

Research Article

Loranthus pulverulentus: A Potent Source of Natural Antioxidants and Alternative Medicine

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This study was designed to evaluate the antioxidant potential of *Loranthus pulverulentus*. Stem bark, leaves, and seeds of *Loranthus pulverulentus* were extracted in methanol:water (90 : 10) and partitioned with n-hexane, chloroform, ethyl acetate, and n-butanol successively using partition chromatography. Total phenolic contents and antioxidant potential were checked using standard protocols. Total phenolic contents of all extracts were determined, using Folin–Ciocalteu reagent, and ranged between 151 ± 2.1 and 396 ± 1.6 for stem bark, 137 ± 0.9 and 430 ± 2.2 for, and 39 ± 0.6 and 231 ± 1.7 for seeds. The antioxidant potential of extracts was evaluated; namely, DPPH, FRAP, and total antioxidant models. The ethyl acetate extract of stem-bark, leaves, and seeds showed the highest activity in DPPH ($94.5 \pm 2.1\%$, $96.30 \pm 0.9\%$, and $92.30 \pm 1.1\%$, IC_{50} $15.9 \pm 0.5 \mu\text{g}$, 14.5 ± 0.8 , and 102.7 ± 1.3 , resp.), FRAP (7.7 ± 0.6 , 7.5 ± 0.7 and 6.6 ± 0.7 , resp.), and total antioxidant (0.95 ± 0.09 , 1.19 ± 0.09 , and 0.686 ± 0.08 , resp.). Strong correlations were observed between total phenols versus total antioxidant activity, DPPH, and FRAP with R^2 values ranging from 0.8185 to 0.9951 (stem-bark), 0.6728 to 0.8648 (leaves), and 0.8658 to 0.9910 (seed) which indicated that phenolic contents are the major constituents responsible for antioxidant activity.

1. Introduction

Plants are important for a healthier life, because they offer us medicines, which are safe, effective and cause no side effects. They play a fundamental role in our lives mainly due to their unusual collection of diverse classes of biochemicals with a variety of biological activities [1, 2]. Many medicinal plants contain antioxidants such as polyphenols, which can play an important role in capturing and neutralizing free radicals and quenching singlet and triplet oxygen. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [3].

Antioxidants are substances which counteract free radicals and prevent the damage caused by them. These can greatly reduce the adverse damage caused by oxidants by crumbling them before they react with biologic targets, preventing chain reactions, or preventing the activation of oxygen to highly reactive products [4]. The use of natural antioxidants

for the treatment of free radical induced pathologies has certain advantages, because these agents produce no side effects, possess low toxicity, and effectively act upon the main factors damaging the vascular system. Plants are known to be rich in biologically active substances (flavonoids, phenolic acids, anthocyanins, ethereal oils, tannins, etc.), many of which exhibit antioxidant activity. In recent years, there has been considerable interest in these biologically active substances, methods of their isolation, and evaluation of activity in natural media. Various nutritional biologically active additives and drug preparations aimed at stimulating metabolic processes in the organism have been created based on plant extracts.

Family Loranthaceae consists of about 74 genera, commonly known as mistletoes and mostly distributed in tropics, and semiparasitic shrubs attached to the hosts by modified roots [5]. The leaves and stem bark of some members of this family have been widely used in traditional medicine as therapeutic herbs [6]. *Loranthus pulverulentus* is a shrub,

commonly called Parwikh and Grunu in Mirpur, Azad Kashmir. *L. pulverulentus* is parasitic on *Dalbergia sissoo* Roxb and *Mallotus philippinensis* Muell. Leaves juice is used for treating diabetes while its plant extract has wound healing properties. This plant is also used to treat other liver diseases, bacterial infections, and hypertension [7].

Pakistan has a strong tradition of herbal remedies, and like most developing countries, its rural population still depends mainly on the indigenous system of medicine for their health-related matters [8]. The present study was undertaken to explore the antioxidant potential of different parts of *L. pulverulentus*.

2. Materials and Methods

2.1. Chemicals and Reagents. Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), FRAP, and gallic acid were purchased from Sigma-Aldrich (USA). All other chemicals and reagents of analytical grade were purchased from Merck (Germany).

