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Original Article

In vitro antioxidant activities of ethanolic extract of *Gmelina asiatica* L. leaves

Augustian Rajam Florence*[1], Cosman Domettila[2], Gnanasingh Seelial Regini Balasingh[1]

[1]Department of Botany, Scott Christian College (Autonomous), Nagercoil– 629 003, Tamilnadu, India.
[2]Assistant Professor Department of Botany, St. Jude's College, Thoothoor- 629 176, Tamilnadu, India.

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ABSTRACT

The present study is aimed to examine the *in vitro* antioxidant activities of *Gmelina asiatica* leaf extracts. The powdered leaves of *G. asiatica* were extracted with ethanol and concentrated using rotary evoporator. The activity of ethanolic extract of *G. asiatica* was assessed against DPPH, OH^-, SO^- and the ABTS radicals were concentrated in the range of 20-100µg/ml. The ethanolic extract resulted high antioxidant activity with the IC_{50} value of DPPH (18.37 ± 0.07), OH^-(14.73 ± 0.09), SO^-(237.82 ± 8.95) and ABTS(12.12 ± 0.01 µg/ml). The values confirmed that ethanolic extract of *G. asiatica* leaves could be an important natural antioxidant agent due to its free radical scavenging activity.

Key words: *Gmelina asiatica*, Free radicals, Antioxidant activity

INTRODUCTION

Free radicals, namely, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damages to lipids, proteins, enzymes and nucleic acids of the cell or tissue injury implicated in the process of ageing. Reactive oxygen species (ROS) are normal by-products of cellular metabolism [1] and may produce oxidative stress and induce various degenerative diseases [2] which can be neutralized by antioxidant defense systems including enzymes and compounds [3]. Free radicals such as superoxide radical (SO^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and lipid peroxide radicals involved in a number of diseases including asthma, cancer, cardiovascular disease, cataracts, diabetes, gastrointestinal inflammatory diseases, liver diseases, macular degeneration, periodontal and other inflammatory diseases [4,5]. Plants are known to be the potential sources of natural antioxidants and they contain many phytochemicals that are used as natural antioxidants such as phenolic diterpenes, flavonoids, tannins, phenolic acids and polyphenols [6,7,8]. Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombic and vasodilatory activities [9,10]. The intakes of natural antioxidants from plants have been associated with low incidence of cancer, cardiovascular diseases, diabetes, and other diseases associated with aging [11]. Today, there are overwhelming interests in finding naturally occurring antioxidants in foods and medicine to replace synthetic antioxidants [12]. Generally, verbenaceae family members are popular in traditional medicine [13]. The plant *G. asiatica* has been used ethnomedicinally as antimicrobial, anti-inflammatory, antioxidant, antihyperglycemic, hypoglycemic, hepatoprotective activity, antipyretic and anticancer activity [14-18]. The objective of the present investigation is aimed to determine the *in vitro* antioxidant activities of ethanolic extract of *G. asiatica* leaves by different radical scavenging methods.

MATERIALS AND METHODS

Collection and Identification: Leaves of *G. asiatica* were collected from Scott Christian College Campus, Nagercoil, Kanyakumari District, South Tamilnadu, India and identified using Gamble and Fisher [19].

Ethanol extract preparation: The healthy and mature leaves were freshly collected and cleaned with distilled water, shade dried and powdered. The powdered samples were soxhlet extracted with ethanol until the solvent was colorless. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator to obtain the

***Corresponding Author Address:** Augustian Rajam Florence, Department of Botany, Scott Christian College (Autonomous), Nagercoil– 629 003, Tamilnadu, India; E-mail: florencedavid28@gmail.com*

extracts and stored in the refrigerator at 4ºC until use.

Determination of Antioxidant Activity

DPPH radical scavenging activity: The antioxidant activity of the sample was determined in terms of hydrogen-donating or radical-scavenging ability, using the stable radical DPPH, according to the method of Blois [20]. The sample extracts were taken at various concentrations (20-100 µg) and the volume was adjusted to 100µL with methanol. Five millilitres of 0.1 mM methanolic solution of DPPH was added and allowed to stand for 20 min at 27˚C. The absorbance of the sample was measured at 517 nm.

