Antioxidant activities and phenolic profile of *Litsea glutinosa* from Pakistan

Syed Mubashar Sabir[1], Basharat Iqbal[1], Muhammad Usman Hameed[1], Saleem Raza[2]

[1]Department of Chemistry, University of Poonch, Rawalakot, Azad Kashmir, Pakistan

[2]College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua 321004, Zhejiang PR, China

*Address of correspondence, ORCID ID: https://orcid.org/0000-0002-3339-366X,

Abstract

The present study was aimed to evaluate the antioxidant and phenolic profile of *Litsea glutinosa* bark which grows in the natural climate of Azad Kashmir. The phenolic compositions were analyzed by high performance liquid chromatography (HPLC) while antioxidant activities were determined by DPPH assay, lipid peroxidation assay, metal chelation and total antioxidant activity by phosphomolybdenum assay. HPLC analysis resulted in the identification of gallic acid, caffeic acid and quercetin. The extract showed high antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay ($IC_{50,}$ 23.1 ± 2.1 µg/ml). The aqueous extract showed its potential to inhibit the TBARS production and brought down the lipid peroxidation in egg yolk phospholipid. The chelating ability of the extract was confirmed by iron chelation assay while, reducing abilities were observed on the phosphomolybdenum assay. It is concluded that extracts obtained from bark of *Litsea glutinosa* from the Kashmir flora have high potential to be utilized as antioxidants due to their rich phenolic compositions.

Key words: *Litsea glutinosa*, antioxidant activity, HPLC analysis, phenolics, flavonoids.

INTRODUCTION:

Litsea glutinosa is a very famous species that remains green throughout the year. The plant

Litsea glutinosa is a member of family Lauraceae. *Litsea glutinosa* (Common name: Indian laurel, Bengali name: Menda pata; Family- Lauraceae) is a moderately sized tree. It is planted in Bangladesh, India, South China and Malaysia [1]. It has been observed that the height of plant ranges from 18 to 20 meter. In past this plant has no importance in the lives of people due to lack of knowledge but recently it has been proved by many researches that, this plant has a lot of importance and due to this, plant is mostly used at domestic level. The plant showed different biological activities such as central analgesic as well as peripheral effect. One of the interesting things is that the extract of this plant expressed dose dependent inhibition of acetic acid that might induce wriggling in mice as compared to saline that is taken as control.

One of the researches has been shown that the methanolic and ethanolic extract of this plant shows a very good antibacterial as well as antifungal activity [2]. It has also been observed that leaf extract of this plant also shows cardiovascular activity [3]. Its bark and leaves are used as antiseptic and mild astringent for bowel diseases and the roots are used in sprains and bruises [4]. The very important compounds those are found in this plant are actinodaphnine, Tannin, quercetin, β-sitosterol, n- methylactinodaphnine, litseferine, Boldine, laurotetanine, and sebiferine. Many others components are also found in this plant [5]. The search of literature has shown that there are few studies with respect to the identification of phenolics and flavonoids from Litsea species [4]. In this paper, the antioxidant and phenolic composition of *Litsea glutinosa* are being reported to justify the popular use of the plant.

MATERIAL AND METHODS

Collection of plant material

The stem bark of *Litsea glutinosa* were collected from district Poonch Azad Jammu and Kashmir. The garden fresh plant material was cleaned with water, dried and put under grinding to obtain powder form.

Preparation of plant extract

The bark of *Litsea glutinosa* was extracted in boiling water for 20 minutes. The extract was filtered and residue was extracted twice and finally the extract was dried in rotary evaporator at low temperature. Extract was diluted to obtain the different extract concentration for experiment.

HPLC analysis

The HPLC analysis of the *Litsea glutinosa* bark was carried out in Glitz Pharma Islamabad, Pakistan. Aqueous extract was dissolved in HPLC grade methanol, after that filtration was done with membrane filter having pore size 0.45 μm and subjected to qualitative as well as quantitative analysis by using Shimadzu HPLC system. Mobile phase combination with ratio of methanol: acetonitrile: water (40:15:45) containing 1 mL of acetic acid was used for the separation of phenolics. Quercetin, gallic acid and caffeic acid standard were used to in order to prepare standard curves. At 257 and 325 nm data was monitored for the identification of phenolic acids while at 356 nm data was monitored for identification of flavonoids. The injection volume of *Litsea glutinosa* extract was 20 μL and the flow rate was maintained as 1 mL/min. Results were authenticated by comparison with retention times and DAD-UV spectra of reference standards.

