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Phytochemical Screening and antioxidant activity of *Glochidion ellipticum*

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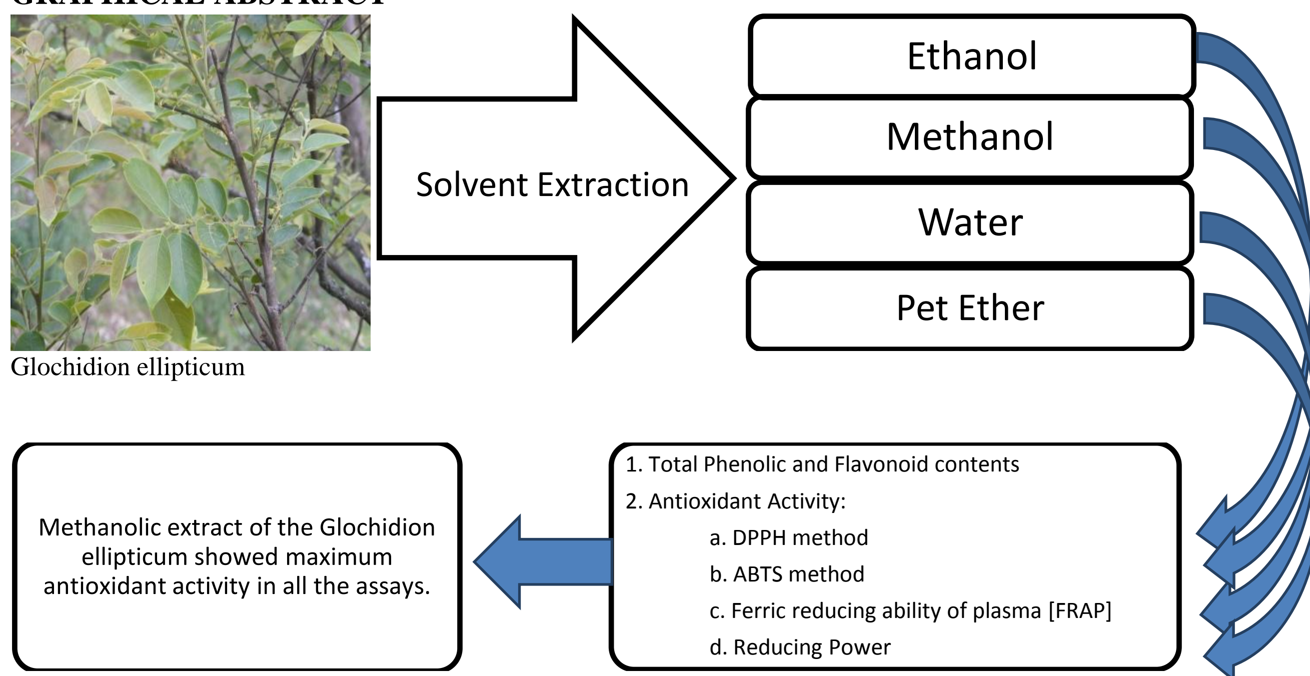
ABSTRACT

Glochidion ellipticum was investigated for its phytochemical content and antioxidant activity. For this purpose, leaves of the plant were selected and phytochemicals were extracted using different solvents viz. methanol, ethanol, aqueous and petroleum ether. The total Phenolic and Flavonoid content was found to be highest in ethanolic extract having 162.2 µg/ml equiv. Ferulic Acid and 135.2µg mL^{-1} equivalent quercetin respectively. The Antioxidant activity of the different extracts was evaluated using DPPH radical scavenging, ABTS radical scavenging, ferric reducing antioxidant power, (FRAP) and Reducing power. Methanolic extract showed the maximum antioxidant activity in all the assays.

GRAPHICAL ABSTRACT

Glochidion ellipticum



Keywords: Antioxidants, Total Phenolic Content, Phytochemicals.

