ISSN (Print 0974-2115) (Online 2349-8552)
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Effect of Preparation conditions on Phenolic Content and Antioxidant Activity of Various Teas and Herbal Teas

Dima Aldiab*
Department of Analytical and Food Chemistry, Faculty of Pharmacy, Tishreen University, Lattakia, Syria
***Corresponding author: E-Mail: dyabdima@yahoo.com**

ABSTRACT

Phenolic compounds are known for their positive effects on health such as antioxidant activity. Teas and Herbal teas are good source of phenolic compounds, however most studies about phenolic compounds use organic solvents for extraction, which is not able to be applied in real life. Therefore, the aim of this study was to compare the phenolic content of different teas and herbal teas by the usage of boiled water and methanol as solvent extraction and then to investigate the effect of grounding of these teas and herbal teas on the phenolic content. Twelve Teas and herbal teas were investigated to determine the best extraction conditions of phenolic compounds. Methanol/water mixture was compared to boiling water in extracting phenolic compounds from teas and herbal teas before and after grounding. The phenolic content was determined using Folin-Ciocalteu method. Results show that methanol/water mixture was superior to boiling water in extracting phenolic compounds from whole plant parts. However, no significant difference was found in most extracts between the two solvents after grounding the samples. The antioxidant activity of infusions of ground teas and herbal teas in boiling water was subsequently determined using FRAP method. Black tea, green tea, mate tea and Damaskus rose had the highest phenolic content and the highest antioxidant activity using these conditions of extraction. Results showed an overall positive correlation between antioxidant activity and phenolic content.

KEY WORDS: Phenolic compounds, Folin-Ciocalteu, Antioxidant activity, FRAP, teas, Herbal teas.

1. INTRODUCTION

Phenolic compounds comprise a large group of molecules with a variety of functions in plant like growth, development, and defense. Their chemical structures contain one or more hydroxyl groups attached directly to an aromatic ring, forming tens of thousands of different compounds (Vermerris and Nicholson, 2006). Phenolic compounds are known as bioactive compounds and health-promoting agents (Nick, 2007). The research in this field led to many conclusions e.g. their ability to reduce the risk of several diseases like hepatitis C, cardiovascular disorders, diabetes, and urinary tract infections (Alam, 2018) as well as their anti-inflammatory properties and their potential to protect against osteoporosis and neurodegenerative diseases upon long term consumption of plant sources rich in phenolic compounds. Phenolic compounds can also become potential therapeutic agents against cancer especially that they are considered as chemo-preventive compounds (Pelle and Compagnone, 2018). The previous health promoting effects of phenolic compounds are based on different positive effects such as antioxidant activity. Phenolic compounds act as antioxidants due to their redox properties (Park, 2018). The antioxidant activity of phenolic compounds can prevent the onset of diseases by slowing down oxidation and scavenging free radicals maintaining the balance between the free radicals and the defense system (Orsine, 2014). They can also delay free radical formation and reduce reactive oxygen species (Mtoloa, 2017) which are harmful to important cellular structures including DNA (Rafehi, 2012), lipids and proteins (Ghasemzadeh, 2018). This balance between oxidants and antioxidants may reduce oxidative stress in the body. It is also reported that these compounds act against major food borne pathogens since they possess antimicrobial activity. Additionally, some food organoleptic properties such as bitterness, astringency, color, flavor and odor can also be attributed to the presence of phenolic compounds (Pelle and Compagnone, 2018). For this reason, research on these functional compounds is prominent in the food science field (Pramai, 2018).

Teas and Herbal teas are good source of phenolic compounds. They are widely consumed as beverage in Syria in form of water infusions. However, most studies about the phenolic content and antioxidant activity of herbal teas use methanol or other organic solvent for extraction, which is not applicable in real life. Additionally, these teas and herbal teas are consumed either grounded or as whole parts which may also affect the amount of extracted phenolic compounds. Thus, the first aim of this study is to compare the yield of phenolic compounds by the use of water and methanol as extraction solvents and then to investigate the effect of grounding on the phenolic content. The third object of this study is to determine the antioxidant activity depending on the best extraction conditions, which gave the maximum phenolic amount.

2. MATERIALS AND METHODS

Plant materials: All dried teas and herbal teas (100 g of each sample) were bought from local markets in Latakia. The used parts were extracted directly [the used parts were bought without grounding except mate tee was slightly ground (table.1)] or were ground with a Mill into powder prior to extraction.