Determination of Phenolic content

Total phenolic content was estimated by using the method of Singleton *et al*., [6].

Determination of total flavonoids

Total flavonoid content as quercetin equivalent/g sample were estimated by the method [7].

***In vitro* lipid peroxidation assay**

The antilipid peroxidative properties of extracts were studied by a method reported by Okhawa et al. [8].

DPPH radical scavenging

DPPH radical scavenging activity of the extract was followed by Hatano et al., [9].

Metal chelating activity

The chelating ability of the extract was analyzed by the method of Puntel et al., [10].

Antioxidant potential assay

The total antioxidant activities of the extract was carried out by phosphomolybdenum method [11].

Statistical analysis

The results were expressed as means ± standard deviation. Statistical analysis was applied as one way ANOVA followed by Duncan Multiple Range test.

RESULTS AND DISCUSSION

HPLC analysis

The phytochemical composition of *Litsea glutinosa* was investigated by HPLC. Several compounds were present in the chemical profile presented by the bark of *Litsea glutinosa.* Retention time was used for identification of caffeic acid, gallic acid and

quercetin and was about 3.80, 6.55 and 3.84 minutes respectively. (Fig. 1). The quantitative analysis showed the highest amount of caffeic acid as 32.29 µg/ml while quercetin showed the least amount of 5.5 µg/ml. Gallic acid was found in the range of 22.1 µg/ml. Present study reveals that total phenolic content as gallic acid equivalent was found to be 59 mg/g of dry extract. The total flavonoid content as quercetin equevalent was found to 38 mg/g dry extract of *Litsea glutinosa*. High pressure liquid chromatography quantitatively showed the presence of phenolic and flavonoids as major components responsible for activity of *Litsea glitinosa* [12].

DPPH Radical Scavenging Activity of extract

Antioxidants has potential to scavenge free radicals. These substances are very helpful to avoid cell damage. With the help of 2,2-Diphenyl-1-Picryl hyrdazyl stable free radical percentage scavenging activity of aqueous extracts of *Litsea glutinosa* was analyzed. Antioxidants show different action and provide protection by quenching singlet oxygen, reduce the substance, possess metal chelation and neutralize the free radicals. The antioxidants act at different stages by preventing, intercepting and repairing the free radicals. In order to find the free radical scavenging activity five concentrations of plant extract 10, 20, 50, 100 and 200 µg/ mL were taken. Earlier studies have reported high DPPH radical scavenging activity of extract [13]. DPPH radical scavenging activity of different extract concentrations of *Litsea glutinosa* are shown in Figure 2. The results have shown that DPPH radical scavenging activity increases with the increase in plant extract concentration. Different extract concentrations of *Litsea glutinosa* showed that they are potential source of antioxidants. Our results were in agreement to Arunodaya *et al.*, [14].

Lipid peroxidation inhibitory effect of extract

The thiobarbituric acid-reactive substances (TBARS) level in an index to lipid

peroxidation and there exist a close resemblance between the substances that shows oxidative damage to DNA and TBARS [15]. lipid peroxidation, a secondary free radical may leads to the damage of bio molecules. The present work showed that the generation of metal catalyzed ROS which involve attack on highly oxidation sensitive components of cell like fatty acids with double bonds, derived lipids, nuclear material and proteins. In comparison to the normal an elevated production of TBARS in ferrous sulphate (10 µM) results with rise of oxidative stress, suggests damage of those closely related group of cells which are overloaded with iron [16]. Because of higher level of production of superoxide in cell organelles like mitochondria and the other part like the cytosol cause great damage. Ferrous ions which actively participate in Fenton reaction is regenerated by reaction of superoxide on reaction with ferric ion [17]. Higher level of iron may damage the liver and kidney through the process of lipid peroxidation. In order to analyze the antioxidant potential of *Litsea glutinosa* we used ferrous sulphate to induce lipid peroxidation in egg yolk phospholipid. Figure 3 shows the anti-lipid peroxidative properties of *Litsea glutinosa*. We observed that plant extract significantly reduced the lipid peroxidation which was induced by ferrous sulphate. These results showed the inhibition of hydroxyl free radical which is primarily responsible for lipid peroxidation. The peroxidative reaction mediated by free radicals could be the possible mechanisms of iron toxicity. The protections offered by the aqueous extracts of *Litsea glutinosa* suggests that they could be beneficial to protect the phospholipids by decreasing the oxidative stress. Significant decrease in lipid peroxidation with the application of plant extracts shows that *L. glutinosa* has the potential to bind with ferrous ion or to free radicals [18].