Hydroxyl radical scavenging activity: The scavenging activity of the sample on hydroxyl radicals was measured according to the method of Klein *et al* [21]. Different concentrations of the extract (20-100 µg) were added 1 mL of iron-ethylenediamine tetraaceticacid (EDTA) solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1mL of dimethyl sulfoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80-90˚C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1 mL of ice-cold trichloroacetic acid (TCA) (17.5% w/v). Three millilitres of Nash reagent (75.0 g of ammonium acetate, 3 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and the volume made up to 1 L with distilled water) was added and the mixture kept undisturbed at room temperature for 15 min. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank.

Superoxide radical scavenging activity: Superoxide radicals were generated by a modified method of Beauchamp and Fridovich [22]. The assay was based on the capacity of the sample to inhibit farmazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT and various concentrations (20-100 µg) of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90s. Immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixture kept under dark condition served as blank.

Free radical scavenging activity on ABTS: The antioxidant activity of the samples was measured by ABTS radical cation depolarization assay according to the method of Re *et al*. (1999) [23]. $ABTS^{\bullet+}$ was produced by the reaction of 7mM ABTS aqueous solution with 2, 4 mM of potassium persulfate under dark condition for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30ºC to give an absorbance at 734 nm of 0.70 ± 0.02. The stock solution of the sample extracts were diluted and add 10-µL aliquots into the assay. It produces 20% and 80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10 µL of sample (10-100µg/ml), absorbance was measured at 734 nm exactly 30 min after the initial mixing. Percentage of DPPH, hydroxyl, superoxide and ABTS radical-scavenging activity of the sample was calculated using the formula:

$$\%\ \text{Inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

All the assays were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

RESULTS AND DISCUSSION

The ethanolic extract of *G. asiatica* leaves showed scavenging activity by inhibiting DPPH, hydroxyl, superoxide and ABTS radical at different concentrations of 20, 40, 60, 80 and 100µg/ml was recorded their percentage and antioxidant activity in the form of IC_{50} values of ethanolic extracts were calculated and shown in Table 1. At all concentrations tested, *G. asiatica* exhibited dose-dependent inhibition was evaluated in all radical scavenging assays. Overall, 80% ethanol extract produced from the leaves exhibited high antioxidant capacity compared to standard quercetin. Among the radical scavenging assays, superoxide radical possessed the highest antioxidant capacity ($IC_{50} =$ 237.82 ± 8.95 µg/ml) and the standard quercetin was IC_{50}=9.30 ± 0.07µg/ml. The present results are in conformity with the works of Selvam *et al*. (2012) in the methanol extract of *Premna serratifolia* leaves and were found to have highest superoxide radical scavenging activity (165.31µg/ml) [43]. In DPPH radical scavenging activity, the *G. asiatica* leaves showed good antioxidant activity and the IC_{50} value of 18.37 ± 0.07 µg/ml compared to quercetin standard IC_{50} (4.42 ± 0.04 µg/ml). The investigations of Merlin and Parthasarathy, (2011), Silvia and Satyanaraya (2014) also confirmed the ethanol extracts of aerial parts of *G. asiatica* and methanolic extract of *G. asiatica* stem respectively and the DPPH radical which are compared to

ascorbic acid [24,18]. The hydroxyl radical scavenging activity of ethanol leaf extract was 14.73 ± 0.09µg/ml and found to be more when compared to that of quercetin standard 6.26 ± 0.18 µg/ml. The highest hydroxyl radical scavenging activity in the methanol extract was reported in *Premna serratifolia* leaf [25]. The ABTS radical (IC_{50} =12.12 ± 0.01 µg/ml) had the lowest antioxidant capacity compared to standard quercetin was 9.39 ± 0.15µg/ml. This study is confirmed with the previous investigations of Arjun *et al*. (2012), Chellappan and Pemiah (2014) in ethanolic extracts of *G. arborea* leaf and *Callicarpa macrophylla* stem bark showed a strong antioxidant activity in ABTS scavenging assay, with IC_{50} values of 0.0306 ± 0.04 mg/ml and 88.33% in 150 µg/ml respectively[26,27].

