

Antioxidant Properties of Various Solvent Extracts of Lemon Verbena (*Lippia Citriodora*) Leaves

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Abstract

Lemon verbena (*Lippia citriodora*) belongs to the Verbenaceae (shahpasand) family. *L. citriodora* is chemical composition of the leaves plant have antimicrobial properties, refrigerant, anti-headaches one-sided, anti-pains nerve and housing, carminative, helping to digestion, relaxation, dizziness, colds therapy, memory Booster and etc. In this study, phenolic compounds of *L. citriodora* leaves were extracted with immersion method by acetone, ethanol, methanol and water. In this method, solvent type was found as an effective factor on the extraction. The highest amount of total phenol compounds was extracted by methanol which was 25/94 (mg Gallic acid per 100 mg of the extract). Among the solvents, acetone extremely had lower efficiency in the extracting phenolic compounds from the leaves of plant. Three complementary assays, reducing power of Fe (III), DPPH radical scavenging and total antioxidant capacity were used for analysis of antioxidant properties of various extracts compared with the synthetic antioxidant, BHT. In all tests, methanolic extract, which had the highest total phenolic compounds, showed the highest antioxidant activity although it was lower than that of BHT. According to the survey results, *L. citriodora* leaves with a good source of natural antioxidants for use in food industry's.

Keywords: *Lippia citriodora* leaves; Reducing power; DPPH scavenging activity; Antioxidant activity.

Introduction

Lemon verbena is from verbenaceae family, *Aloysia genesis*, and *A. citriodora* species. Extract of lemon verbena contains phenyl perionoides, isoverbasosides and verbasoside as the main phenolic combination. These combinations can be a reliable source of antioxidants, anti-arthritis, anticancer and antibacterial that can be used for synthesis of nutraceutical formulations (Cruz et al., 2003). Strong antioxidant effect of phenol combinations in the extract of verbena can attenuate or stop activity of superoxide, hydroxyl, and hypochloric radicals (Valentao et al., 2002). In addition, antioxidant capacity values are comparable to those of a commercial antioxidant drink based on green tea (Abderrahim et al., 2011). The phenolic compounds of lemon leaves were extracted by water and ethanol solvents, and the obtained extracts were tested on the rate to examine antioxidant activity. The results showed that the antioxidant activity of

ethanol extract was higher than that of the aqueous extract, which is probably due to higher content of phenolic compounds extracted by ethanol solvent (El-Hawary *et al.*, 2012). The combined supplement containing standardized lemon verbena extract and fish oil omega-3 fatty acid reduced symptoms of pain and arthritis caused by free radical among athletes who use antioxidant rich foods. (Caturla *et al.*, 2011). Examination of the antioxidant effect of lemon verbena extract on students' lymphocytes during aerobic exercises showed that phenol and proinoid content and verbascoside play a notable role in reducing glutathione and redox status in lymphocytes and protecting plasma and red blood cells from oxidative damage (Carrera-Quintanar *et al.*, 2010). Considering absence of a study on extraction of phenol combinations from verbena using different solvents and measuring content of the phenol combinations and on antioxidant effects of different extracts on reducing Fe(III), controlling DPPH radicals, and antioxidant characteristics, the present study is an attempt to determine phenol content and antioxidative activity of the extracts and find a safe and healthy product.

Material and methods

Materials

Lemon verbena (*L. citriodora*) leaves were harvested in April 2013 from the talae sefid gonbadco. The leaves were washed and dried in a hot air oven at 45 C for 6 h. The dried material was ground to a fine powder, passed through a 63-mesh sieve and kept in an air-tight container at 4 C until further use. All chemicals and solvents used in this study were obtained from the companies Merck and Titrachem high purity.

Extraction of antioxidants from Lemon verbena leaves

The dried leaves of lemon verbena (15 g) were extracted overnight with 100 ml each of methanol, ethanol, acetone or water, respectively, in a mechanical shaker at room temperature. Each extract was filtered with Whatman No. 1 filter paper. The filtrate obtained from methanol, ethanol and acetone was evaporated to dryness at 40 C in a rotary evaporator and the water extract was freeze-dried. The dried sample of each extract was weighed to determine the yield of soluble constituents and stored at 4 C until use.

