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Antioxidant activity of various extracts of *Givotia moluccana* (L.) Sreem

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Abstract
The natural antioxidants present in the plants which are of great human interest that help to prevent or treat many diseases are phenols and flavonoids. This article is focused at estimation of total Phenolic, Flavonoid content and Antioxidant activity of Hexane, Ethyl acetate, Acetone, Methanol and Ethanol extracts of *Givotia moluccana* (L.) Sreem. Total phenol and flavonoid content for the various solvent extracts of leaves was carried out using Folin-Ciocalteau method and flavonoid content by Aluminium chloride colorimetric method. Antioxidant properties of leaf extracts were determined by DPPH, Hydrogen peroxide scavenging capacity and Ferrous reducing antioxidant capacity assay. The plant showed good phenolic content 181.34±2.24 mg GAE/gr. Ext. and flavonoid content 96.27±1.42 mg CE/gr. Ext. for methanol extract compared to other extracts. The Antioxidant activity of methanolic extract showed highest activity by various methods used.

Keywords: *Givotia moluccana*, phenolic content, flavonoid content, DPPH, scavenging capacity, antioxidant activity

1. Introduction
Medicinal plants have been used by man for centuries to manage diseases and have a host of antioxidant complexes [1]. Medicinal plants are well known for their antioxidant and antimicrobial properties that prevent food degradation and alteration as they are rich in phenolic substances, usually referred to as polyphenols [2], which are ubiquitous components of plants. Their importance in the safeguarding of health, and the protection from coronary heart disease and cancer, has recently been established, thus constituting them as functional food preservatives [2]. Different parts of plants appear to have desired comparative advantages; hence there is growing interest in natural antioxidants [3]. The search for novel natural antioxidants has greatly increased. It is certainly not known which constituents of plant are associated in reducing the risk of chronic diseases, but antioxidants appear to play a key role in the protective effect of plant medicine [4]. The antioxidant contents of medicinal plants may contribute to the protection they offer from disease. Consuming of natural antioxidants has been observed to save man's life from degenerative disorders [5]. Many studies have shown that natural antioxidants in plants are usually derived from polyphenols, flavonoids, and proanthocyanidins [6]. The plants having high phenolic compounds contents were proved to be a good source of powerful antioxidants [7]. The antioxidant activities of the extracts are positively correlated with their contents of polyphenols, flavonoids, or proanthocyanidins [8] play a vital role for preventing cell damage caused by oxidative destruction as a result of free radical generation [9]. It is known that the antioxidant properties depend on the type of solvent used in the extraction [10] as different solvents extract different type of constituents into it.
The present study demonstrates the antioxidant activity of *Givotia moluccana* species of *Givotia* occurring in peninsular India and Sri Lanka and it is based on the examination of herbarium specimens [11]. The hydroalcoholic extract of *G. moluccana* showed the presence of major secondary metabolites [12, 13]. The silver nanoparticles of the extract showed antimicrobial activity [14]. The seeds of *G. moluccana* have been reported to be rich in oil used to lubricate fine machinery. In addition, its endosperm extracts have been used to improve the digestion of children by Palliyar tribes of Tamilnadu [15] and its decoction of various parts like fruits, flowers, stem and leaves were used to treat rheumatism, dandruff and wounds by Irula tribal communities [16] and identified as one of the significant medicinal plants of Seshachalam Biosphere Reserve in Eastern Ghats [17]. *G. moluccana* was prioritized in to the category of the highest conservation priority by Non-Timber Forest Products (NTFPs) survey [18]. Other species *Givotia madagascariensis* showed significant antitumor activities [19].

2. Materials and Methods

2.1 Preparation of extract

Plant material was collected from Manguru forest, Telangana. The leaves were shade dried for 14days and powdered. 30g of plant material was extracted with hexane, ethyl acetate, acetone, methanol and ethanol (100ml each) using soxhlet apparatus until clear solvent was observed in siphon tube of soxhlet. The excess solvent was evaporated using rota evaporator and the extracts were dried [20].