Sample preparation: Dry plant materials (1 g) were weighed into a test tube. A total of 12 ml of a mixture of methanol/water (80, 20%) was added. Tubes were sonicated for 25 min at room temperature. The mixture was centrifuged at 2000 rpm for 5 min, and supernatants were collected. For aqueous extract, 1 g of dry plant materials were weighed into a test tube then 12 ml of freshly boiling water were added and left for three min at room temperature. The samples were then cooled in ice, filtered and diluted to proper concentration prior to analysis.

Determination of phenolic content: Total phenolic content was determined using Folin-Ciocalteu reagent. First, 0.1 ml of aqueous or methanol extracts was mixed with two ml of freshly prepared sodium carbonate solution 2% (w/v) and allowed to stand for 5 min. 0.1 ml of Folin-Denis' reagent (1:1 diluted with distilled water) was added to the mixture and left at room temperature for 30 min. A blank solution was prepared like samples; however, 0.1 ml of distilled water was added instead of the sample. A standard curve was prepared from Gallic acid in concentrations range (0.1-0.8 g/L); the absorbance was read at 750 nm for both Gallic acid and extracts. The equation was $y=1.311x+0.0220$ and R^2 was 0.997. The total phenolic content was expressed as milligrams of Gallic acid equivalents (GAE) per 1g of dried herb. The experiments were carried out in duplicate.

Ferric reducing antioxidant power (FRAP) assay: The total antioxidant activity of a sample was determined using the ferric reducing ability as a measure of antioxidant power. An antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (v/v/v). 1 ml of FRAP reagent was added to 100 µl of extracts. The absorbance was taken at 593 nm after 5 min. Standard curve was prepared using different concentrations of $FeSO_4$ in concentrations between (100-650 µM). The equation was $y=1.0014x-0.0051$ and R^2 was 0.997. The results were expressed as micromole Fe^{2+}/1Gram. All solutions were used on the day of preparation.

Statistical Analysis: All the results were presented as means ± standard deviations. The differences between phenolic extracts were determined by applying Student's t-test.

3. RESULTS AND DISCUSSION

Extraction procedure was first applied on teas and herbal teas without grounding as the dried parts were bought. Results are shown in table.1. The Lowest levels of phenolic compounds were found in leaves of thymus, rosemary, salvia, marjoram, as well as hyssop and chamomile flowers, while the highest levels were found in leaves of black, green and mate tea. As seen Table.1, the phenolic contents in methanol/water extracts was higher than those in boiled water in most of teas and herbal teas infusions indicating that methanol/water mixture can extract phenolic compounds from teas and herbal teas better than boiling water. Koutelidakis (2016), arrived similar findings by studying different types of herbal teas. In their study, methanol extracts had higher phenolic content than boiling water infusions due to the ability of methanol to extract lipophilic phenolic compounds as well as hydrophilic ones. However, methanol extracts are not applicable to consumers in real life, which necessitate the increase of phenolic yield by other procedures like grounding, since grounding increase the surface area available for maximum extraction.

Table.1. Differences in phenolic content of teas and herbal teas according to the extracting solvent when samples are extracted without grounding

Sample	Phenolic content mg (GAE)/g in methanol/water extract	Phenolic content mg (GAE)/g in boiling water extract
Thymus (leaves)	2.86 ± 0.21	2.37 ± 0.15*
Melisa (leaves)	20.02 ± 0.47	19.04 ± 0.158
Rosemary (leaves)	2.75 ± 0.08	1.34 ± 0.1*
Mate tea (leaves)	42.97 ±1.51	41.04 ± 0.99
Hyssop (The above part)	5.71 ± 3.05	2.75 ± 0.1*
Damascus rose (Petaliferous)	36.24 ± 0.61	25.14 ± 2.24*
Green tea (leaves)	50.62 ± 0.09	43.04 ± 3.36*
Salvia (leaves)	3.89 ± 0.15	2.57 ± 0.37*
Marjoram (leaves)	4.93 ± 0.07	2.85 ± 0.23*
Peppermint (leaves)	30.72 ± 1.0	20.76 ± 0.99*
Chamomile (Flowers)	2.8 ± 0.13	2.51 ± 0.16
Black tea (leaves)	62.14 ± 0.24	59.57 ± 0.95*