INTRODUCTION

Plants are large source of bioactive molecules having antioxidant activity, which are effective in various diseases [1]. There is renewed interest in traditional medicine and an increase in demands for more drugs from plant sources. This change is mainly due to the wide spread belief that green medicine is safe and more dependable than synthetic drugs known for their adverse effects [2,3]. Hence, there is a need to substitute them from the naturally occurring antioxidants [4, 5]. The various mechanisms at the cellular levels generate potentially reactive derivatives of oxygen, (ROS). ROS are various forms of activated oxygen, which include free radicals such as superoxide ions (O^{2-}), hydroxyl radical (OH^-), as well as non-free radical species (H_2O_2) [6]. However, increase in ROS production or inadequate antioxidants can affect and induce oxidative damage to various biomolecules, proteins, lipids and DNA [7]. The bioactive molecules which can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions are known as Antioxidants [8, 9]. Reactive oxygen species are detoxified by antioxidants. The plant derived compounds can scavenge these reactive chemical species and minimise their oxidative damage. These antioxidants such as ascorbic acid, carotenoids, amino acids, peptides, polyphenols, flavonoids are important components for both human and animal diets and can be consumed safely, simultaneously exhibiting wide range of biological effects [10, 11]. The ability of the compound to scavenge the reactive species is termed as its antioxidant activity.

The genus Glochidion of the family Euphorbiaceae has many representative species in India and most of them contain constituents like triterpenoids saponins, sesquiterpenoids, glycosides and alkaloids. Therefore, it can be considered that these plants may possess a lot of medicinal value which may be beneficial for the human well-being [12]. The present work evaluates the phytochemical constituents, total phenolic, flavonoid content and antioxidant activity of the different extracts obtained from *Glochidion ellipticum*.

MATERIALS AND METHODS

Leaves of *Glochidion ellipticum* were collected from Mahabaleswar, Maharashtra. The leaves were rinsed with water, shade dried and finally powdered. About 5g powdered plant material was extracted with Ethanol, Methanol, water and Pet. ether (60-80) in Soxhlet apparatus. The successive hot extraction process was carried out for 8 hrs. The solvent was evaporated and obtained powder was used for analysis.

Determination of Ash value

Total Ash: 0.5g of powdered plant material was taken in tarred silica crucible, and then it was subjected to incineration in muffle furnace for 2 h at 450°C. The procedure was repeated till constant weight of the ash was obtained. Total Ash was expressed as % w/w [13].

Acid insoluble Ash: The Total Ash was boiled with 15mL of 2N Hydrochloric acid for 15min. The residue was filtered using Whatman paper with hot water washings. The paper was incinerated in muffle furnace at 450°C for 2 h. The procedure was repeated till constant weight of the ash was obtained. Acid insoluble Ash was expressed as % w/w [13].

Water soluble Ash: The total Ash was boiled with 15mL of distilled water for 15min. The residue was filtered by Whatman paper and incinerated. Constant weight was recorded, the difference between the total ash value and the insoluble ash value gives the water soluble ash expressed as %w/w [14].

Total Phenolic: Total phenolic content was measured by using Folin-Ciocalteu method [15]. Ferulic Acid was used as reference standard (50-250µL mL^{-1}) for plotting calibration curve. 250µL of extract was mixed with 2mL of water and 250µL of freshly prepared 1N Folin-Ciocalteu reagent and was neutralized with 3mL of 5% Na_2CO_3. The reaction mixture was then incubated for 150 minutes in dark at room

temperature. The absorbance of the resulting blue colour was measured at 765nm on UV -visible spectrophotometer. The total phenolic content was expressed as µg mL^{-1} equivalent Ferulic Acid.

Flavonoid Content: The total Flavonoid content was estimated by aluminium chloride ($AlCl_3$) method using quercetin as standard. 0.5mL of plant extract was mixed with 2mL of distilled water, 0.15mL of $NaNO_2$ was added and the solution was incubated for 5min. 0.15mL of $AlCl_3$ (10%) was added and again incubated for 2min. then 1mL of 1M NaOH was added. The absorbance of the solution was measured at 510 nm. The results are expressed as µg mL^{-1} equivalent quercetin [16].

Estimation of Antioxidant Activity

Scavenging effect of DPPH (1, 1 Diphenyl, 2-picrylhydrazyl) radical: The method given by Abdul et al [17] with some modifications was used to test the DPPH scavenging activity with slight modifications. 200µL of extract was mixed with 1mL 0.05mM DPPH solution in methanol and the total volume was made to 5.2mL by addition of methanol to the mixture. The reaction mixture was incubated for 30 min in dark at room temperature. The absorbance was measured at 517nm on UV-visible spectrophotometer. The radical scavenging activity was measured as decrease in the absorbance of DPPH and % scavenging was calculated by using the following equation.

$$\% \text{ scavenging} = [(A_o - A_t) / A_o] \times 100 \quad ..1$$

Where A_o is the absorbance of the control reaction (containing all reagents except the test compound), and A_t is the absorbance of the test compound.