Metal chelating activity of extract

Litsea glutinosa extract showed the potential of metal chelation as the extract was rich

in phenolics. In metal catalyzed redox reaction the most prominent intermediate are ROS. Metal which contain partially filled orbitals like ferrous ion showed remarkable trend in generation of free radicals by picking up or removal of electrons. Formation of ROS must be controlled with use of substances which has ability to donate electrons to metal ion result in formation chelate complex [19]. Ferrous ion related oxidative stress in liver find better treatment with the application of chelating agent. In order to assess the chelating ability of plant extract iron chelation assay was carried. Chelating ability of *Litsea glutinosa* was found to be significant (Figure 4). These results encourages the use of *Litsea glutinosa* in iron overload. Present study on metal chelating ability of *Litsea glutinosa* was found in good agreement with the results of published data [20].

Total antioxidant activity of extract

Phosphomolybdenum assay was performed in order to evaluate the total antioxidant activity which include both water soluble and fat soluble antioxidants. *Litsea glutinosa* extract reveals the ability to terminate the chain reaction by donation of electrons resulted in the formation of stabilized products. Molybdenum was reduced from +VI oxidation state to +V oxidation state by treatment with plant extract. The total antioxidant activity was experessed as ascorbic acid equivalent [21]. *Litsea glutinosa* total antioxidant activity is shown in Figure 5. All the extract concentrations have shown their abilities to reduce the molybdenum ions in a concentration dependent manner. Reducing ability of compounds is shown by earlier studies [22].

Conclusions:

Important phenolic compounds were detected in the bark of the plant. High DPPH radical scavenging activity, inhibition of lipid peroxidation and metal chelation activities encourages the use the plant in different diseases arising from oxidative stress. These

results have shown that *L. glutinosa* is a good source of antioxidants and could be effectively utilized in food and pharmaceutical industries with additional income.

Conflict of Interest statement: None declared.

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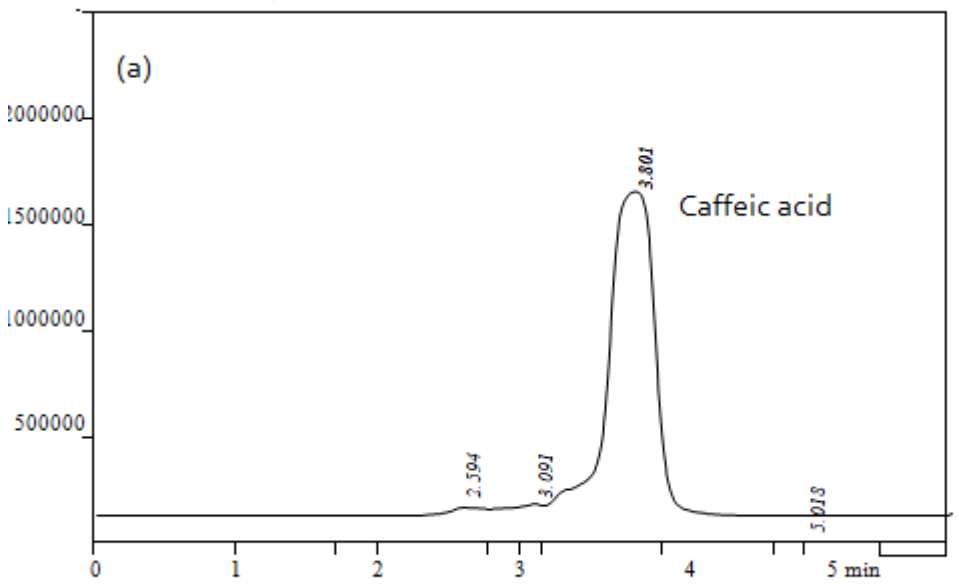
Figures:


