

Research article

Preliminary phytochemical analysis and *in vitro* antioxidant activity of *Glochidion ellipticum* Wight (Phyllanthaceae)Vinayaka K.S.¹, Raghavendra L.S. Hallur², Prashith Kekuda T.R.³¹Plant Biology Laboratory, Department of Botany, Sri Venkatramana Swamy College, Vidyagiri, Bantwal 574 211, Dakshina Kannada, Karnataka, India²Center for Biotechnology, Pravara Institute of Medical Sciences (Deemed to be University), Loni, 413736 Rahata Taluk, Ahmednagar District, Maharashtra, India³Department of Microbiology, SRNMN College of Applied Sciences, Shivamogga, 577201, Shivamogga Karnataka, India

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Corresponding author: **Prashith Kekuda T.R.** Email: p.kekuda@gmail.com**ABSTRACT**

Introduction and Aim: Reactive oxygen species are implicated in the pathophysiology of several human ailments. Antioxidants from plants are shown to be promising in terms of their health benefits. *Glochidion ellipticum* Wight is belonging to the family Phyllanthaceae. This study investigated the antioxidant potential of solvent extracts of *G. ellipticum* leaves *in vitro*.

Materials and Methods: Sequential extraction of the shade dried leaf powder was carried out by maceration using petroleum ether, chloroform and methanol solvents. The solvent extracts were subjected to preliminary phytochemical analysis. DPPH, ABTS and Ferric reducing assays were performed to investigate *in vitro* antioxidant activity of solvent extracts. Total phenolic and flavonoid content of extracts was estimated by Folin-Ciocalteu reagent and Aluminium chloride colorimetric estimation method, respectively.

Results: Preliminary phytochemical analysis of solvent extracts revealed the presence of phytoconstituents viz. flavonoids, saponins, terpenoids and tannins in the leaf material. The solvent extracts scavenged both DPPH and ABTS radicals in a concentration-dependent manner with marked and least activity being shown by methanol extract and petroleum ether extract, respectively. In ferric reducing assay also, methanol extract showed marked activity followed by chloroform and petroleum ether extracts. Total phenolic and flavonoid content was highest in methanol and least in petroleum ether extract.

Conclusion: The radical scavenging and reducing abilities of extracts observed in this study could be attributed to the presence of secondary metabolites detected in the plant as it is well established that the polyphenolic compounds including flavonoids are excellent antioxidants. A direct correlation was observed between the content of phenolics and flavonoids and the antioxidant activity of extracts. The plant appears to be suitable for developing novel formulations that can be used to manage oxidative damage.

Keywords: *Glochidion ellipticum*; free radicals; DPPH; ABTS; ferric reducing; total phenolic, total flavonoid.

INTRODUCTION

Reactive oxygen species (ROS) include free radicals such as superoxide radical, hydroxyl radical and non-radicals such as singlet oxygen and hydrogen peroxide. ROS are the derivatives of oxygen metabolism and are constantly produced. In excess, these ROS contribute to cellular damage by affecting carbohydrates, proteins, lipids and nucleic acids. Free radicals and other ROS are implicated in several diseases or disorders such as cancer, aging, neurodegenerative diseases and cardiovascular diseases. The cells of aerobic organisms are equipped with antioxidant protection systems that include enzymatic and non-enzymatic defence mechanisms (1-4). However, the body needs to extract antioxidants in the form of a diet, especially during pathophysiological conditions.

Antioxidants from herbs, fruits and vegetables are shown to protect against the deleterious effects of ROS. Polyphenolic compounds in plants are shown to be excellent antioxidants (5-7).

Glochidion ellipticum Wight belonging to the family Phyllanthaceae is a medium sized tree. The plant is used ethnomedicinally (8-10). It is reported that *G. ellipticum* exhibit anthelmintic (11), anti-inflammatory (12), anticancer (13), antioxidant (14) and antidiabetic activity (15). In a recent study, Hossen *et al.*, (16) revealed the efficacy of leaf extracts of *G. ellipticum* to exhibit inhibitory activity against LPS induced inflammation of RAW 264.7 macrophage cells and dextran sulfate sodium induced acute colitis. To the best of our knowledge, details on the antioxidant activity of *G. ellipticum* leaves are scarce. Hence, in the present study, we carried out *in*

in vitro antioxidant activity of solvent extracts of *G. ellipticum* leaves.