Table 1. Antioxidant effect of *Gmelina asiatica* leaf extract at different concentrations

DPPH radical scavenging activity					
Leaf extract of *Gmelina asiatica*			**Quercetin**		
Concentration (µg)	Percentage activity (%)	IC_{50}	Concentration (µg)	Percentage activity (%)	IC_{50}
20	36.18 ± 0.62	18.37 ± 0.07	4	29.56 ± 0.47	4.42 ± 0.04
40	52.64 ± 0.22		8	40.27 ± 0.70	
60	68.16 ± 1.45		12	53.45 ± 0.54	
80	94.40 ± o.17		16	71.50 ± 1.09	
100	92.56 ± 0.17		20	88.00 ± 0.41	
Superoxide radical scavenging activity					
20	1.93 ± 0.23	237.82 ± 8.95	10	32.27 ± 0.46	9.03 ± 0.07
40	3.60 ± 0.33		20	42.56 ± 0.64	
60	4.51 ± 0.09		30	55.24 ± 0.52	
80	5.57 ± 0.23		40	72.60 ± 1.04	
100	6.33 ± 0.23		50	88.46 ± 0.39	
Hydroxyl radical scavenging activity					
20	58.27 ± 1.05	14.73 ± 0.09	4	14.29 ± 2.99	6.26 ± 0.18
40	59.83 ± 0.65		8	21.29 ± 0.50	
60	66.23 ± 1.04		12	32.70 ± 4.10	
80	69.18 ± 0.98		16	36.32 ± 0.46	
100	81.30 ± 1.04		20	40.39 ± 0.95	
ABTS radical scavenging activity					
20	26.32 ± 0. 19	12.12 ± 0.01	10	32.76±3.76	9.39 ± 0.15
40	50.38 ± 0.59		20	51.21±3.85	
60	61.94 ± 0.32		30	67.66±4.07	
80	82.93 ± 0.24		40	84.26±0.84	
100	95.21 ± 0.50		50	99.33±0.08	

Figure 1. DPPH radical scavenging activity of *Gmelina asiatica* leaf extract

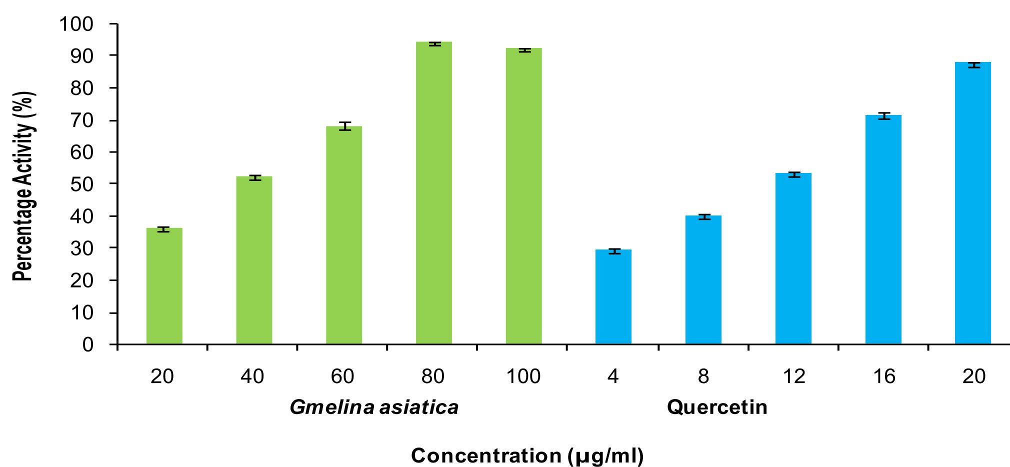


Figure 2. Superoxide radical scavenging activity of *Gmelina asiatica* leaf extract