(a)
2000000
1500000
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Caffeic acid
2.594
3.091
5.018
0
1
2
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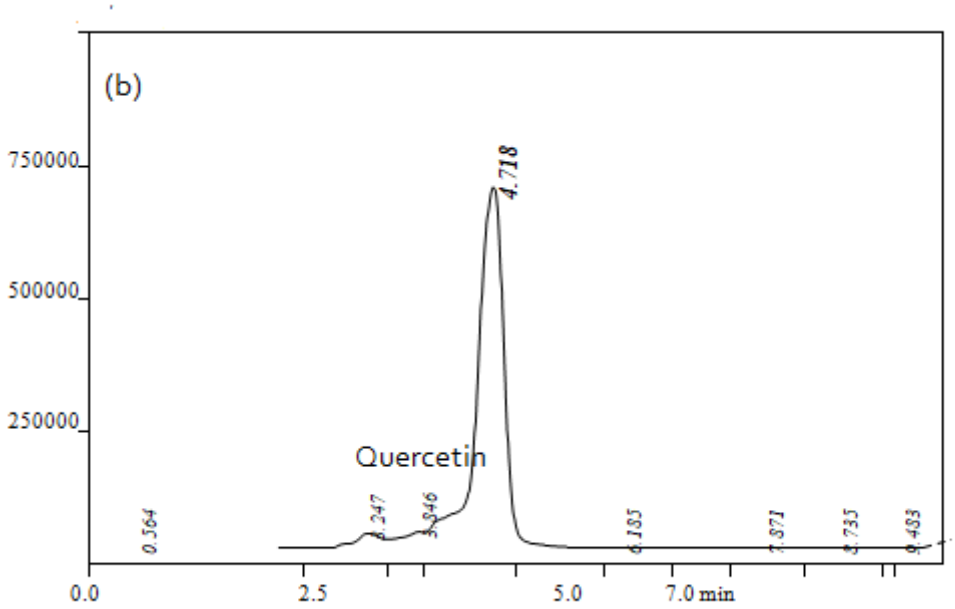
4
5 min


(b)
750000
500000
250000
4.718
Quercetin
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3.946
6.135
7.871
8.735
9.483
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2.5
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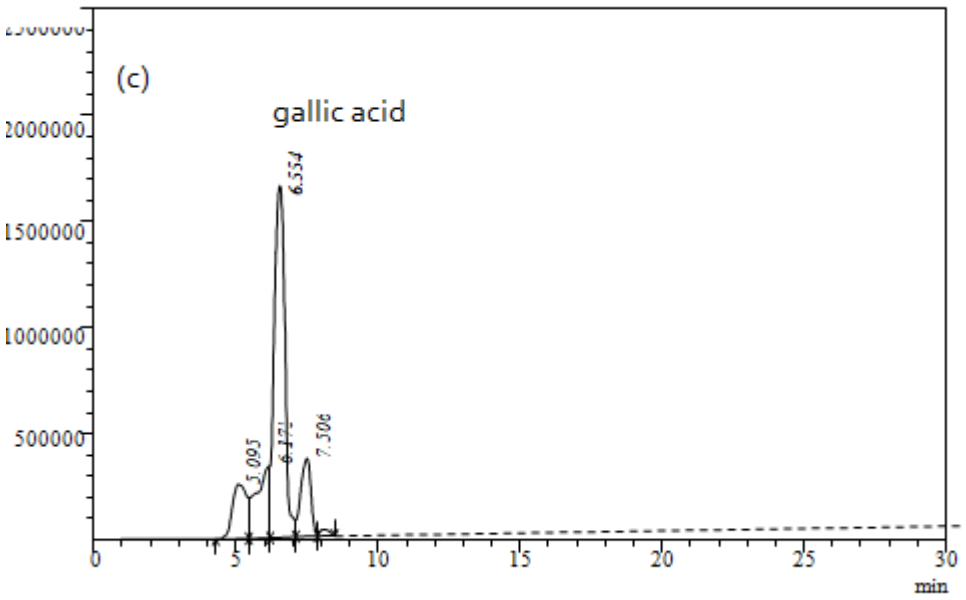

Figure 1. HPLC analysis of *Litsea glutinosa* extract obtained from bark (a). Chromatogram showing the presence of caffeic acid (b). Chromatogram showing the presence of quercetin (c). Chromatogram showing the presence of gallic acid.