2.2. Plant Material. *Loranthus pulverulentus* was collected from Azad Kashmir Valley, Pakistan, and was identified by Dr. Zaheer-ud-Din Khan where a voucher specimen was deposited at Sultan Herbarium, Department of Botany, GC University, Lahore, Pakistan.

2.3. Extraction. Stem bark, leaves, and seeds were shade-dried, powdered, and extracted by soaking in methanol:water (90:10) at room temperature (300 gm each). The crude extract was filtered through Whatman filter paper number 40, concentrated using rotary evaporator at reduced pressure, and partitioned with n-hexane, chloroform, ethyl acetate, and n-butanol.

2.4. Determination of Total Phenolics. Folin-Ciocalteu reagent was used to determine total phenolics in the plant extracts [9]. 300 μ L (1 mg/mL) of each sample was mixed with 200 μ L of Folin-Ciocalteu reagent and 0.8 mL of 10% sodium carbonate solution. The mixture was allowed to stand for 30 minutes, and the absorbance was measured at 765 nm against a blank which contained 300 μ L of methanol in place of sample. The total phenolic contents were expressed as mg gallic acid equivalents/g of extract. Correlation studies between total phenolic content and antioxidant activities in the DPPH, FRAP, and phosphomolybdate assays were also performed.

2.5. DPPH Radical Scavenging Assay. The radical scavenging ability of different extracts was measured using the method of Shahwar et al. 2010 [9]. Methanolic solution (0.2 mL) of all extracts at various concentrations (25–300 μ g/mL) was added to DPPH (0.025 g/L) and kept in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using UV/VIS spectrophotometer. The % scavenging of radical was determined by the following formula:

$$\begin{aligned} \text{\%age DPPH inhibition} \\ = \frac{\text{Abs. of blank} - \text{Abs. of sample}}{\text{Abs. of blank}} \times 100. \end{aligned} \quad (1)$$

2.6. Ferric Reducing Antioxidant Power. Ferric reducing antioxidant power (FRAP) of the extracts was carried out using the method of Shahwar et al. 2010 [10]. 150 μ L of FRAP (Fe^{III} -TPTZ) reagent and different concentrations (50–200 μ g/mL) of samples were mixed. Read the absorbance at 592 nm after 8 min of the addition of reagent.

2.7. Total Antioxidant Capacity by Phosphomolybdate Method. The total antioxidant capacity of the plant extracts was evaluated by the method of Shahwar et al. 2010 [10]. An aliquot of 0.2 mL (500 μ g/mL) of the sample solution was mixed with 2.0 mL of the reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was heated at 95°C for 60 min, and absorbance was measured at 695 nm against a blank containing 2 mL of reagent solution.

2.8. Statistical Analysis. All assays were carried out in triplet form, and standard deviation (\pm SD) was calculated using computer program MS Excel.

3. Results and Discussion

Loranthus pulverulentus was collected from Azad Kashmir. The stem bark, leaves, and seeds were separated, shade-dried, powdered, extracted with methanol:water (9:1), and partitioned successively with n-hexane, chloroform, ethyl acetate, and n-butanol. All the extracts were evaluated for antioxidant potential through various models, and the results are shown in Table 1 and Figures 1–3.

3.1. Total Phenolic Contents. Total phenolic contents in the plant extract of different parts of the plant were calculated using FC method and expressed as mg gallic acid equivalents/g of extract. Our results showed that *L. pulverulentus* has a high amount of total phenols in stem bark (151 ± 2.1 to 396 ± 1.6 GAE/g of extract) and leaves (137 ± 0.9 to 430 ± 2.2 GAE/g of extract), while the total phenols of seed extract ranged from 39 ± 0.6 to 231 ± 1.7 GAE/g of extract. Among the various solvent extracts of bark, leaves, and seeds, ethyl acetate extract of stem bark leaves and seed extracts contained the highest contents of phenols, while n-hexane and aqueous extract of these parts showed less quantity of phenolics (Table 1). Phenolic compounds occur in plants in a large quantity and exert a number of activities; one of them is antioxidant activity. They act as antioxidants through various mechanisms such as hydrogen donors and singlet oxygen quenchers [11].