* Significance differences

Some consumer grind teas and herbals teas before consumption or may also buy them as tea bags and herbal tea bags. Therefore, in the next step of this study, samples were ground before extraction to investigate the effect of grounding on the yield of phenolic compounds especially in aqueous extracts. Table.2, shows the results of methanol/water extracts and aqueous extracts after milling the samples. It is noticed that in both extracts, the phenolic

content increased significantly especially in samples of thymus, rosemary, hyssop, salvia marjoram and chamomile, which indicates the importance of decreasing particle size for better extraction (Kasparaviciene, 2017). What is also worth mentioning is that no significance differences between the phenolic content in the methanol/water extracts and the aqueous extracts in the majority of teas and herbal tea infusions. This finding suggests that grinding enhances the ability of boiled water to extract phenolic compounds to a point that it becomes as efficient as methanol/water mixture, and even better in some samples like peppermint (Mariotti-Celis, 2018).

Table.2. Differences in phenolic content of teas and herbal teas according to the extracting solvent when samples are extracted after grounding

Ground sample	phenolic content mg (GAE)/g in methanol/water extract	phenolic content mg (GAE)/g in boiling water extract
Thymus	9.14 ± 0.96	9.43 ± 0.07
Melisa	28.36 ± 1.77	27.38 ± 0.87
Rosemary	7.07 ± 0.01	7.28 ± 0.79
Mate tea	47.32 ± 0.32	46.96 ± 0.22
Hyssop	7.91 ± 0.10	7.84 ± 0.28
Damaskus rose (Petaliferous)	42.48 ± 0.62	42.38 ± 0.51
Green tea	57.18 ± 0.92	46.44 ± 0.18*
Salvia	9.58 ± 0.24	7.61 ± 0.12*
Marjoram	11.48 ± 0.05	10.27 ± 0.01*
Peppermint	28.98 ± 0.87	39.26 ± 0.35
Chamomile	3.7 ± 0.12	3.8 ± 0.15
Black tea	71.14 ± 0.80	69.57 ± 0.95

* Significance differences

Black tea had the highest phenolic content compared to other infusions regardless the conditions of preparation as solvent or grounding. Black tea is the most consumed beverage around the world after water. Its phenolic content is responsible for its antioxidant activity (Camargo, 2016). In the present study, tea was prepared to mimic the consumer's preparation method, thus, the extraction time was set to three minutes. Koch (2017), proved that brewing tea for two minutes was enough to extract most phenolic compounds, as prolonged extraction time did not lead to a significant change in extraction.

It was noticed that grinding petaliferous of Damascus rose increased the phenolic content in both methanol/ water and boiled water extracts however, this increase was more notable in aqueous extracts. Damascus rose is a common flower in Syria, additionally it is found in Europe and Middle East countries, Iran and Turkey. Many positive effects are related to Damascus rose extracts like anti-inflammatory, antioxidant and antibacterial activities (Ulusoy, 2009). Usually, Damaskus rose is used to obtain essential oil, which has antibacterial, antioxidant, and skin wound healing properties (Fahimi, 2016). The present study showed that Damaskus rose infusion is a good source of phenolic compounds. Another study arrived at similar conclusion (Baydar and Gokturk Baydar, 2017). This finding suggest highlights the importance of drinking more of this herbal tea as a good source of phenolic compounds.

Since grinding plant material prior to extraction increased the phenolic content in the infusion and since these teas and herbal teas are consumed only with water, the optimum extraction conditions for phenolic content were selected (ground plant materials and boiling water). Then, the antioxidant activity of these infusions were determined according to FRAP method depending on the selected extraction conditions. Results are shown in table.3.

Table.3. Antioxidant activity of ground teas and herbal teas extracted with boiled water

Boiled water extracts of ground samples	FRAP micromole Fe^{+2}/g
Thymus	103.95 ± 1.32
Melisa	41.80 ± 0.68
Rosemary	101.16 ± 2.23
Mate tea	449.35 ± 4.52
Hyssop	43.95 ± 2.78
Damascus rose	297.9 ± 3.59
Green tea	503.64 ± 5.79
Salvia	33.23 ± 1
Marjoram	76.09 ± 2.92
Peppermint	110.37 ± 5.47
Chamomile	46.09 ± 3.1
Black tea	296.5 ± 6.11

Antioxidant activity according to FRAP ranged between 33.23 to 503.64 micromole Fe^{+2}/g. Green tea possessed the highest antioxidant activity among all other infusions even higher than black tea infusion, which has the highest phenolic content. In addition to phenolic compounds, green tea contains vitamins (ascorbic acid) and minerals (Cr, Mn, Se and Zn) which also increase the antioxidant activity (Atalay and Erge, 2017). Green tea has many health benefits: It has antibiotic, antiviral, anti-inflammatory and anti-carcinogenic properties. These health benefits can be attributed to the phenolic compounds (Lee, 2016).