2.2 Determination of the total phenolic content assay

An aliquot (1 ml) of *G. moluccana* leaf extract or a standard solution of Gallic acid (50, 100, 150, 200 and 250 µg/ml) was diluted to 10ml with distilled deionised water [21]. 1ml of the Folin-Ciocalteau phenol reagent was added to the mixture, shaken and allowed to stand for 5min. After 5 min, 4ml of 7% Na_2CO_3 solution added to above mixture. The solution was diluted to 25ml with distilled water and mixed [22]. After incubation of 90 min at room temperature, absorbance against the prepared reagent blank determined at 750 nm with an UV-Visible spectrometer. This data was expressed in mg. GAE/g. Extract.

2.3 Determination of the total flavonoid Assay

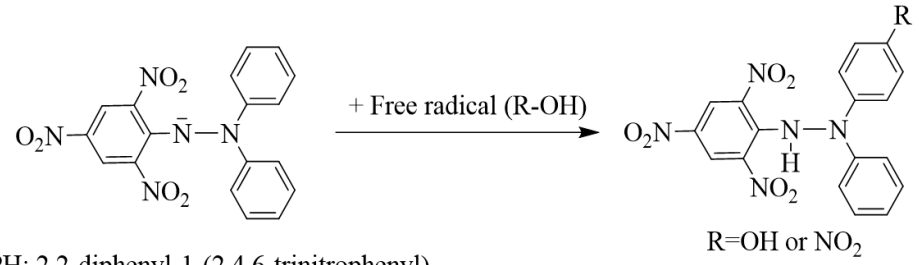
An aliquot (2.5 ml) of extract or a standard (+)-catechin (100, 200, 400, 600, 800 and 1000 µg /ml) was introduced to 10 ml of distilled deionized water to a 25 ml of volumetric flask [23]. To that added 5 % $NaNO_2$ of 0.75 ml, 10 % $AlCl_3$ 0.75 ml, incubated for 5 min. Later 5 ml 1M NaOH was added and diluted to 25ml. Agitated and absorbance measured at 510nm in UV-VIS Spectrophotometer. The data was expressed in mg. CE/g Extract.

2.4 In-vitro antioxidant activity

2.4.1 DPPH radical scavenging assay

2.4.1.1 Principle

DPPH is nitrogen-centered free radical, produces a purple solution in methanol. In radical form, it shows maximum absorbance at 517 nm that disappears on reduction by an antioxidant. This can be noticed as color change from purple to yellow in a spectrophotometer. The fast electron-transfer from the phenoxide anions of the phenolic compounds to the DPPH radicals is a possible mechanism for their reducing capacity.



DPPH: 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazin-1-ide

1(4-Hydroxyphenyl)-1-phenyl-2-picryl hydrazine
1(4-Nitrophenyl)-1-phenyl-2-picryl hydrazine

2.4.1.2 Method

Antioxidant activity of different solvents extracts of *G. moluccana* were determined by DPPH radical scavenging method [24]. DPPH radicals have an absorption maximum at 517 nm, which disappears on reduction by antioxidant component. The DPPH solution in methanol (0.1 m M) was prepared and 1ml of this solution mixed with 2.5 mL of test compounds solution. The samples were incubated at room temperature for 30min in dark chamber and then the decrease in absorbance was measured at 517 nm (As). A blank sample containing 1ml DPPH and 4ml methanol was prepared and absorbance measured (Ab). Radical scavenging was calculated using following formula.

Percentage of inhibition= [(Ab-As)/Ab] x 100

2.4.2 Ferrous reducing antioxidant power assay

2.4.2.1 Principle

The principle states the increase in absorbance of the reaction mixtures, as the absorbance increases the antioxidant activity increases. The constituent with antioxidant character in the sample produces a colored complex with potassium ferricyanide, ferric chloride and trichloroacetic acid that is measured at 700 nm by UV-Spectrophotometer.

2.4.2.2 Method

The Fe2+ can be assessed by measuring the formation of Perl's Prussian blue at 700 nm. 0.25 ml*G. Moluccana* extracts and standard solution at different concentration (12.5–150 µg/ml), 0.625 ml of potassium buffer (0.2 M) and 0.625 ml of 1% potassium ferricyanide solution were added into the test tubes and incubated at 50°C for 20 min to complete the reaction. 10 % trichloro acetic acid solution of 0.625 ml was added to it. The whole mixture was centrifuged for 10 min at 3000 rpm; 1.8 ml supernatant was withdrawn from the test tubes and mixed with 1.8 ml of distilled water and 0.36 ml of 0.1% ferric chloride ($FeCl_3$) solution. A blank solution was prepared and incubation was done similarly, its absorbance was measured at 700 nm. This was done in triplicate for each concentration [25].