3.2. Antioxidant Activities. Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems. Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation endpoint. ABTS⁺ or DPPH radical scavenging methods are common spectrophotometric procedures for determining the

TABLE 1: Total phenolic contents and antioxidant activities of aerial parts of *L. pulverulentus*.

Parts	Extract	Total phenols ^a	DPPH (%)	IC ₅₀ (μg)	FRAP ^b (μM)	Total antioxidant ^b
Stem-bark	LBM	302 ± 1.1	83.24 ± 1.4	68.2 ± 0.9	6.5 ± 0.4	0.677 ± 0.05
	LBH	165 ± 0.9	55.49 ± 1.6	32.5 ± 0.6	4.1 ± 0.5	0.595 ± 0.06
	LBC	309 ± 1.5	88.04 ± 0.7	22.1 ± 0.5	6.5 ± 0.9	0.750 ± 0.04
	LBE	396 ± 1.6	94.50 ± 2.1	15.9 ± 0.5	7.7 ± 0.6	0.952 ± 0.09
	LBB	344 ± 0.8	91.22 ± 1.5	17.4 ± 1.1	6.9 ± 0.4	0.717 ± 0.08
	LBA	151 ± 2.1	45.30 ± 0.6	46.3 ± 1.8	4.0 ± 0.2	0.365 ± 0.03
Leave	LLM	264 ± 1.3	95.50 ± 1.1	16.6 ± 0.7	7.2 ± 0.8	0.886 ± 0.08
	LLH	137 ± 0.9	94.30 ± 0.7	20.2 ± 1.0	4.2 ± 0.4	0.603 ± 0.05
	LLC	210 ± 1.4	95.40 ± 1.8	32.7 ± 1.4	6.1 ± 0.3	0.818 ± 0.07
	LLE	430 ± 2.2	96.30 ± 0.9	14.5 ± 0.8	7.5 ± 0.7	1.190 ± 0.09
	LLB	278 ± 1.6	95.10 ± 1.3	14.8 ± 0.5	7.3 ± 0.6	0.780 ± 0.04
	LLA	197 ± 0.7	94.90 ± 1.4	18.5 ± 0.4	6.1 ± 0.5	0.579 ± 0.04
Seed	LSM	81 ± 1.5	49.60 ± 0.7	201.3 ± 1.1	2.4 ± 0.8	0.403 ± 0.05
	LSH	62 ± 0.6	10.40 ± 0.5	455.8 ± 0.9	2.3 ± 0.5	0.354 ± 0.02
	LSC	179 ± 1.1	85.00 ± 0.8	117.5 ± 0.9	5.3 ± 0.8	0.651 ± 0.08
	LSE	231 ± 1.7	92.30 ± 1.1	102.7 ± 1.3	6.6 ± 0.7	0.686 ± 0.08
	LSB	171 ± 1.3	93.80 ± 1.5	105.4 ± 1.5	5.4 ± 0.2	0.559 ± 0.04
	LSA	39 ± 0.6	21.50 ± 0.4	465 ± 2.2	1.5 ± 0.1	0.157 ± 0.01
Gallic acid ^c	—	—	94.31 ± 0.9	14 ± 0.2	7.5 ± 0.1	—

^a mg GAE/g of extract, ^b equivalent to FeSO₄·7H₂O, and ^c positive control.

antioxidant capacities of components. These chromogens (the violet DPPH radical and the blue green ABTS radical cation) are easy to use, have a high sensitivity, and allow for rapid analysis of the antioxidant activity of a large number of samples. These assays have been applied to determine the antioxidant activity of pure components [12–14]. DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances [15]. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow-colored diphenyl-picrylhydrazyl. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the nonradical form DPPH-H by the reaction. DPPH is usually used as a reagent to evaluate the free radical scavenging activity of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [16].