Mate tea is a popular beverage in South America (Borre, 2010), as well as in Syria. Mate tea had the second highest antioxidant activity even though it ranked third in phenolic content. This can be attributed to a variety of bioactive compounds including phenolic compounds (e.g. phenolic acids like caffeic acid and chlorogenic acid) and methylxanthines like caffeine and theobromine (Gerkea, 2017). Grujic (2012), stated that aqueous extracts of mate has an excellent antioxidant activity due to its high phenolic content. In the present study mate tea had a comparable antioxidant activity to green tea, this has been reported in other studies that mate has comparable or superior antioxidant activity as green tea (Peres, 2013).

It is worth mentioning that, usually, an increase in phenolic content leads to an increase in antioxidant activity. This is true because phenolic compounds account for the majority of antioxidant activity. However, the infusion that had the highest phenolic content did not possess the highest antioxidant activity (black tea infusion). This can be explained by the differences in types of phenolic compounds among these infusions. Different phenolic compounds have different antioxidant activity (Baruah, 2011). So, the positive correlation between phenolic content and antioxidant activity will be more pronounced within the same infusion and not between different teas and herbal teas infusion since they have also different phenolic compounds. This explains that the positive correlation (R^2 = 0.58) between phenolic content and antioxidant activity shown in Figure.1 is not as high as different studies show.

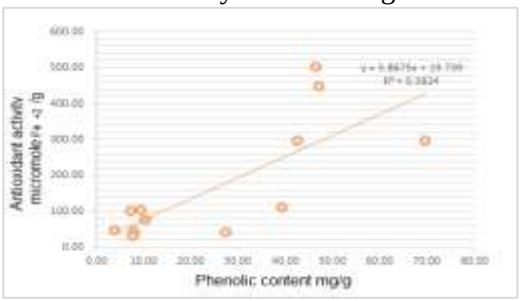

Figure.1. Correlation between phenolic content and antioxidant activity of various teas and herbal teas infusions in boiling water

4. CONCLUSION

Since it is not applicable for consumers to use methanol in herbal infusions, another approach was identified to get the maximum amount of phenolic content using boiled water. Grinding teas and herbal teas prior to preparation reduced the superiority of methanol as an extracting medium, making boiling water almost just as good and, in some cases, even better in extracting phenolic compounds. Black tea, green tea, mate tea and Damaskus rose had the highest phenolic content and the highest antioxidant activity using these conditions of extraction, which suggests that consuming bigger amounts of their infusions could leads to health-promoting effects.

REFERENCES

Alam Z, Roncal J, Pena-Castillo L, Genetic variation associated with healthy traits and environmental conditions in Vaccinium vitis-idaea, BMC Genomics, 19 (4), 2018, 1-13.

Atalay D, Erge H, Determination of some Physical and Chemical Properties of White, Green and Black Teas (Camellia Sinensis), Gida the Journal of Food, 42 (5), 2017, 494-504.

Baruah J.B, Chemistry of Phenolic Compounds: State of the Art, New York: Nova Science Publishers Inc., 2011.

Baydar H, Gokturk Baydar N, Essential Oils and Phenolic Compounds, Antiradical and Antioxidant Activities of Distillation Products, Journal of Agricultural Sciences, 23, 2017, 1-9.

Borre G, Kaiser S, Pavei C, Da Silva F, Bassani V, Ortega G, Comparison of Methylxanthine, Phenolics and Saponin Contents in Leaves, Branches and Unripe Fruits from Ilex Paraguariensis A. St Hil (Mate), Journal of Liquid Chromatography and Related Technologies, 33, 2010, 362–374.

Camargo L, Pedroso L, Vendrame S, Mainardes R, Khalil N, Antioxidant and antifungal activities of Camellia sinensis (L.) Kuntze leaves obtained by different forms of production, Brazillian Journal of Microbiology, 76 (2), 2016, 428-434.