MATERIALS AND METHODS

Collection and extraction

The plant material *G. ellipticum* were collected at Haniya, Hosanagara Taluk, Karnataka in December 2020 and identified based on its characteristics by referring to standard flora. The leaves were separated and cleaned using water. The leaves were dried under shade and powdered in a blender. A known quantity of leaf powder (50g) was subjected to a sequential extraction process using solvents of different polarity viz., petroleum ether, chloroform and methanol by maceration process (17). The solvent extracts were filtered and evaporated to obtain crude extracts. The yield and colour of extracts was noted.

Preliminary phytochemical analysis

The crude solvent extracts were screened for detection of phytochemicals viz., alkaloids, flavonoids, terpenoids, steroids, glycosides, tannins and saponins by standard phytochemical analysis (18,19).

Antiradical activity of solvent extracts

Two *in vitro* assays namely DPPH [2,2-diphenyl-1-picrylhydrazyl] and ABTS⁺ [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging assay was used to investigate the antiradical nature of solvent extracts.

DPPH radical scavenging assay

The method employed by Raghavendra *et al.*, (17) was used to investigate DPPH radical scavenging activity of different concentrations of solvent extracts and the reference standard Ascorbic acid (3.12 to 100 µg/ml). The absorbance of reaction mixtures was measured spectrophotometrically at 520nm. IC₅₀ values for extracts and ascorbic acid were calculated.

ABTS radical scavenging assay

The ability of different concentrations of solvent extracts (3.12 to 100 µg/ml) against ABTS⁺ was tested by following the method of Raghavendra *et al.*, (17). Ascorbic acid was used as standard. The absorbance of reaction mixtures was read at 730nm spectrophotometrically. IC₅₀ values for extracts and ascorbic acid were calculated.

Ferric reducing assay

The reducing capacity of different concentrations of solvent extracts and ascorbic acid (3.12 to 100 µg/ml) was evaluated by following the method described in the study by Poornima *et al.*, (2). The absorbance of reaction mixtures was read at 700nm spectrophotometrically. An increase in the

absorbance with increase in concentration indicates reducing capacity.

Total phenolic content (TPC) of solvent extracts

The protocol employed by Pavithra *et al.* (20) was used to estimate the TPC of solvent extracts of *G. ellipticum*. Gallic acid was used as standard. The TPC was expressed as mg Gallic acid equivalents (GAE)/100g of dry extract.

Total flavonoid content (TFC) of solvent extracts

The content of total flavonoids in solvent extracts of *G. ellipticum* was estimated by the Aluminium chloride colorimetric estimation method as described in the study of Pavithra *et al.*, (20). Catechin was used as standard. The TFC was expressed as mg Catechin equivalents (CE)/100g of dry extract.

Statistical analysis

The experiment was conducted in triplicates and the data were presented as Mean ± SD. The IC₅₀ values were calculated using regression equation ($Y = a + bX$; where, X= concentration; Y= % Inhibition) using Microsoft Excel 2010. IC₅₀ value denotes the concentration of extract/standard required to scavenge 50% of free radicals.

RESULTS

Phytochemicals detected in the solvent extracts

Table 1 shows the details on the yield of extract obtained. The highest yield was obtained in methanol followed by chloroform and petroleum ether solvent. Preliminary phytochemical analysis of methanol extract revealed the presence of alkaloids, saponins, tannins, terpenoids and flavonoids. Chloroform extract showed the presence of steroids and terpenoids while terpenoids were detected in petroleum ether extract (Table 1).