The radical scavenging ability of extracts was checked by using DPPH as a free radical, and it was noted from results that all extracts of stem bark exerted remarkable antiradical activity and ranged from 45.30 ± 0.6% to 94.50 ± 2.1%, with ethyl acetate extract showing maximum activity as shown in Figure 1. All extracts of leave are very effective against DPPH assay, while in seed, n-butanol extract 93.80 ± 1.5% and n-hexane extract showed 10.40 ± 0.5% activity as shown in Figure 1. Ethyl acetate extracts of stem bark and leaves showed the least IC₅₀ values 15.9 ± 0.5 μg and 14.5 ± 0.8 μg with 94.5 ± 2.1 and 96.30 ± 0.9% DPPH inhibition, respectively (Table 1 and Figure 2).

Free radicals such as oxygen, superoxide, and hydroxyl are biologically important substances which are naturally released from human tissues. The highly reactive radicals

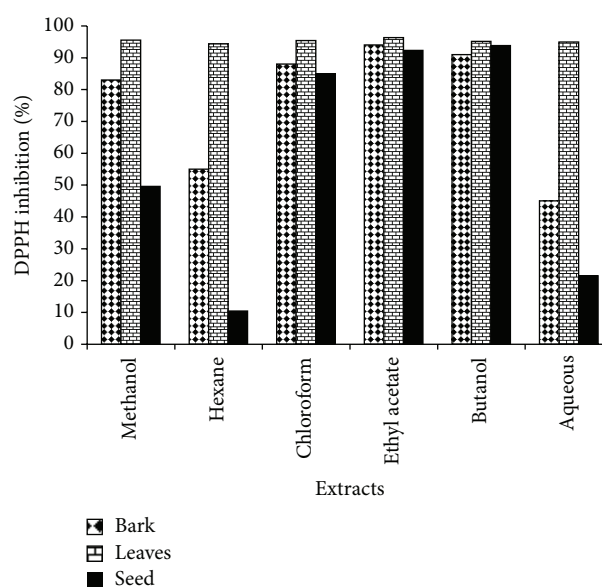


FIGURE 1: DPPH inhibition potential of different extracts of *L. pulverulentus*.

can cause oxidative damage to DNA, lipids, and proteins [17, 18]. Therefore, free radicals result in many disorders like cancer, cardiovascular diseases, and diabetes mellitus [19, 20]. Flavonoid and phenolic compounds and the plant extracts rich in these two type of compounds exhibit significant free radical scavenging activity and antioxidant activity. The method of DPPH free radical scavenging can be used to

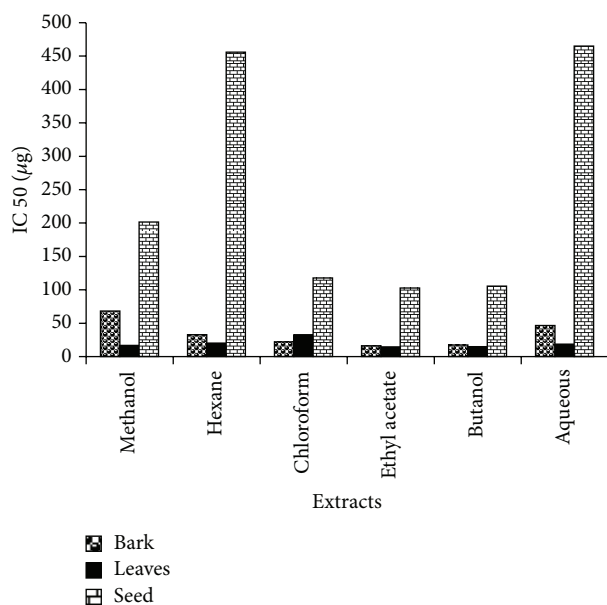


FIGURE 2: IC₅₀ µg values of different extracts of *L. pulverulentus*.

evaluate the antioxidant activity of specific compounds or extracts. Phenolic compounds are the principal antioxidant constituents of natural products and are composed of phenolic acids and flavonoids, which are potent radical terminators [21] by donating hydrogen to radicals. The high potential of polyphenols to scavenge free radicals may be because of their hydroxyl groups [22]. DPPH forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in the determination of radical scavenging activity of natural products [23, 24].