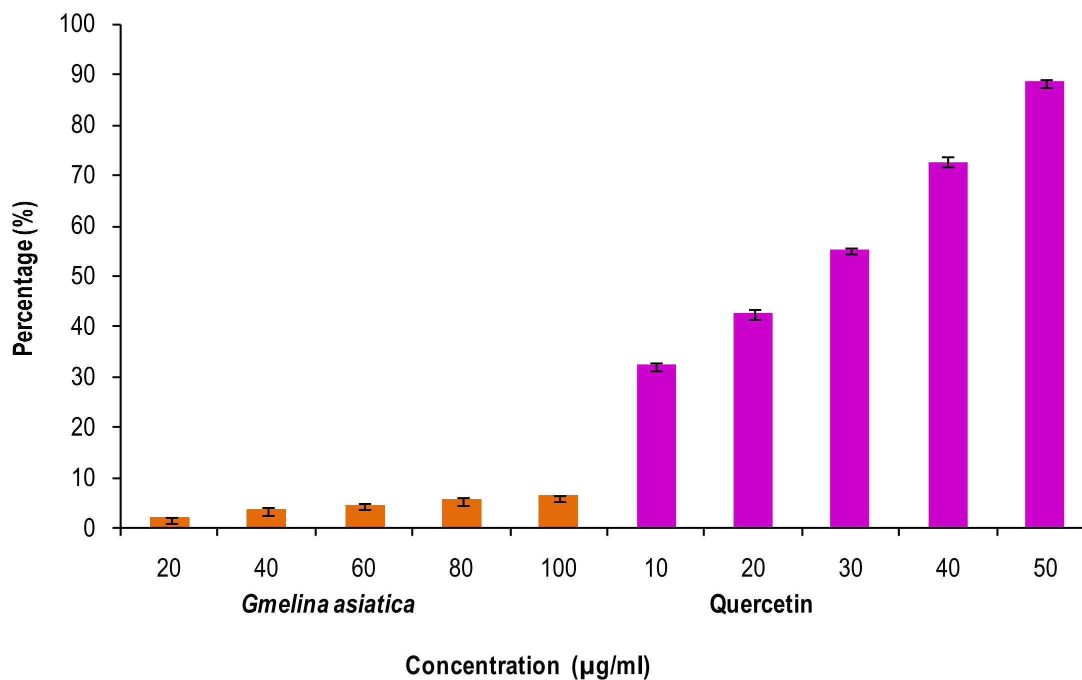


Figure 3. Hydroxyl radical scavenging activity of *Gmelina asiatica* leaf extract