Table 1: Extract yield and phytochemistry of the methanol, chloroform and petroleum ether

Parameter	Solvents		
	Methanol	Chloroform	Petroleum ether
Yield (% w/w)	8.49	5.18	2.66
Color	Dark green	Brownish green	Greenish yellow
Alkaloids	+	-	-
Steroids	-	+	-
Saponins	+	-	-
Tannins	+	-	-
Glycosides	-	-	-
Terpenoids	+	+	+
Flavonoids	+	-	-

“+” Present; “-” Absent

DPPH radical scavenging activity of solvent extracts

Figure 1 shows the scavenging ability of solvent extracts against DPPH free radicals. In this study, the concentration-dependent scavenging activity of solvent extracts was observed. Among extracts, methanol extract (with an IC_{50} value of 14.40 $\mu\text{g/ml}$) was shown to be more effective in scavenging radicals followed by chloroform (IC_{50} value 28.74 $\mu\text{g/ml}$) and petroleum ether extract (IC_{50} value 70.72 $\mu\text{g/ml}$). Scavenging activity of 50% and higher was observed at extract concentrations 12.50, 25 and 100 $\mu\text{g/ml}$ of methanol extract, chloroform extract and petroleum ether extract, respectively (Fig. 1). 100% scavenging of DPPH radicals was shown by only methanol extract at the highest concentration used. The reference standard ascorbic acid was effective in scavenging DPPH radicals' dose-

dependently and exhibited stronger activity (IC_{50} value 2.46 $\mu\text{g/ml}$) than solvent extracts.

ABTS radical scavenging activity of solvent extracts

The radical scavenging ability of extracts was tested by ABTS assay and the result obtained was concentration dependent (Fig. 2). Among extracts, methanol extract displayed stronger scavenging activity with an IC_{50} value of 12.73 $\mu\text{g/ml}$ while petroleum ether extract revealed the least scavenging activity (IC_{50} value 22.51 $\mu\text{g/ml}$). Ascorbic acid scavenged ABTS radicals dose-dependently with an IC_{50} value of 2.05 $\mu\text{g/ml}$ and the activity observed was higher than that of all three solvent extracts. Methanol, chloroform, and petroleum ether extracts have scavenged ABTS radicals to 50% and higher at concentrations 12.50, 25 and 50 $\mu\text{g/ml}$, respectively (Fig. 2).