The reducing power of the extracts was determined using standard protocols such as FRAP and phosphomolybdate assays. All extracts showed remarkable reducing behavior, and it was deduced from the results that ethyl acetate extract of all the three plant parts exhibited the highest activities in the two antioxidant assays: FRAP (7.7 ± 0.6 , 7.5 ± 0.7 , and $6.6 \pm 0.7\%$, resp.) and total antioxidant activity (0.952 ± 0.09 , 1.190 ± 0.09 , and 0.686 ± 0.08 , resp.) as shown in Table 1 and Figure 3. FRAP and total antioxidant activity were measured at different concentrations of extract to check the dose effect on the activities. It was observed from results that both assays are concentration dependent (increasing the concentration of the extract enhanced the ability to reduce the ferric and molybdenum ion).

The correlation studies between total phenols and DPPH as well as FRAP and total antioxidant revealed a strong correlation between phenolics and antioxidant activities of the stem bark, leaves, and seed extracts of *L. pulverulentus* with $R^2 = 0.9506$ (stem bark), 0.8413 (leaves), and 0.8658 (seeds) for DPPH, 0.8185 (stem bark), 0.8648 (leaves), and 0.9131 (seeds) for total antioxidant, and 0.9951 (stem bark), 0.6728 (leaves), and 0.991 (seeds) for FRAP as shown in Figures 4, 5, 6, 7, 8, 9, 10, 11, and 12.

Several studies have been conducted in the past that natural antioxidants in medicinal and dietary plants were

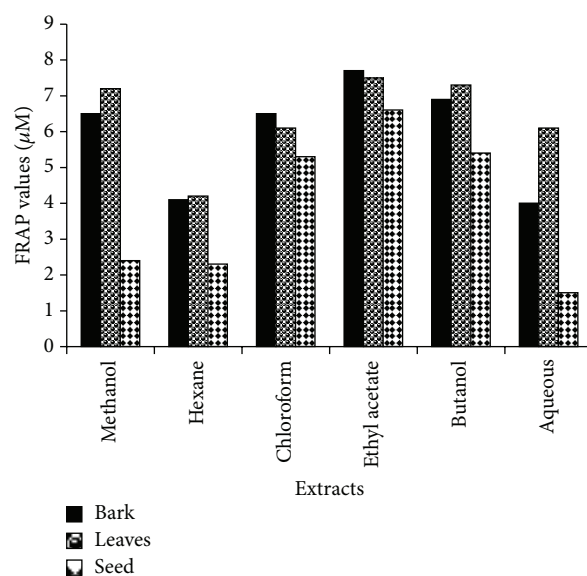


FIGURE 3: FRAP of different extracts of *L. pulverulentus*.

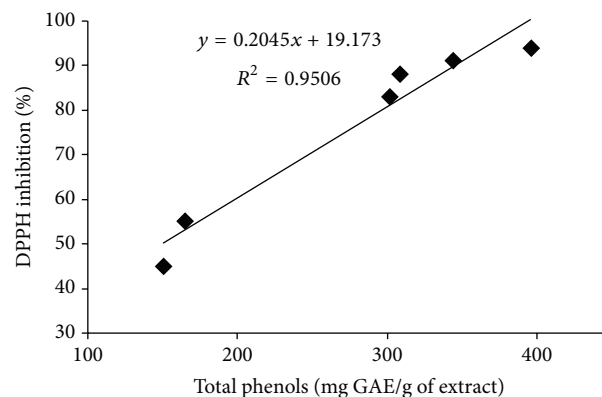


FIGURE 4: Correlation between total phenols and DPPH scavenging activity of bark extracts of *L. pulverulentus*.

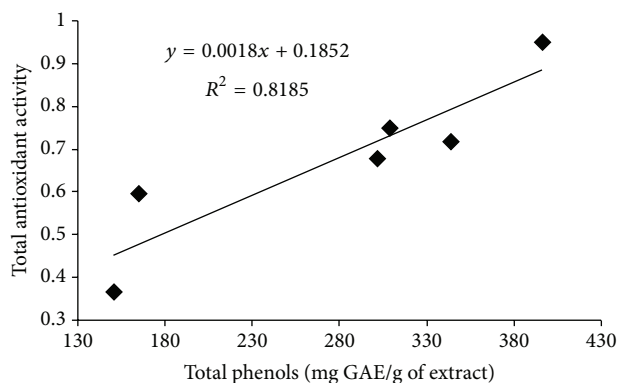


FIGURE 5: Correlation between total phenols and total antioxidant activity of bark extracts of *L. pulverulentus*.

