32.10	3.27	7.14	**36.41**
2	50	64.12	13.90	3.79	**23.31**
3	75	96.3	20.91	17.41	**24.27**
4	100	128.4	25.11	3.68	**18.99**
5	125	160.5	25.11	9.59	**44.59**

Table 1: Affect of various extracts *G.resinifera* on DPPH radicals.

The *In-Vitro* Anti-oxidant activity using DPPH method was observed for various extracts and they were to be **(**36.41, 23.31, 24.27, 18.99, 44.59% at 25, 50, 75, 100 & 1250 µg/ mL). From the obtained above results it was showed that the % inhibition rate by antioxidant principles on DPPH free radicals found to be more in stem Aq. Methanol extract with 44.59% at 50 µg/ mL concentration when compared to other extracts *G.resinifera* with respect to ascorbic acid reference standard whose percentage inhibition was (97.23% at 50µg/ mL). The Order of *in-vitro* anti-oxidant activity for *G.resinifera* plant extracts by DPPH method is

Aq.Methanol > Ethyl acetate > Methanol > Hexane

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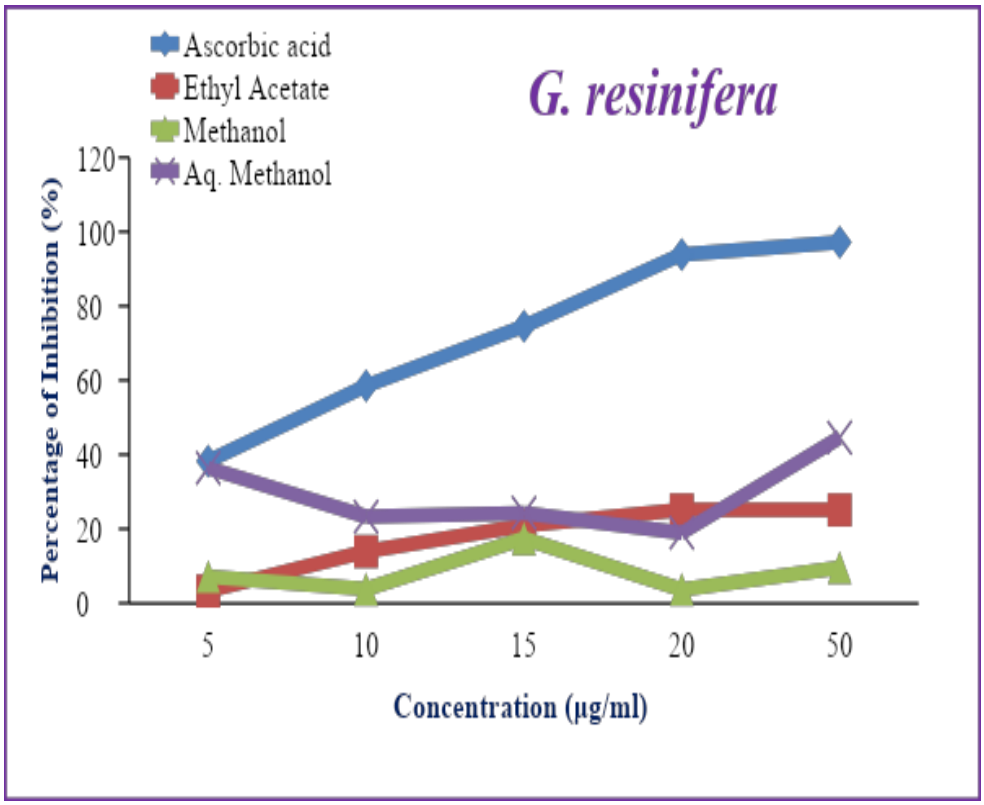

Figure 1: Graph of Extracts by DPPH method

Total Phenolic Content

The total phenolic content of *G.resinifera* stem extract was estimated by Folin-Ciocalteu assay method. The plant leaf extract (Hexane, Ethyl acetate, Methanol and 30% aq. Methanolic extracts) the total phenolic content found to be 15.38, 33.27, 37.86, 43.60 mg respectively as Gallic acid equivalents/100 grams of extract and is depicted in (Table-3). Standard graph of Gallic acid was depicted in (Table 2, Fig2).

S. No	Concentration (µg/ml)	Absorbance (nm)
	0	0
2	75	0.32
3	150	0.61
4	225	0.906
5	300	1.228
6	375	1.601

Table 2: Absorbance values of Gallic acid standard

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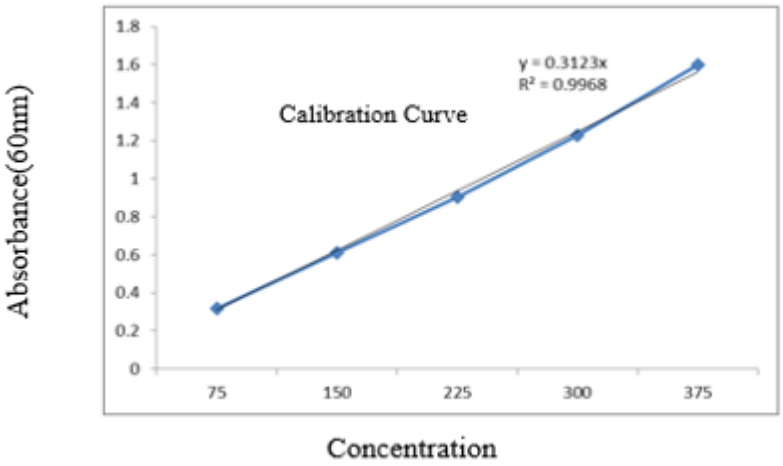

Figure 2: Standard graph of Gallic acid.