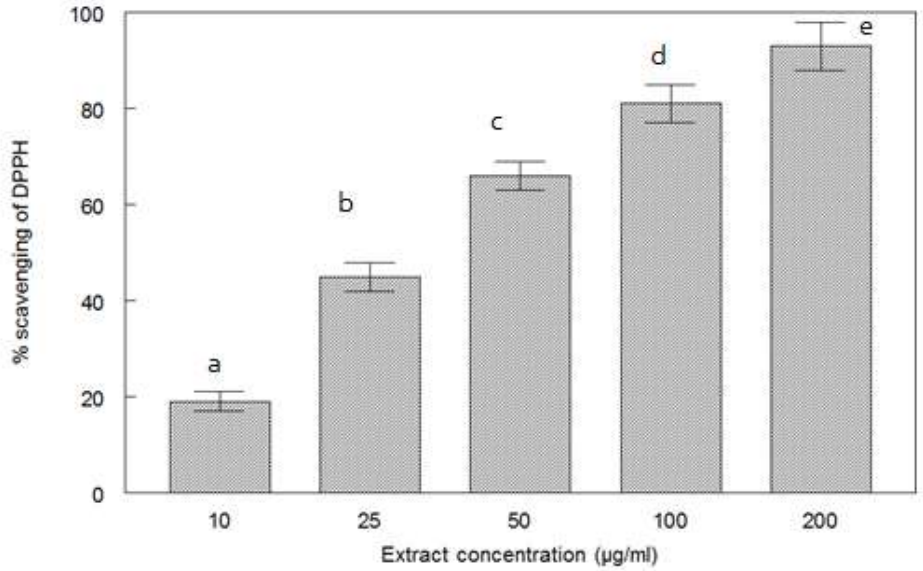

Figure 2. DPPH radical scavenging activity of aqueous extract obtained from bark of *Litsea glutinosa*. Values are means±SD (n=3).Values in figures which share different letters are significantly (p<0.05) different from each other by DMRT.

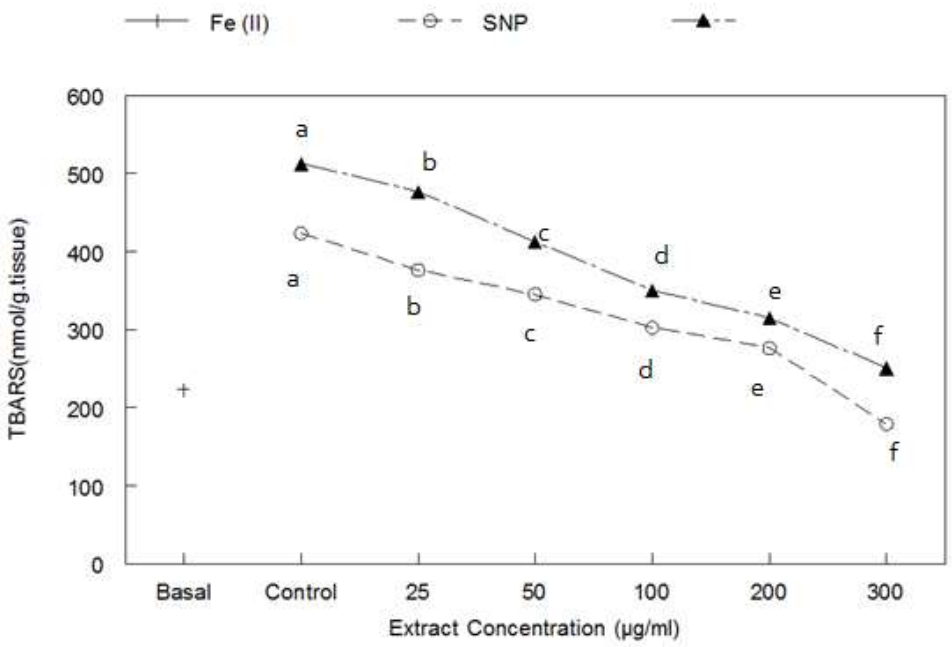

Figure 3. Inhibitory effect of *Litsea glutinosa* on lipid peroxidation induced by 10 µM Fe(II) and 5 µM sodium nitroprusside in egg yolk phospholipid. Values represent the means of three separate experiments in duplicate ± SD. $p<0.05$ is significantly different from control by DMRT. Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.