2.4.3 Hydrogen peroxide scavenging assay

2.4.3.1 Principle

Hydrogen peroxide is a weak oxidizing agent having capability to inactivate a few enzymes by oxidation. It may react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical. The ability of test compounds to neutralize the hydroxyl radical is determined.

2.4.3.2Method

H_2O_2 (40 mM) solution was prepared in phosphate buffer (pH 7.4). *G. moluccana* extracts (100 µg/ml) were added to above solution and absorbance was measured at 230nm after 10 min against a blank solution containing phosphate buffer without H_2O_2. The percentage H_2O_2scavenging of extracts and ascorbic acid were calculated by formula [26]

% H_2O_2 Scavenged = [(Ac – Ae)/Ac] x 100

Where Ac is the absorbance of the control and Ae is the absorbance of the *G. moluccana* extracts or ascorbic acid

3. Results and Discussion

3.1 Determination of total phenolic content (TPC)

TPC of various *G. moluccana* extracts was calculated from calibration curve. Standard graph of Gallic acid with different concentrations was given in Fig-1. TPC of *G. moluccana* leaves equivalent to134.56±3.22, 153.89±2.84, 162.48±1.28, 181.34±2.24, 173.12±3.12 for hexane, ethyl acetate, acetone, methanol and ethanol extracts respectively of Gallic acid (GAE/g). Highest content was observed in methanolic extract. The TPC values were tabulated in table 1 and graphical representation was given in figure 3.

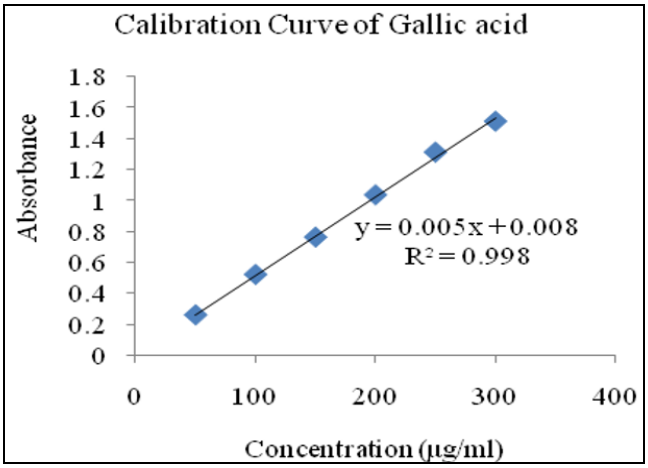


Fig 1: Calibration curve of Gallic acid

3.2 Determination of the total flavonoid content (TFC)

The TFC of Hexane, Ethyl acetate, Acetone, Methanol and Ethanol extracts of *G. moluccana* leaves of 1mg equivalent to28.13±2.28, 58.45±1.12, 65.13±2.62, 96.27±1.42, 92.58±0.62 /100g equivalents respectively of Catechin was Detected and depicted in table1. Standard graph of Catechin depicted in figure 2 and graphical representation of TFC in Fig 3.

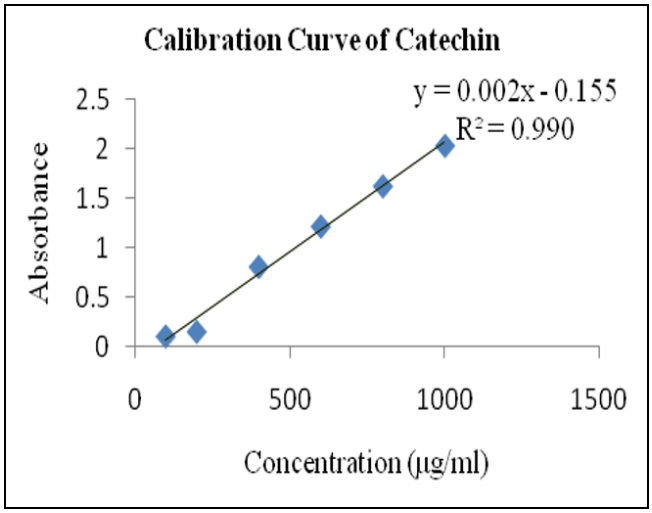


Fig 2: Calibration curve of Standard Catechin

Table 1: Total Phenolic and Flavonoid content of *G. moluccana* extracts.