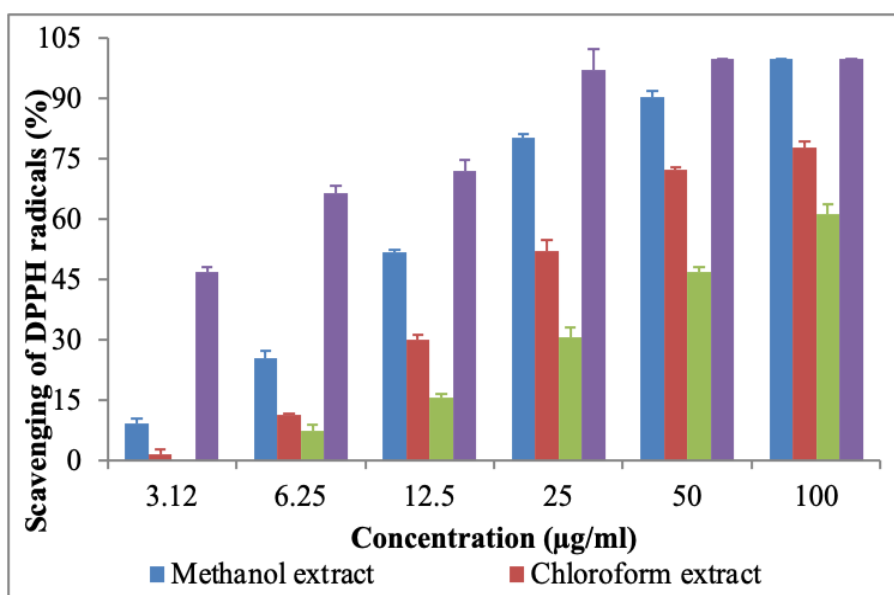


Fig. 1: Scavenging of DPPH radicals (%) by solvent extracts

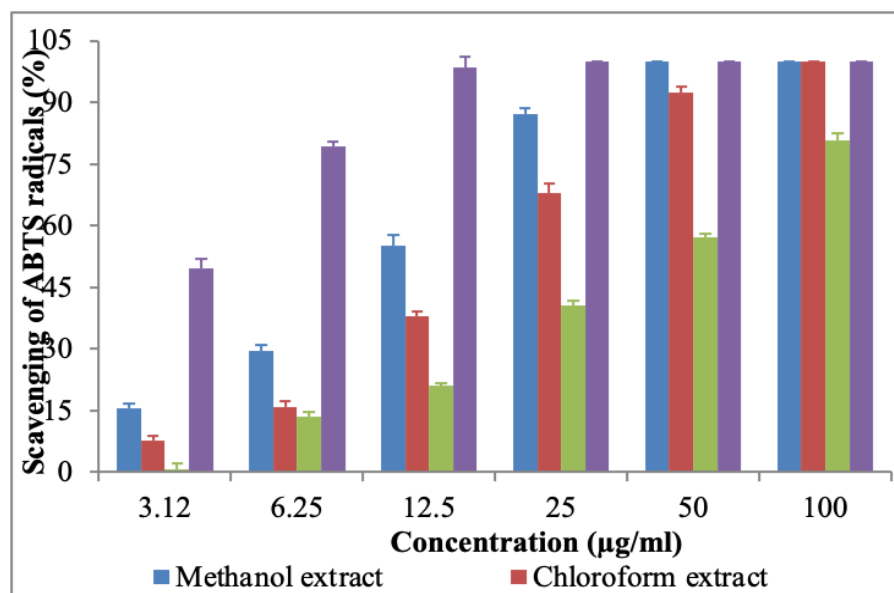


Fig. 2: Scavenging of ABTS radicals (%) by solvent extracts

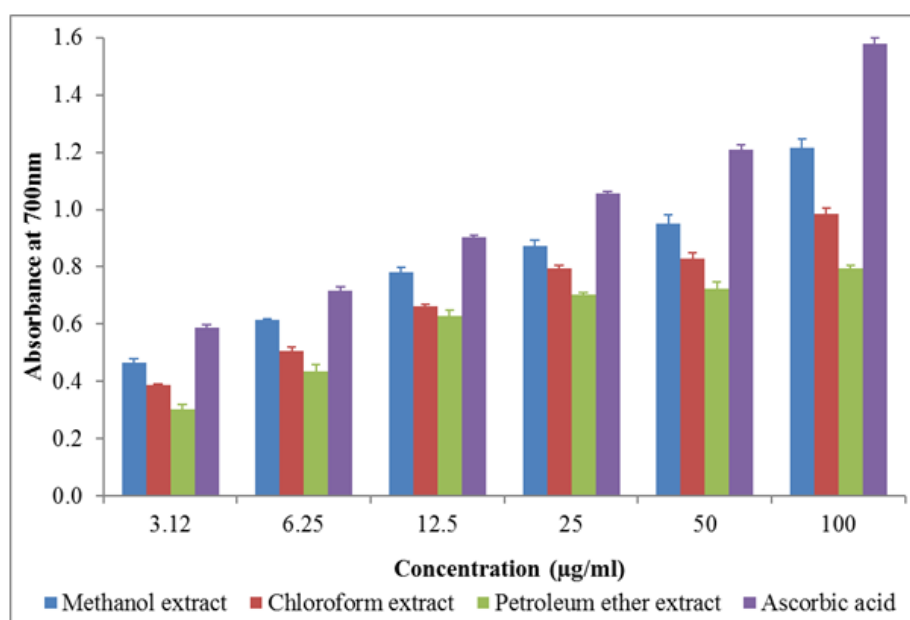


Fig. 3: Ferric reducing activity of solvent extracts

Ferric reducing activity of solvent extracts

The result of reducing potential of solvent extracts is shown in Fig. 3. The extracts were effective in terms of displaying reducing potential as indicated by an increase in the absorbance with increase in concentration. Here also, methanol extract displayed stronger activity followed by chloroform and petroleum ether extracts.

TPC and TFC of solvent extracts

Table 2 shows the content of TPC and TFC in solvent extracts of *G. ellipticum*. The content of both phenolics and flavonoids was highest in methanol extract followed by chloroform and petroleum ether extracts.