S. No	(Extracts)	mg/100 gm Gallic acid equivalents
1	Hexane	15.38
2	Ethyl acetate	33.27
3	Methanol	37.86
4	**Aq.Methanol**	**43.60**

Table 3: mg/100gm Gallic acid equivalents of different extracts of *G.resinifera*

Based on the results obtained, the total Phenolic content showed the presence of high phenolic content in stem Aq.methanolic extract with (43.60mg GAE/gr. Ext.) compared to other extracts. The Order of Phenolic content of plant *G.resinifera* stem extract

Aq.MeOH > MeOH > EtOAc > Hexane

Total Flavonoid Content

Total Flavonoid content of *G.resinifera* stem extract (Hexane, Ethyl acetate, Methanol and aq. Methanol) were found to be 20.18, 64.43, 120.74and 158.62mg QE/100 gram extract respectively and was depicted in (Table-5). Standard graph of Quercetin is depicted in (Table-4, Fig-3).

S. No	Concentration (µg/mL)	Absorbance	
		Quercetin	Catechin
1	0	0	0
2	200	0.18	0.16
3	400	0.38	0.36
4	600	0.57	0.53
5	800	0.76	1.74
6	1000	0.98	1.95

Table 4: Absorbance values of Flavonoid content using Quercetin and Catechin as standards.

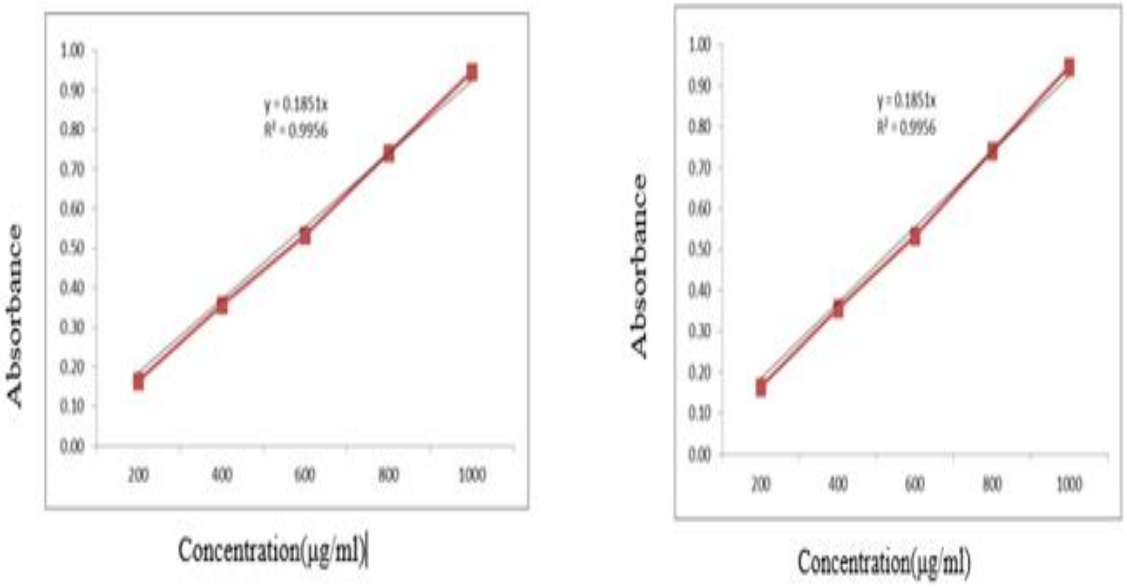

Figure 3: Standards graph of Quercetin and Catechin

Total flavonoid content of *G.resinifera* stem extract (Hexane, Ethyl acetate, Methanol and aq. Methanol) were found to be 5.65, 34.08, 44.02 and 55.63 mg CE/100 gram extract respectively and was depicted in (Table-5). Standard graph of Catechin depicted in (Table-4, Fig-3).

From the obtained overall results which were depicted in (Table-5 & Fig-3) it was inferred that the total Flavonoid content was found to be more in Aq.methanolic extract with 55.63mg CE/ 100 gm extract.

The Order of Flavonoid content of plant extracts using Catechin as standard.

Aq.MeOH > MeOH > Ethyl acetate > Hexane

Extract	Flavonoid Content	
	mg Catechin/gr. Extract	**mg Quercetin/gr. Extract**
Hexane	5.65	20.18
Ethyl acetate	34.08	64.43
Methanol	44.02	120.74
Aq.methanol	**55.63**	**158.06**

Table 5: mg/100gm Quercetin and Catechin equivalents of Hexane, Ethyl acetate, Methanol and 30% Aq. methanol extracts of *G.resinifera* stem

SUMMARY AND CONCLUSION

From the overall studies conducted on various extracts of *G.resinifera*, the antioxidant activity performed using DPPH method showed stem Aq. Methanolic extract as the most active one with percentage inhibition rate of about 44.59 µg/ml at 125 µg/mL concentration indicating the presence of more number of electron rich chemical constituents. Similarly the total phenolic content performed by Folin-Ciocalteu assay method using Gallic acid as a standard showed that the stem Aq.methanolic extract contains high phenolic content of about 43.60 mg GAE/ 100 gr. extract. Likewise the total Flavonoidal content performed by aluminium chloride colorimetric assay method using Quercetin and Catechin as a standards visualized that the stem Aq.methanolic extract contains high amount of Flavonoidal content in Quercetin standard method (with 158.06 mg QE/100gr Extract) where as in Catechin standard method, the stem Aq.methanolic extract showed high amount of Flavonoid content with (55.63mg CE/100gr Extract).

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