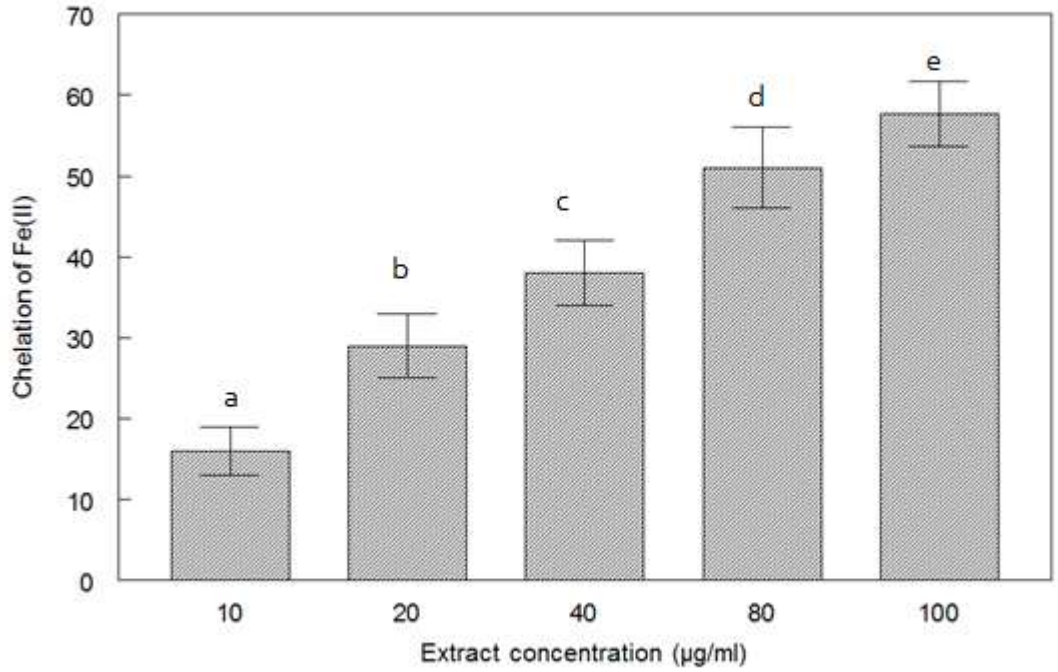

Figure 4. Iron chelating abilities of *Litsea glutinosa* bark extract. Values are means±SD (n=3). Values in figures which share different letters are significantly (p<0.05) different from each other by DMRT.

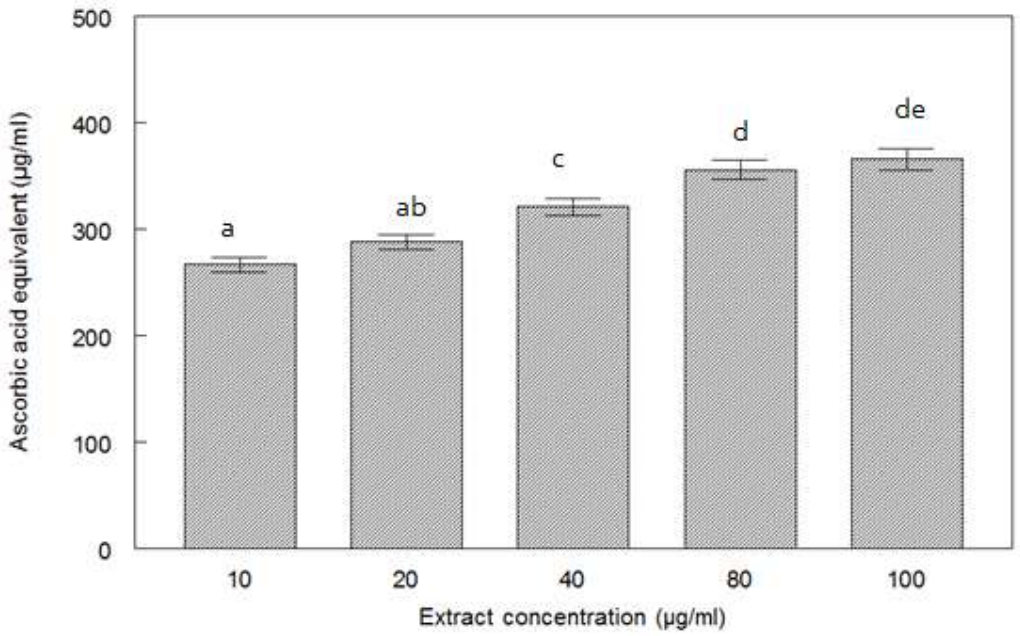

Figure 5. Total antioxidant activity of *Litsea glutinosa* bark extract. Values are means±SD (n=3). Values in figures which share different letters are significantly (p<0.05) different from each other by DMRT.