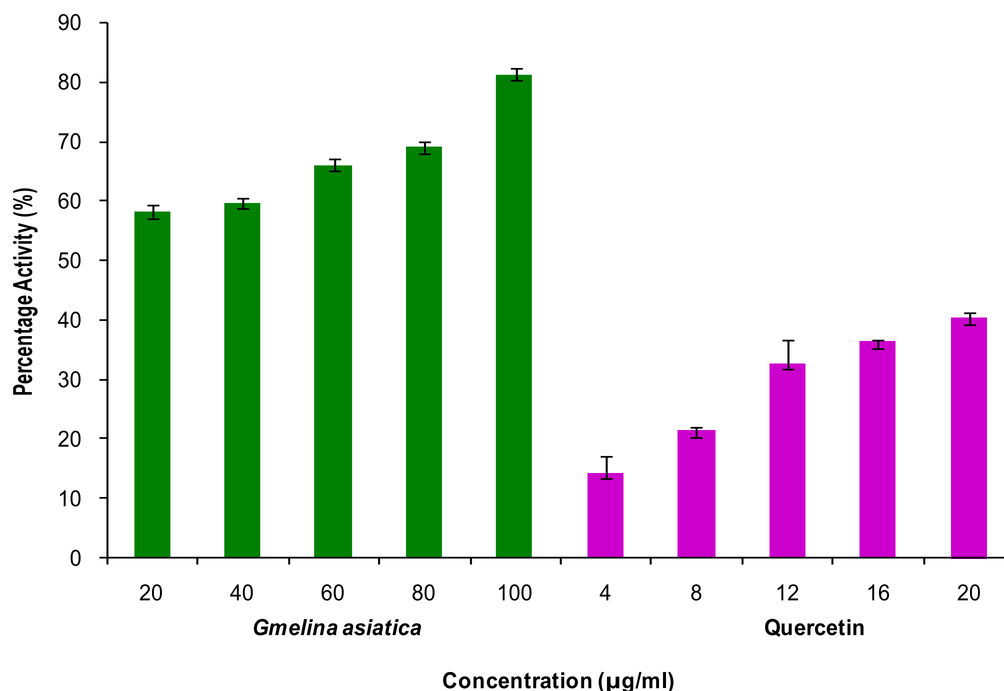
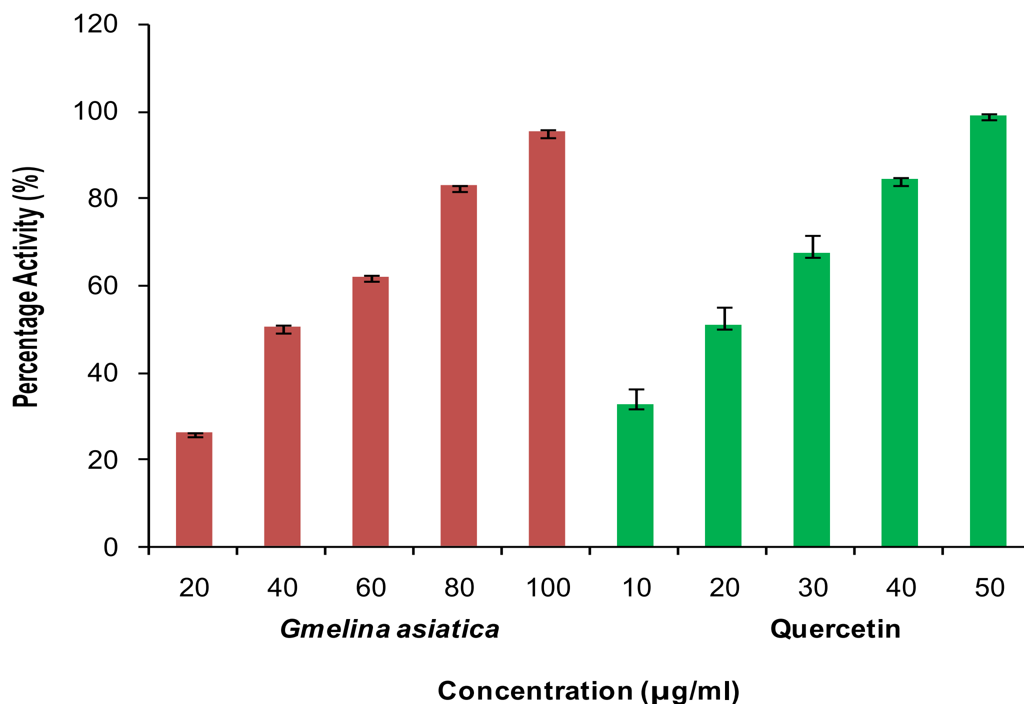


Figure 4. ABTS radical scavenging activity of *Gmelina asiatica* leaf extract



CONCLUSION

In conclusion, the results from these *in vitro* experiments further support the view that the plant *G. asiatica* is a source of naturally occurring antioxidant product. The potential of these easily accessible sources of natural antioxidants should be explored by the pharmaceutical and medical industries. For this reason, further work should be performed to isolate and identify the antioxidative components of tested plant.

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