Ascorbic acid was used as the standard

ABTS Radical analysis: The assay procedure as described by Kriengsak et al. [18] was used. The stock solution was prepared by mixing 7.4 mM $ABTS^{\bullet+}$ solution and 2.6mM of potassium persulfate in 1:1 ratio. The mixture was allowed to react for 24 hr in dark at room temperature. The solution was diluted with methanol to obtain an absorbance of 0.7 units at 734nm using spectrophotometer. 0.1 mL of plant extract was allowed to react with 3.9 mL of freshly prepared $ABTS^{\bullet+}$ solution for 30min in dark at room temperature. Standard curve was obtained using different concentration of Trolox. The results are expressed as µg mL^{-1} equivalent Trolox. ABTS scavenging was calculated using the following equation.

$$\text{ABTS scavenging activity} = [(A_o - A_t) / A_o] \times 100 \quad ..2$$

Where A_o is the absorbance of the control reaction (containing all reagents except the test compound), and A_t is the absorbance of the test compound

Ferric reducing ability of plasma (FRAP): The antioxidant activity was estimated using procedure by Marzanna et al [19] with slight modifications. The FRAP reagent contains 2.5mL of 10mm TPTZ (2, 4, 6-tripyrudyl-s-triazine) solution in 40mM HCl, 2.5 mL of 20mM $FeCl_3.6H_2O$ and 0.3mM sodium acetate buffer of pH 3.6 [20]. 3mL of FRAP reagent was mixed with 1mL of standard and incubated for 15 min in dark at room temperature and absorbance was measured at 593nm. The calibration curve was prepared using standard methanolic solution of Trolox and expressed as µg mL^{-1} equivalent Trolox.

Reducing power: The reducing power of the extract was determined using Gallic acid as a standard. 1mL of sample was mixed with 0.5ml of phosphate buffer (0.2M) pH 6.6, 0.5 mL of potassium ferricyanide, 0.1 mL of 10% trichloroacetic acid. After incubation for 15min in dark at room temperature 0.1 mL of 0.1 % of ferric chloride was added. The absorbance was measured at 700nm. An increase in absorbance was used as measure of the reducing power [21].

RESULTS AND DISCUSSION

Weight of the powder obtained after Soxhlet extraction was found to be 51, 65, 27 and 17mg g^{-1} dry weight of sample using Ethanol, methanol, water and Pet ether as solvents respectively. The total ash value of the leaves was found to be 9.7%, while 3.5% was found to be water soluble and 0.95% acid insoluble ash.

Qualitative Phytochemical analysis: Qualitative analysis of phytochemicals was carried out by different tests [22] and the results are shown in table 1.

Table 1. Qualitative Phytochemical analysis of *Glochidion Ellipticum*

	Glochidion ellipticum			
Tests	Ethanolic Extract	Methanolic Extract	Aqueous Extract	Pet. Ether Extract
Phenolic	+	+	+	+
Flavonoid	+	+	+	+
Tannins	+	+	+	+
Terpenoids	+	+	+	-
Saponins	+	+	+	-
Glycosides	+	+	+	-
Alkaloids	+	+	+	-
Anthroquinone	+	+	+	-

Note: (+) indicates present, (-) indicates absent

The various tests confirms the presence of phenolics, flavonoids, Tannins, terpenoids, Saponins, Glycosides, Alkaloids, Anthraquinone in ethanolic, methanolic and Water extracts of the plant, on the other hand pet ether extract shows only presence of phenolic, flavonoids and Tanis.