Estimation of total phenolics

Total phenolic content of each extract was determined by the Folin-Ciocalteu micro-method (Slinkard & Singleton, 1977). Briefly, 20 µl of extract solution were mixed with 1.16 ml distilled water and 100 µl of Folin Ciocalteu reagent, followed by addition of 300 µl of Na₂CO₃ solution (20%) after 1 min and before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40 C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents using the following linear equation based on the calibration curve:

$$Y = 0.001X + 0.022 \quad R^2 = 0.9982$$

where Y is the absorbance and X is concentration as Gallic acid equivalents (µg/ml).

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the method of Shimada (1992). Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 50-300 µg of dried extract and). The mixture was then vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity(\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

Reducing power of Fe (III)

The ability of extracts to reduce iron (III) was assessed by the method of (Yildirim *et al.*, 2001). The dried extract (50-500 μg) in 1 ml of the corresponding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferri cyanide ($\text{K}_3\text{Fe}(\text{CN})_6$; 10 g l^{-1}), then the mixture was incubated at 50 C for 30 min. After incubation, 2.5 ml of trichloroacetic acid (100 g l^{-1}) were added and the mixture was centrifuged at 1650g for 10 min. Finally, 2.5 ml of the supernatant solution were mixed with 2.5 ml of distilled water and 0.5 ml of FeCl_3 ($\mu\text{g l}^{-1}$) and the absorbance was measured at 700 nm. High absorbance indicates high reducing power.

Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto *et al.*, 1999). An aliquot of 0.1 ml of sample solution (containing 50-500 μg of dried extract in corresponding solvent and synthetic antioxidant BHT was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples.

Methodology, data analysis, sampling

The data were collected through a fully-random plan and analyzed by ANOVA. Each test was conducted with 3 replications. Comparison of the mean points was done by Duncan's multi-range test ($P < 0.005$) in SPSS. The diagrams were drawn in EXCEL.

Results and discussion

Extract yield and total phenolics

Table 1 lists mean extraction performance based on solvent and the effect of the solvent on total phenol content in the extraction. Variance analysis revealed that the solvent had significant effect ($P < 0.05$) on essence extraction. Clearly, methanol has the highest extract extraction performance followed by ethanol and acetone. Maximum extraction 10.07 (Grams of extract per 100 g of dried leaves) was done by aqua extract and minimum extraction 3.43 (Grams of extract per 100 g of dried leaves) was achieved by acetone. Type of solvent was found to be significantly effective ($P < 0.05$) on total phenol content of the extracted extract. In a descending order, the extracted extract by methanol, ethanol, acetone, and water had the highest phenol content. In spite of high extraction performance, aqueous solvent extracted less phenolic compound comparing with methanolic solvent.

DPPH radical scavenging activity

Variance analysis results in figure 1 show that regarding capacity to control free radicals, methanol extract of Lemon verbena is significantly different from BHT synthesis antioxidant ($p < 0.05$). In addition, type and concentration of ethanolic, acetonic, and aqueous essences were significantly effective on controlling free radicals ($p < 0.05$). Furthermore, this characteristic increased along with increase in concentration of the extracts. At concentration range 50-300 $\mu\text{g/ml}$, highest capacity was observed in BHT synthesis antioxidant, methanolic extract, ethanolic, acetoneic, and aqueous extract in a descending order. This is in agreement with the reports of Ting & chi-tang (2005), Arabshahi-Delouee & Urooj (2007), and Yao & Qing (2007) that methanol is a widely used and effective solvent for extraction of antioxidants. that methanol is a widely used and effective solvent for extraction of antioxidants.