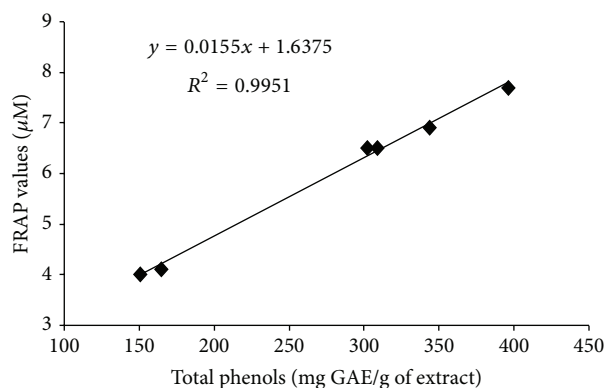


FIGURE 6: Correlation between total phenols and FRAP of bark extracts of *Loranthus pulverulentus*.

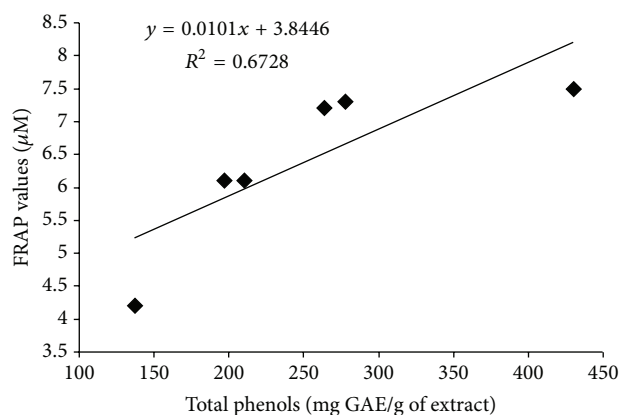


FIGURE 9: Correlation between total phenols and FRAP of leaf extracts of *Loranthus pulverulentus*.

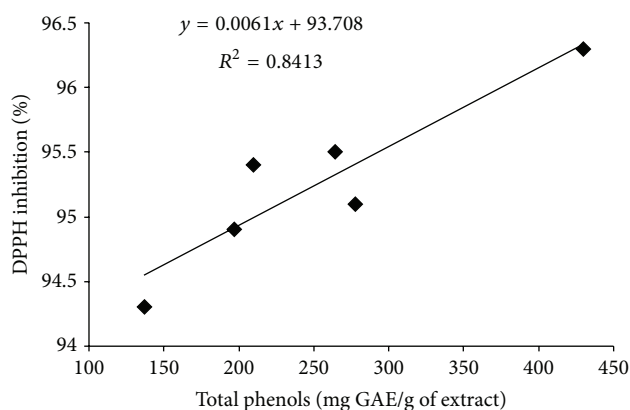


FIGURE 7: Correlation between total phenols and DPPH scavenging activity of leaf extracts of *Loranthus pulverulentus*.

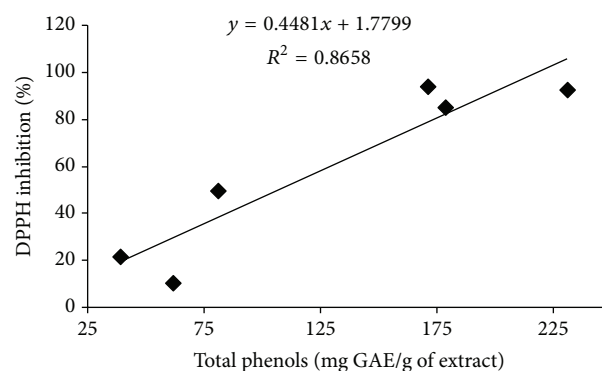


FIGURE 10: Correlation between total phenols and DPPH scavenging activity of seed extracts of *Loranthus pulverulentus*.

