

**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EXTRACTION OF LIPPICITRIFLORA LEAF AND EVALUATION OF ITS PHENOLIC COMPOUNDS

MOHAMMAD MEHDI NEMAT SHAHI ^{*1}, AMIR HOSSEIN ELHAMI RAD ², AHMAD
PEDRAM NIA ², NAFISE NEMAT SHAHI ³

1. Young Researchers and Elite Club, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran.
2. Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University,
Sabzevar, Iran.
3. Ph.D Student in Biology - Plant Physiology, Faculty of Basic Sciences, Ferdowsi University
of Mashhad, Mashhad, Iran

***Corresponding Author: m.nematshahi67@gmail.com**

ABSTRACT

Many plants and fruits such as the Lippia Citriodora leaf are rich in antioxidant compounds such as phenolic compounds, which can be used in healthy diets. These compounds are as powerful antioxidants can preserve the biological systems from the dangers of free radicals. In this study, at first, the methanolic extract of Lippia Citriodora leaf was extracted by maceration method with methanol at the mixing ratio of 1:10 (weight-volume). Then, the phenolic compounds of the extract of Lippia Citriodora leaf was studied in different concentrations (50, 100, 200, 400, 800, 1600 ppm) by the Folin-Ciocalteu method and compared with the 200 ppm of synthetic antioxidant of BHT. One-way ANOVA method and Mstat c software were used to analyze the data. The results showed that the amount of phenolic compounds, in compared to control sample, increased significantly by increasing the concentration of the extract of Lippia Citriodora leaf, so, only the concentration of 350 and 400 ppm had more phenolic compounds than 200 ppm of BHT ($P < 0.05$). This was due to the much further tocopherol and phenolic compounds of the extract. According to the results, the methanolic extract of Lippia Citriodora leaf can be used as a low cost and available plant antioxidant source in the food industry after performing supplementary tests.

Key words: Lippia Citriodora Leaf, Phenolic Compounds, Extraction, Antioxidant Activity.

INTRODUCTION

Oxidation and producing free radicals and peroxides lead to the destruction of food lipids which affect the nutritional quality (fatty acids and fat-soluble vitamins) and food organoleptic characteristics (color, odor, taste, tissue) adversely. In addition, many of the products of lipid oxidation are considered as the dangerous factors for human health (1). In recent years, the use of synthetic antioxidants such as BHT, BHA and TBHQ as other chemical additives has been limited due to their potential toxicity and carcinogenicity. Today, most of the research on this area have focused on the use of new and safe antioxidants from plant, animal and food sources. The phenolic compounds, with several different mechanisms, prevent the lipid oxidation. The most important function of these compounds associated with oxidation is making free radicals inactive and forming Metal Byonne Complex (2). Lemon verbena is a species of flowering plant in the verbena family Verbenaceae. It emits a powerful scent reminiscent of lemon. Its scientific name is Lippia Citriodora and its English name is Lemon Verbena (3). It is native to Argentina and Chile and also it grows in the North of Iran well. It is sensitive to cold and the temperature

below 0 C. Its shrub grows to 1.5-2 m high. It has 8 to 10-cm-long, glossy, pointed leaves. Its useful parts are its leaves. In herbal medicine, it is used in the treatment of indigestion, distention, nervous pain, heart palpitations, dizziness, cold and improving memory. This herb is collected 2 to 3 times a year and usually in the months of July, August, October. Then, its leafy branches are cut and dried naturally. It is reproduced by cutting the branch or layering (3).

Kamkar et al. (2010) determined the antioxidant activity of the Dill (*Anethum Graveolens*) extract by using two methods of 2,2-diphenyl-1-picrylhydrazyl based on the percentages of inhibitory activity of free radicals and inhibitory activity of linoleic acid oxidation in the system of beta-carotene-linoleic acid. The results showed that the antioxidant power of ethanolic extract was significantly more than the antioxidant power of the essence ($p < 0.05$) and it can be due to the different amount of their phenolic compounds (4). The studies have indicated that no research had performed on the antioxidant activity of the extract of Lippia Citriodora leaf, so this study has been performed on the use of plant oxidant of

Lippia Citriodora leaf, which is the native products of our country (Iran), as a suitable alternative of synthetic antioxidants.