Fe⁺²(III) reduction power

Figure 2 illustrates a comparison of mean absorption capacity of different concentrations of methanolic, ethanolic, acetic, and aqueous extracts of Lemon verbena and BHT synthesis antioxidant at wavelength 700nm. This characteristic is an indicator of reduction power of Fe⁺²(III). Variance analysis indicated a significant difference ($p < 0.05$) between variety of extracts, and BHT synthesis antioxidant regarding Fe⁺²(III) reduction power. As pictured, BHT synthesis antioxidant has the highest Fe⁺²(III) reduction power at 50-500 $\mu\text{g/ml}$. There is a direct relationship between reduction power of the extracts and total phenolic content in them, so that methanolic extract with highest phenol content had the highest reduction power and aqueous essence with lowest phenol content also had lowest reduction power. Increase in concentration, in all cases, increased absorption power of the extract solutions.

Total antioxidant capacity

Mean absorption capacity of the aqueous, ethanolic, methanolic, acetic, and BHT synthesis antioxidant are compared in figure 3. Variance analysis results showed that there is significant difference ($p < 0.05$) among different extracts of Lemon verbena and between them and BHT synthesis antioxidant regarding the absorption capacity. As the results showed, regardless of the concentration of the extracts (50-50ppm) BHT had the highest antioxidant absorption capacity following by methanol extract. In addition, absorption capacity increased with increase in concentration of the extract.

Conclusions

The results obtained in this study showed that methanolic extract demonstrated the most efficient extraction regarding phenolic compounds, reducing power of Fe (III), DPPH radical scavenging, and total antioxidant capacity. Given that lemon leaves contain large amounts of phenolic compounds, then its antioxidant potential is high. Stability of edible oils in oxidative environment increases by adding phenolic extracts of the plant. Due to the adverse effects of synthetic antioxidants on human health, lemon juice is recommended as a replacement for these compounds in the foods containing fats.

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Table 1. extract yield and total phenolic contents of different solvent extracts from *L.citriodora* leaves

Sample	Yield	Total phenolics
Methanol extract	10.03 ^a ± 0.2	25.94 ^a ± 0.215
Ethanol extract	9.53 ^b ± 0.5	23.75 ^b ± 0.32
Acetone extract	3.43 ^c ± 0.37	18.06 ^c ± 0.18
Water extract	10.07 ^a ± 0.36	16.58 ^d ± 0.29

Values in the same column followed by different letters are significantly different ($p < 0.05$).mg of extract per 100 mg of dried leaves;grams of gallic acid per 100 mg (dry weight) of extract