Table 2: Content of total phenolics and flavonoids in the solvent extracts

Solvent extract	Total phenolics (mg GAE/100g DEW)	Total Flavonoids (mg CE/100g DEW)
Methanol	252.67 ± 8.81	179.58 ± 4.76
Chloroform	136.34 ± 11.75	78.70 ± 7.92
Petroleum ether	25.86 ± 5.30	14.71 ± 1.45

GAE: Gallic acid equivalent; CE: Catechin Equivalent; DEW: Dry extract weight

DISCUSSION

The extraction was carried out using various solvents and methanol was shown to be promising with respect to the highest yield being obtained. Rathod and Rajurkar (14) also obtained a high yield in case of methanol solvent. In our study, methanol extract was shown to contain all phytochemicals tested except steroids. A previous GC-MS study on the leaves of *G. ellipticum* revealed several phytochemical groups including alkaloids and

terpenoids (21). The study by Jawarkar and Kane (22) revealed the presence of flavonoids, diterpenoids, tannins and steroids in the leaves of *G. ellipticum*.

DPPH is stable, nitrogen centered, ready to use organic free radical that shows maximum absorption at 520nm in an alcoholic solution. The assay involving scavenging of DPPH radicals is one of the widely used *in vitro* antiradical assays being used to evaluate antiradical nature of plant extracts (2,23,24). In this study, we measured the absorbance of DPPH radical solution in the presence of varying concentrations of solvent extracts of *G. ellipticum* leaves and the result obtained showed concentration dependent activity. The scavenging activity of extracts was in the order methanol extract > chloroform extract > petroleum ether extract. In a similar study by Rathod and Rajurkar (14), the methanolic extract of *G. ellipticum* leaves displayed stronger scavenging of DPPH radicals when compared to other solvent extracts.

Unlike DPPH assay, the ABTS radical scavenging assay requires the generation of ABTS radicals that is accomplished by mixing ABTS stock solution with potassium persulfate followed by incubation in dark for 16 hours. The resulting ABTS radical solution (bluish-green) is diluted in water and used (17,23,25). The present study revealed the potent efficacy of solvent extracts of *G. ellipticum* to display concentration dependent scavenging activity against ABTS radicals. Here also, methanol extract displayed stronger scavenging activity. The study of Rathod and Rajurkar (14) also highlighted potent scavenging activity of methanolic extract of leaves of *G. ellipticum* against ABTS radicals.

Reducing capacity of a substance is considered as a significant indicator of antioxidant capacity. In this

assay, the substances (antioxidants) react with potassium ferricyanide and form potassium ferrocyanide that further reacts with ferric chloride to produce a Prussian blue colored complex that shows absorption maxima at 700nm. Ferric reducing assay is widely used to evaluate the antioxidant capacity of botanical extracts (2,25). The solvent extracts of *G. ellipticum* were shown to be effective reducing agents in this study. The reducing assay revealed an increase in absorbance of reaction mixtures with an increase in the concentration of solvent extracts indicating the reducing capability of extracts. Among extracts, methanol extract displayed stronger reducing activity followed by chloroform and petroleum ether extracts. In a similar study, Rathod and Rajurkar (14) also observed stronger reducing capacity of methanol extract of *G. ellipticum* leaves when compared to other solvent extracts.

Polyphenolic compounds including flavonoids are widely distributed in different parts of the plant such as leaves, roots, flowers and seeds. These compounds have gained more significance due in part to their potential to exhibit several bioactivities including antioxidant activity. Phenolic and flavonoid compounds are promising with respect to scavenging and reducing activities (20,24). FCR method is widely used to investigate the TPC of plant extracts (20,24). The methanol extract was shown to contain high TPC while petroleum ether extract contained a low quantity of TPC. Aluminium chloride colorimetric estimation is routinely used to estimate the content of total flavonoids in plant extracts (20,24). The TFC was highest and least in methanol and petroleum ether extracts, respectively. In this study, the extent of scavenging and reducing activity was shown to be directly proportional to the TPC and TFC of solvent extracts as methanol extract showing the highest antioxidant activity contained high TPC and TFC. A similar kind of result was observed in the study by Rathod and Rajurkar (14) in which TPC and TFC was highest and least in methanol and petroleum ether extract, respectively.

CONCLUSION

The solvent extracts, in particular methanol and chloroform extracts were shown to be potent scavenging and reducing agents in this study. A positive correlation between the phenolic content and flavonoid content of extracts and the antioxidant activity was observed. The leaves of *G. ellipticum* seem to be an important natural source of antioxidants that can be used to alleviate radical induced oxidative damage.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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