MATERIALS AND METHODS

Extraction of Lippia Citriodora leaf by maceration method

In this study, a variety of the Lippia Citriodora's leaf were gathered from the trees of Gorgan City and their waste parts were removed and they were dried immediately after washing. In order to extract, cleaned leaved were powdered by mill (Kenwood, model 100 CG) and then sifted and mixed with the solvent of ethanol as a mixing ratio of 1:10 (weight-volume) and put in hotplate with the rate of 250 rpm for 24 hours at ambient temperature and then under vacuum, it was filtrated by Buchner funnel with a Whatman filter paper No. 1. After that, it was condensed by rotary evaporator (LABORATA4000) at 35 C and finally, the extracts were dried under vacuum at 40 C by dryer and kept in container, which is enclosed and impervious to air, at 4 C. All materials used in this study were provided from Merck and Sigma companied with high purity (5).

Measurement of phenolic compounds

Plot a calibration curve

First, the standard solution of Gallic acid in ethanol with various concentrations in the range of 0.04 to 0.4 mg per ml was prepared.

Then, 0.5 ml of the standard solution of Gallic acid was mixed with 2.5 ml of the Folin-Ciocalteu reagent (to prepare the Folin-Ciocalteu reagent, the concentrate was diluted with distilled water at a ratio of 1 to 10) by 50 ml volumetric flasks. After 10 minutes, 2 ml of sodium carbonate solution 7.5% was added to them and the final volume was completed with pure water. After one hour at ambient temperature, its absorption was read at the wavelength of 765 nm and absorption curve was plotted to the Gallic acid concentration (mg/ml) and the following equation was obtained with the explanation correlation of 0.99:

$$Y = 1/0776X^2 + 0/2644X + 0/0099$$

(1)

Where X is the amount of absorption at the wavelength of 765 nm and Y is the amount of phenolic compounds (mg/ml) (6).

Measurement of phenolic compounds sample

First, the standard solutions of the extract of Lippia Citriodora leaf were prepared with a solvent (methanol), which has extracted it, with various concentrations in the range of 50 to 400 ppm and also the control sample was prepared. Then, 0.5 ml of the standard solution of Gallic acid was mixed with 2.5 ml of normal Folin-Ciocalteu reagent 0.2 (to prepare the Folin-Ciocalteu reagent, the

concentrate was diluted with distilled water at a ratio of 1 to 10) by 50 ml volumetric flasks. After 10 minutes, 2 ml of sodium carbonate solution 7.5% was added to them and the final volume was completed with methanol. After two hours at ambient temperature, its absorption was read at the wavelength of 765 nm. The total amount of phenolic compounds was measured based on Gallic acid (%) by the calibration equation (as a standard for Gallic acid) (6).

Statistical analysis

To analyze the results, one-way ANOVA method was used. The data was analyzed by statistical software of Mstat c. The averages were compared with each other and control sample by LSD-test at the alpha level of 0.05. The Microsoft Excel software was used to plot the charts.

RESULTS AND DISCUSSION

The phenolic compounds of the extract of Lippia Citriodora leaf

Since phenols and phenolic compounds have been widely found in the food and plant products and due to the fact that the phenolic compounds are the main cause of the medicinal plants antioxidant properties, the phenolic compounds of the extract of Lippia Citriodora leaf were measured (7). The effect of various concentration of the extract of Lippia Citriodora leaf on the amount of its

phenolic compounds was measured by Folin test and the standard curve equation of Gallic acid. The results were shown in Figure 1. The results showed that the phenolic compounds of the extract of Lippia Citriodora leaf has increased by increasing the concentration of extract from 100 to 400 ppm and it leads to the increase of antioxidant property. Based on the results, the antioxidant compounds has increased from 5.9 ppm in the concentration of 100 ppm to 17.2 ppm in the concentration of 400 ppm of the extract of Lippia Citriodora leaf by increasing the different concentrations of the extract. This difference in all concentrations was significant in compared with a sample of 200 ppm of synthetic antioxidant of BHT with 12.6 ppm of phenolic compounds at the level of 95% ($P < 0.05$). As can be seen in Figure 1, a sample of 200 ppm of synthetic antioxidant of BHT had less phenolic and antioxidant compounds than the samples of 350 and 400 ppm of the extract of Lippia Citriodora leaf that this difference was statistically significant. Therefore, based on this parameter, the concentration of 400 ppm had the most phenolic compounds.