Quantitative Analysis of Total Phenolic and Flavonoid Content

Total Phenolic Content: The Total Phenolic content was estimated by Folin-Ciocalteu method. The exact chemical nature of FC reagent is not known, but believed to have hetreopolyphosphotungstates-molybdates. The transfer of electrons between antioxidants and molybdenum which is reduced in the complex, leading to blue species can be measured spectrophotometrically. Total Phenolic Content ranged from 71.39 µg mL^{-1} to 162.2µg mL^{-1} equivalent Ferulic acid. Ethanolic plant extract showed the maximum concentration of 162.2 µg mL^{-1} equivalent Ferulic acid, followed by methanol and water extracts with concentration of 136.7 and 130.1 µg mL^{-1} equivalent Ferulic acid respectively whereas, pet ether extract showed the least concentration of Total phenolic of 71.39 µg mL^{-1} equivalent Ferulic acid.

Total Flavonoid Content: The Flavonoid content of the extracts was determined by the reaction with sodium nitrite followed by coloured flavonoid-aluminium complex formation using Aluminium chloride. The coloured complex was measured spectrophotometrically at 510 nm. The ethanolic extract had the maximum flavonoid content of 135.2 µg mL^{-1} equivalent Rutin. The methanolic extract showed a concentration of 125.6 µg mL^{-1}equivalent Rutin, Water extract showed 85 µg mL^{-1} equivalent Rutin and pet ether extract had the least of 18.16 µg mL^{-1} equivalent Rutin. The Total phenolic and Flavonoid content in the various extracts are shown graphically in Figure 1.

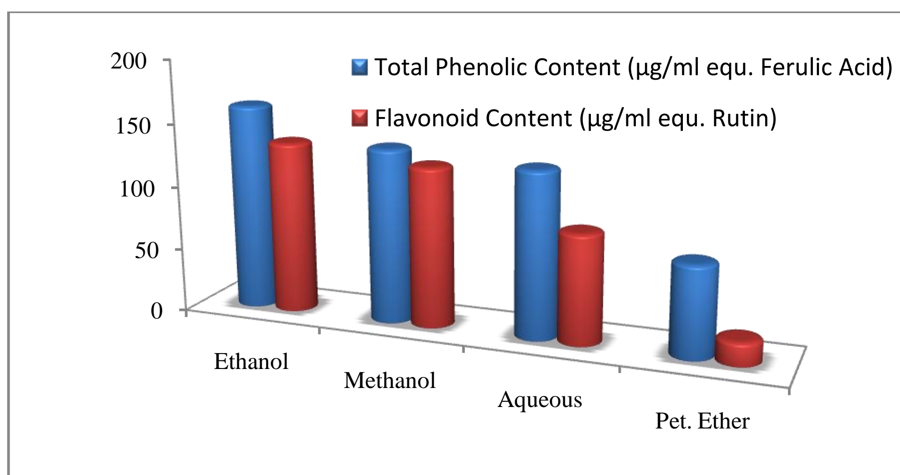


Figure 1 Graphical representation of Total Phenolic and Flavonoid content in various solvent extracts

Antioxidant Activity: The antioxidant activity of the various extracts was determined using DPPH radical scavenging, ABTS radical scavenging, FRAP (ferric reducing antioxidant power), reducing power assay.

DPPH Radical Scavenging Activity: DPPH assay method involves the estimation of decrease in absorbance of DPPH at 516nm, by reduction of free radical DPPH by plant extracts. the extend of the reaction depends upon the hydrogen donating ability of the antioxidants of the plant extracts [3].

ABTS Radical Scavenging Activity: the assay permits the measurement of antioxidant activity by measuring the radical cation as the percentage inhibition of absorbance at 734nm.

FRAP (Ferric Reducing ability of Plasma): FRAP assay is one of the most rapid test and very useful for routine analysis. The ability of the antioxidant to reduce Fe^{3+} to Fe^{2+} forming an intense blue colour Fe^{2+}-TPTZ complex having maximum absorption at 593nm.

Reducing Power: In the reducing assay, the antioxidants present reduces the Fe^{3+} / ferricyanide complex to the ferrous form [23]. The increase in the absorbance shows increase in reducing power of the extracts. The values of antioxidant activity are presented in table 2

Table 2 Antioxidant activity of various extracts in different Assay

Assay	DPPH radical % inhibition	ABTS radical % Inhibition	FRAP vales µg/ml equi. Trolox	Reducing Power Absorbance
Extracts				
Ethanol	47.1	39.2	558	0.507
Methanol	52.5	62.8	485	0.553
Water	35.1	36.7	532	0.509
Pet ether	6.8	11.4	-	0.377

An examination of table 2 reveals that methanolic extract shows highest antioxidant activity in different assay as compared to other solvent extracts. Further examination shows that pet ether extract has the lowest antioxidant activity. It can be revealed that methanol is the best solvent for the extraction of phytochemicals.