Sample	Phenolic Content mg GAE/g. Gm Extract	Total Flavonoid Content mg CA/g. Gm Extract
Hexane extract	134.56±3.22	28.13±2.28
Ethyl acetate extract	153.89±2.84	58.45±1.12
Acetone extract	162.48±1.28	65.13±2.62
Methanol extract	181.34±2.24	96.27±1.42
Ethanol extract	173.12±3.12	92.58±0.62

Data represents a mean±SD (n = 3)

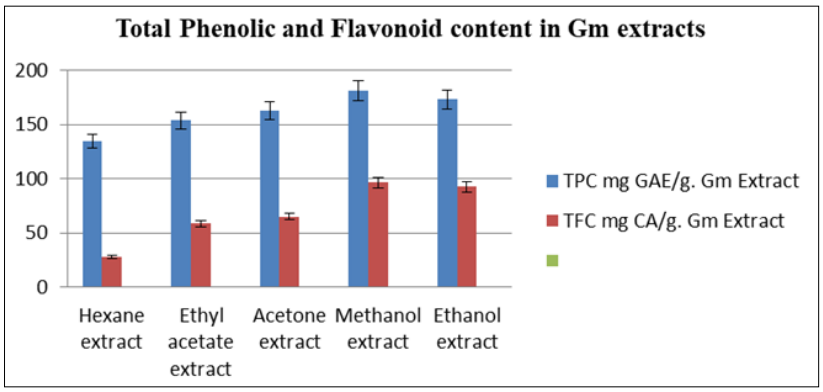


Fig 3: Bar diagram of TFC of Ps leaf extracts

3.3 *In-vitro* antioxidant activity

3.3.1 DPPH radical scavenging assay

The assay determines the ability of extracts to reduce DPPH radical to corresponding Hydrazine by converting unpaired electrons to pair once. In the present study inhibition of DPPH radical indicates that extracts cause reduction in DPPH radical. Percentage scavenging of DPPH examined at various concentrations was shown in table-2, fig-4 using Ascorbic Acid as standard and illustrates the effect of hexane (HE), ethylacetae (EAE), acetone (AE), methanol (ME) and ethanol (EE) extracts on DPPH radicals.

Table 2: Effect of various extracts of *G. moluccana* leaves on DPPH radicals

S. No	Concentration (mg/ml)	Percentage Inhibition (%)					
		Ascorbic acid	Hexane extract	Ethyl Acetate extract	Acetone extract	Methanol extract	Ethanol extract
1	2	58.23±0.11	11±0.1	15±0.16	19±0.17	29±0.13	25±0.12
2	4	75.63±0.13	16±0.13	22±0.13	21±0.15	48±0.12	42±0.14
3	6	88.11±0.15	21±0.12	30±0.12	32±0.16	69±0.11	65±0.11
4	8	94.55±0.14	26±0.14	34±0.11	43±0.14	74±0.16	70±0.13
5	10	98.9±0.13	34±0.12	45±0.17	53±0.13	88±0.14	82±0.16
IC_{50}		2.67	14.47	10.41	9.25	4.84	5.09

Data represents a mean ± SD (n = 3)