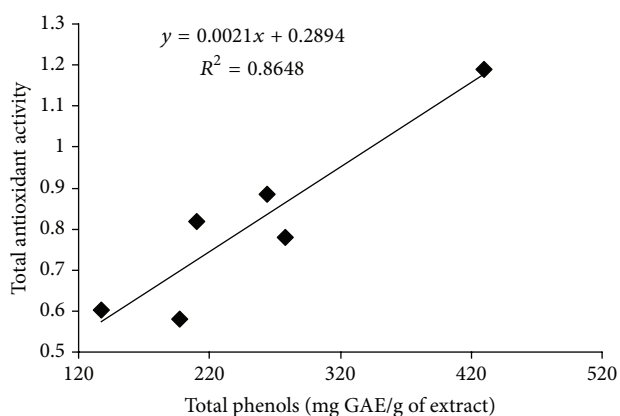


FIGURE 8: Correlation between total phenols and total antioxidant activity of leaf extracts of *Loranthus pulverulentus*.

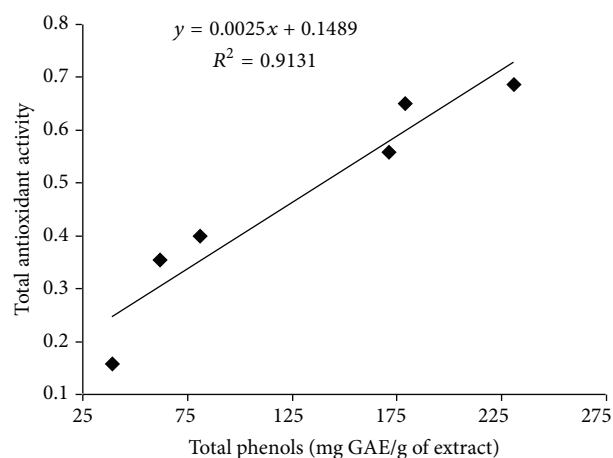


FIGURE 11: Correlation between total phenols and total antioxidant activity of seed extracts of *Loranthus pulverulentus*.

closely related to their biofunctionalities, such as the prevention or suppression of aging and many diseases associated with oxidative stress: cancer, cardiovascular diseases, rheumatoid arthritis, autoimmune diseases, and AIDS. Thus, antioxidant capacity is widely used as a parameter to characterize food or medicinal plants. Phenolic compounds have

antioxidant properties because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen and hydroxyl radicals [25]. The compounds responsible for such antioxidants activity can be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders [26–28]. Therefore, research to identify

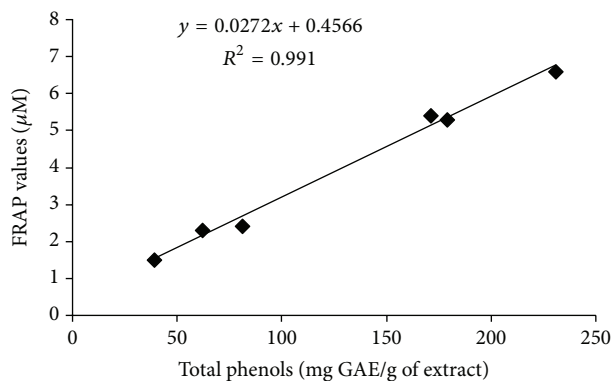


FIGURE 12: Correlation between total phenols and FRAP of seed extracts of *Loranthus pulverulentus*.

antioxidative compounds is an important issue. Although it remains unclear which of the compounds of medicinal plants are the active ones, polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid peroxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies isolating polyphenols and evaluating their antioxidative effects have rarely been carried out.

4. Conclusion

It is well known that free radicals are the principal cause of several diseases, including Parkinson's disease, coronary heart disease, cancer, and Alzheimer's disease. This study demonstrated that *Loranthus pulverulentus* has high phenolic contents and excellent antioxidant activity. It would be interesting to further investigate the potential effectiveness of this plant for treating diseases caused by the overproduction of free radicals.

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