EC_{50} values represent the effective concentration of sample extract. This value for each assay was calculated by plotting the graph of % inhibition as a function of sample extract concentration. The corresponding graphs are shown in the figures 2 to 4.

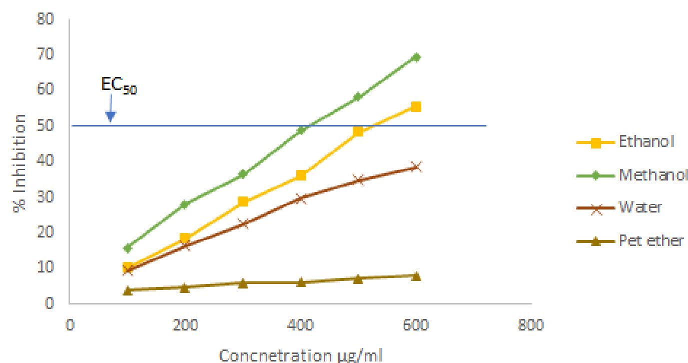


Figure 2. % Inhibition of DPPH radical different solvent extracts at various concentrations

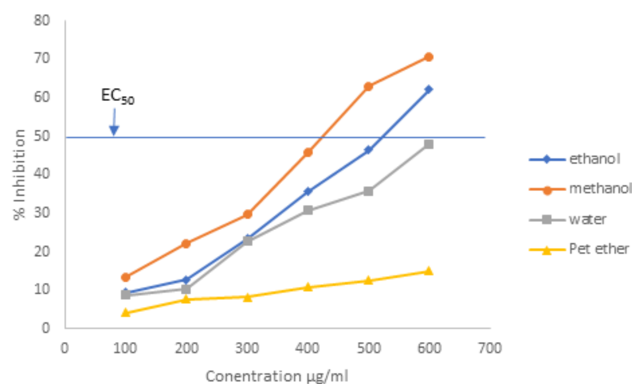


Figure 3. ABTS radical scavenging activity of different extracts at various concentrations

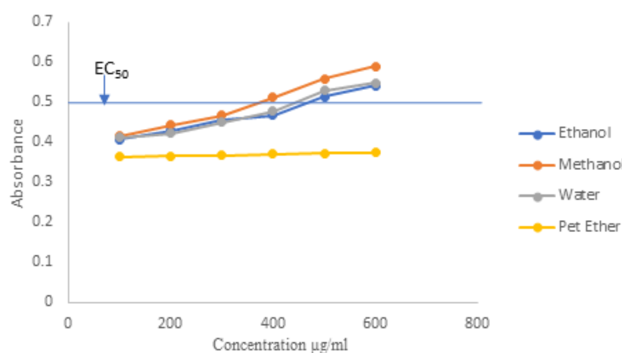


Figure 4. Reducing Power ability of different extracts at various concentrations

The obtained EC_{50} values are shown in the table 3.

Table 3. EC_{50} values of extracts in different assay

Assay / Extract	DPPH EC_{50} µg mL^{-1}	ABTS EC_{50} µg m L^{-1} eq. Trolox	FRAP EC_{50} µg m L^{-1} eq. Trolox	Reducing power EC_{50} µg m L^{-1} eq. Gallic Acid
Ethanol	534	520	553	581
Methanol	418	425	465	441
Water	766	648	528	543
Standard	168	127	132	132

As can be seen from the table 3 methanol shows the lowest EC_{50} values indicating high antioxidant power. Pet ether extract is having the lowest antioxidant power.

APPLICATIONS

The study reveals that extracts of the plant have active ingredient compounds showing high antioxidant activity, thus the plant will have good medicinal value useful for its medicinal applications.

CONCLUSIONS

The results show that the methanolic extract of the leaves of *Glochidion ellipticum* exhibits maximum antioxidant activity. The potent antioxidant activity in methanolic extracts shows that most of the active components are extracted in methanol, though ethanolic extract showed the maximum total phenolic content. However, more study is required to identify the potential medicinal properties and active ingredient of the plant.

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