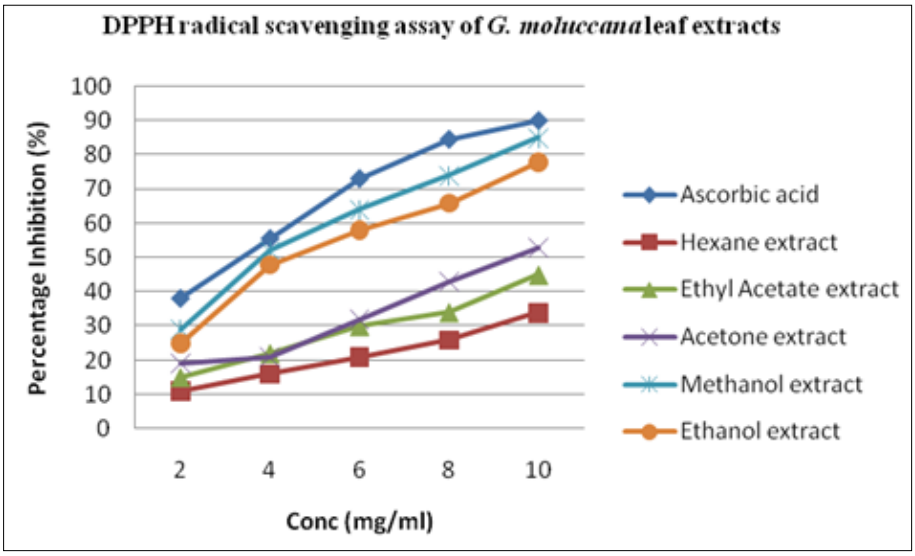


Fig 4: DPPH radical scavenging assay of different solvent extracts of *G. moluccana* leaves

The Anti-oxidant activity of *G. moluccana* using DPPH method was observed more in methanolic extract compared to other solvent extracts. Increase in concentration showed increase in scavenging activity. Order of Anti-oxidant activity of plant extracts by DPPH method ME> EE > AE > EAE> HE

3.3.2 Hydrogen peroxide scavenging capacity

In this study it was identified that various extracts of *G. moluccana* shown H_2O_2 scavenging activity in dose dependent manner. Ascorbic acid was standard and the percentage scavenging activity was compared with hexane, ethyl acetate, acetone, methanol and ethanol extracts. The results were tabulated in table 3 and figure 5.

Table 3: Hydrogen peroxide scavenging capacity of *G. moluccana*

S. No	Conc. (mg/ml)	Percentage Inhibition (%)					
		Ascorbic acid	Hexane extract	Ethyl Acetate extract	Acetone extract	Methanol extract	Ethanol extract
1	2	41.83±0.12	9.11±0.16	15.35±0.14	16.88±0.12	21.36±0.15	20.18±0.16
2	4	63.63±0.14	10.21±0.17	20.86±0.16	21.04±0.13	43.02±0.14	42.16±0.12
3	6	74.56±0.15	15.42±0.12	31.09±0.13	32.18±0.14	58.89±0.12	56.45±0.17
4	8	85.95±0.17	20.51±0.14	42.16±0.12	43.38±0.12	74.96±0.17	69.18±0.13
5	10	96.56±0.14	30.98±0.13	53.1±0.13	54.06±0.15	85.98±0.16	82.67±0.16
IC_{50}		2.51	14.47	9.41	9.19	4.25	4.95

Data represents a mean ± SD (n = 3)

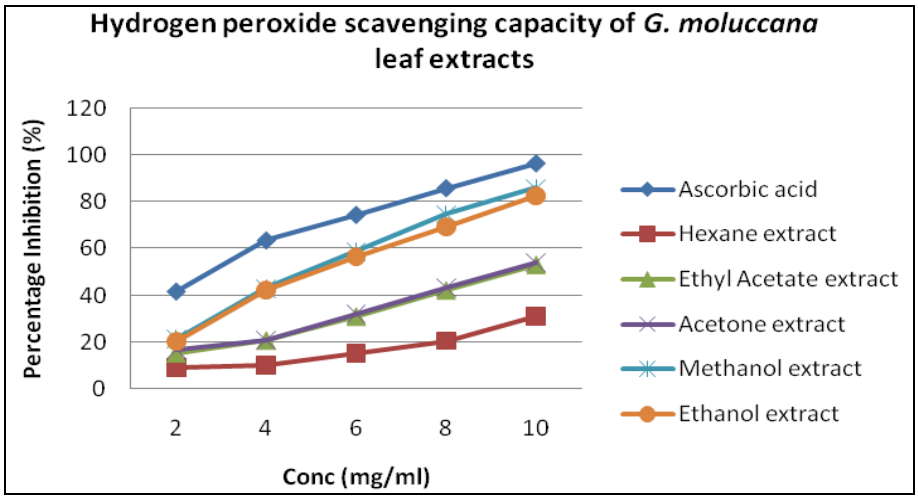


Fig 5: Graph showing hydrogen peroxide scavenging assay of *G. moluccana* leaf extracts.