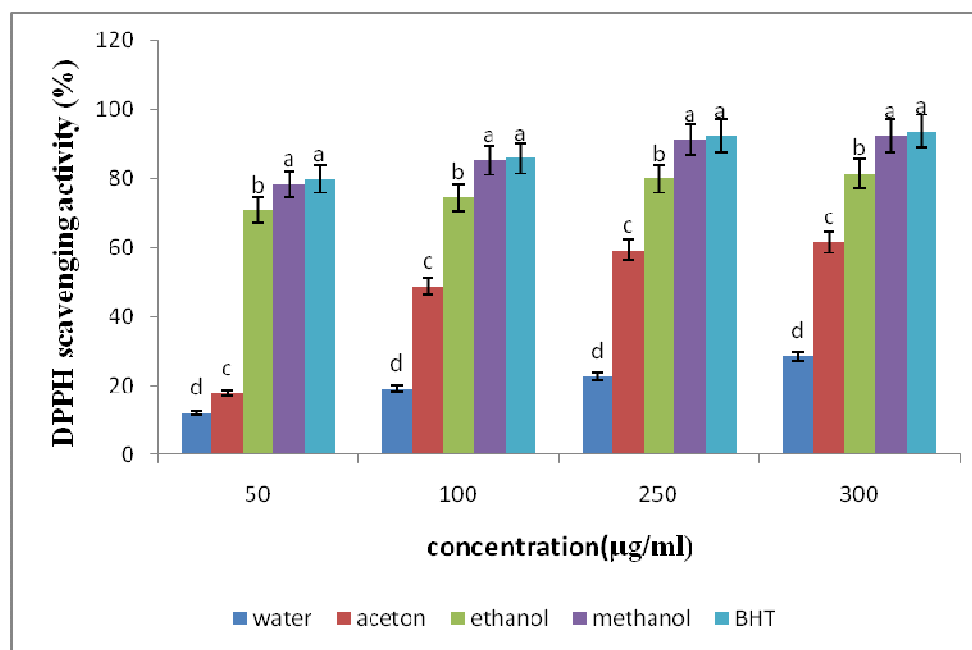


Fig1. DPPH scavenging activities of methanol, ethanol, acetone and water extract of *L.citriodora* leaves. BHT were used as positive controls. Percentag radical scavenging capacity relative to control.

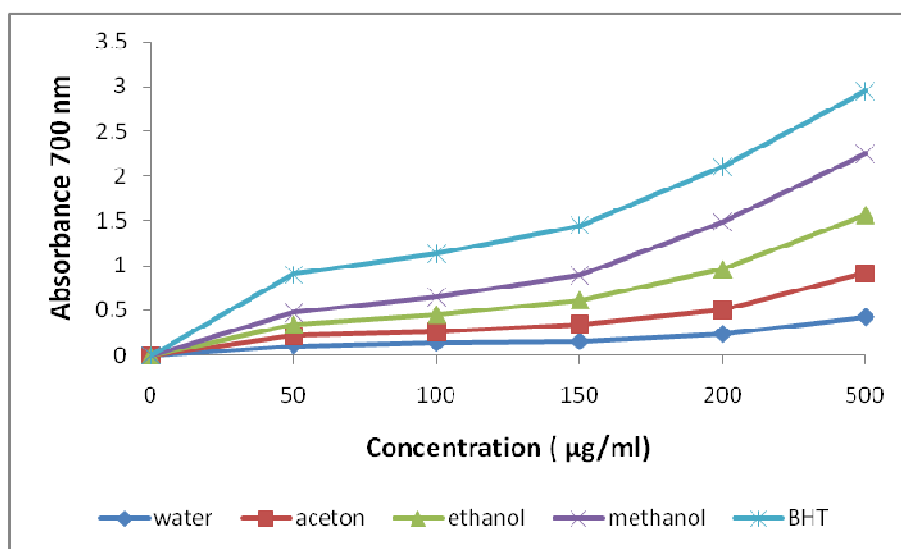


Fig2. Reducing powers of methanol, ethanol, acetone and water extracts of *L.citriodora* leaves.

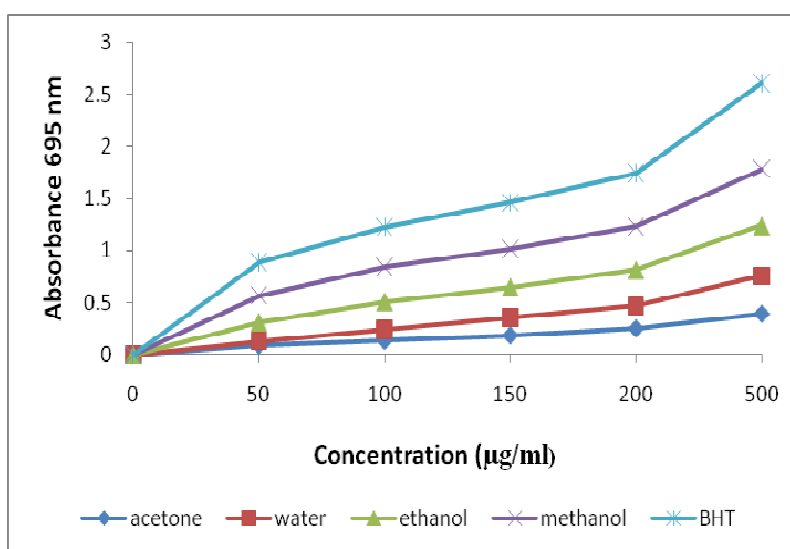


Fig3. Total antioxidant activities of methanol, ethanol, acetone and water extracts of *L.citriodora* leaves. BHT was used as positive control.