In a similar study, Unver et al. (2009) studied the antioxidant activity and the amount of total phenol of methanolic extract of several plants and concluded that there is direct correlation between the amount of total phenol and

antioxidant activity of these plants extracts, as Mentha Piperita had showed high amount of total phenol (493 mg of Gallic acid per gr) and antioxidant activity ($IC_{50}=0.23$ mg/ml) and

Capparis Ovate had showed low amount of total phenol (185 mg of Gallic acid per gr) and antioxidant activity ($IC_{50}=4.08$ mg/ml) (8).

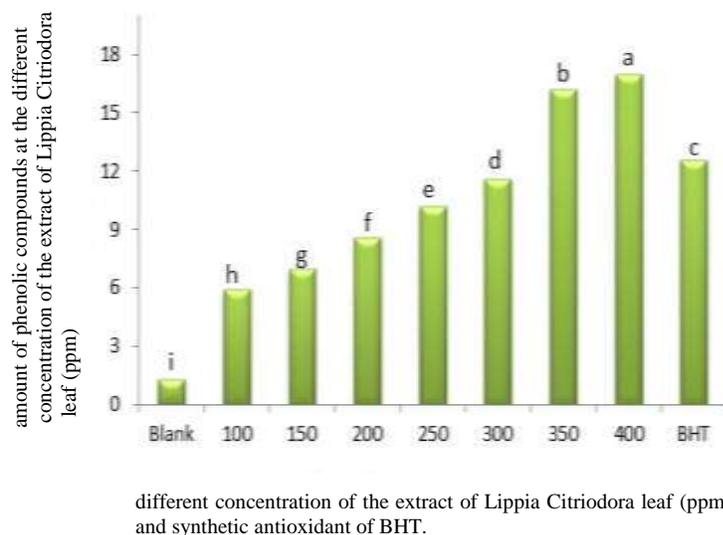


Figure1. The changes of phenolic compounds at the different concentrations of the extract of Lippia Citriodora leaf

CONCLUSION

Since the synthetic antioxidants have undesirable effects on the human body, they have been gradually removed from the list of consumption antioxidants, so, it is necessary to investigate the sources of natural antioxidants to use instead of synthetic compounds. In this study, the antioxidant properties of the extract of Lippia Citriodora leaf as a natural antioxidant were investigated in different concentration by measuring phenolic compounds and comparing with synthetic antioxidant of BHT. The results showed that the extract of Lippia Citriodora

leaf has the good antioxidant effects. Compared with the similar studies of other researchers, its antioxidant power is less than some cases and is more than some other cases. In total, inconsistency in the results of the research can be associated with a variety of chemical compounds found in plants, their different reaction mechanisms and kinetics in selected methods. The measured antioxidant capacity of a sample is in relation with the used method and an oxidizing agent. Therefore, the extract of Lippia Citriodora leaf can be used in food and pharmaceutical industries after performing additional tests.

REFERENCES

- [1] Prior, R.L. and Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *Horticulture Science*. 35: 588-592.
- [2] pokorny,J, Yanishlieva, N and Gordon,M.(2001). *Antioxidants in Food* CRC Press.380p.
- [3] Zargari, A. (1990) “Medicinal Plants”, Volume IV, Fourth Edition, Tehran University Press.
- [4] Kamkar,A, Jebelli,A, Javan, F. A, Kamalinejad.(2010). The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil , *Food and Chemical Toxicology*, Volume 48, Issue ,7Pages 1796-1800.
- [5] Nemat Shahi, M. (2013). “Investigating the antioxidant effect of *Laurus Nobilis* L leaf extract on the stability of canola oil during storage”, Master Thesis, Islamic Azad University of Sabzevar, Record number: 1392.
- [6] Stoilova, I, Krastanov, A, Stoyanova, A, Denev, P, Gargova, S.(2007). Antioxidant activity of ginger extract (*Zingiber officinale*), *Food Chemistry*, 102: 764-70.
- [7] Lee, S.J, Umamo, K, Shibamoto, T and Lee, K.G.(2005).Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties.*Food Chem.*; 91: 131 - 7.
- [8] Unver, A , Arslan, D, Ozcan, M.M and Akbulut, M. (2009). Phenolic content and antioxidant activity of some spices, *World Appl, Sci, J. 6*: 373 - 7.