Hydrogen peroxide scavenging activity was observed to be more in methanolic extract than other extracts. Order of Anti-oxidant activity of plant extracts ME > EE > AE > EAE> HE

3.3.3 Ferrous reducing antioxidant capacity assay

Here the reducing power of extracts (Fe^{3+} to Fe^{2+}) was compared with Ascorbic acid. In this assay the reductive capability of the extracts increased in increasing concentrations. The absorbance values at 700 nm were tabulated (Table 4) and the graphical representation was given the Figure 6. Increase in the absorbance indicates increase in the antioxidant activity.

Table 4: Ferrous reducing antioxidant capacity assay of *G. moluccana* leaf extracts.

S.no	Conc (mg/ml)	Absorbance					
		Ascorbic acid	Hexane extract	Ethyl Acetate extract	Acetone extract	Methanol extract	Ethanol extract
1	2	0.19±0.01	0.11±0.02	0.15±0.06	0.15±0.05	0.24±0.02	0.24±0.03
2	4	0.36±0.01	0.18±0.04	0.22±0.01	0.26±0.04	0.38±0.03	0.32±0.01
3	6	0.59±0.02	0.24±0.02	0.34±0.02	0.41±0.03	0.56±0.01	0.52±0.02
4	8	0.78±0.03	0.32±0.01	0.52±0.03	0.58±0.01	0.78±0.02	0.76±0.03
5	10	0.95±0.01	0.38±0.01	0.62±0.04	0.69±0.02	0.92±0.01	0.90±0.01

Data represents a mean ± SD (n = 3)

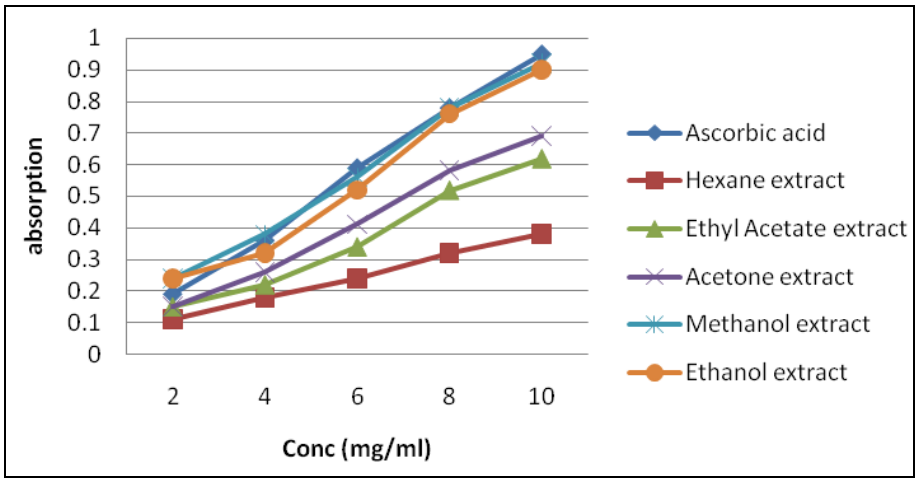


Fig 6: Graph showing Ferrous reducing antioxidant capacity assay of *G. moluccana* leaf extracts

4. Conclusion

Based on the present study it was concluded that *G. moluccana* leaves has considerable amounts of flavonoids and phenols and has good antioxidant activity which can act against free radicals to reduce their negative effects on various organs in the human body. All the extracts showed the presence of phenols and flavonoids but in various amounts. The order of total phenolic and flavonoid content by various extracts of *G. moluccana* was Hexane extract> Ethyl acetate extract>Acetone extract> Ethanol extract> Methanol extract. The methanolic extract showed highest amount of phenols 181.34±2.2 and flavonoids 96.27±1.42. The antioxidant activity by various methods showed good results with emphasis on methanolic extract as it showed highest activity compared to other extracts. However further investigation of various pharmacological activities and isolation and characterization of potent phytochemicals is necessary to give a detailed profile of the plant.

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