Fahimi S, Mortazavi S, Abdollahi M, Hajimehdipoor H, Formulation of a Traditionally used Polyherbal Product For Burn Healing And Hptlc Fingerprinting Of Its Phenolic Contents, Iranian Journal Of Pharmaceutical Research, 15 (1), 2016, 95-105.

Gerke I, Hamerski F, Scheer A, Siluab V, Clarification of Crude Extract of Yerba Mate (Ilex Paraguariensis) By Membrane Processes, Analysis of Fouling and Loss of Bioactive Compounds, Food and Bioproducts Processing, 10 (2), 2017, 204-212.

Ghasemzadeh A, Karbalaii M, Jaafar H, Rahmat A, Phytochemical constituents, antioxidant activity and antiproliferative properties of black, red and brown rice bran, Chemistry Central Journal, 12 (17), 2018, 1-13.

Grujic N, Lepojevic Z, Srdjenovic B, Vladic J, Sudji J, Effects of Different Extraction Methods and Conditions on The Phenolic Composition of Mate Tea Extracts, Molecules, 17, 2012, 2518-2528.

Kasparaviciene G, Jurgelevic J, Kalveniene Z, Velziene S, Savickas A, Kazlauskiene D, Evaluation of extraction factors influence on total phenolic content and antioxidant activity of Melissa officinalis L, leaves extracts, Chemija, 28 (1), 2017, 58–63.

Koch W, Kukula-Koch W, Głowniak K, Catechin Composition and Antioxidant Activity of Black Teas in Relation to Brewing Time, Journal of AOAC International, 100 (6), 2017, 1694-1699.

Koutelidakis A, Andritsos N, Kabolis D, Kapsokefalou M, Drosinos E, Komaitis M, Antioxidant and Antimicrobial Properties of Tea and Aromatic Plant Extracts Against Bacterial Foodborne Pathogens, A Comparative Evaluation, Current Topics in Nutraceutical Research, 14 (2), 2016, 133-142.

Lee B, Nam T, Park N, Chun O, Koo S, Kim D, Estimated daily intake of phenolics and antioxidants from green tea consumption in the Korean diet, International Journal of Food Sciences and Nutrition, 67 (3), 2016, 344–352.

Madeira J, Macedo G, Simultaneous Extraction and Biotransformation Process to Obtain High Bioactivity Phenolic Compounds from Brazilian Citrus Residues, Biotechnol. Prog., 31 (5), 2015, 1273-1279.

Mariotti-Celis M, Martinez-Cifuentes M, Huaman-Castilla N, Vargas-González M, Pedreschi F, Perez-Correa J, The Antioxidant and Safety Properties of Spent Coffee Ground Extracts Impacted by the Combined Hot Pressurized Liquid Extraction-Resin Purification Process, Molecules, 23 (21), 2018, 1-11.

Mtoloa M, Gerranob A, Me J, Effect of simulated gastrointestinal digestion on the phenolic compound content and *in vitro* antioxidant capacity of processed Cowpea (V.unguiculata) cultivars, Journal of Food, 15 (3), 2017, 391–399.

Nick K, Anastasia M, Antonia C, Maria S.I, Nikolaos K.A, Retention and distribution of natural antioxidants (a-tocopherol, polyphenols and terpenic acids) after shallow frying of vegetables in virgin olive oil, LWT-Food Science and Technology, 2007, 40.

Orsine J.V, Novaes M, Asquieri E, Canete R, Determination of Chemical Antioxidants and Phenolic Compounds in the Brazilian Mushroom Agaricus sylvaticus, West Indian Medical Journal, 63 (2), 2014, 142.

Park H, Sung J, Kim B, Ha S, Kim Y, Effect of Degree of Rice Milling on Antioxidant Components and Capacities, Italian Journal of Food Science, 30, 2018, 50-60.

Pelle F, Compagnone D, Nanomaterial-Based Sensing and Biosensing of Phenolic Compounds and Related Antioxidant Capacity in Food, Sensors, 18 (462), 2018, 1-32.

Peres R, Tonin F, Tavares M, Rodriguez-Amaya D, HPLC-DAD-ESI/MS Identification and Quantification of Phenolic Compounds In Ilex Paraguariensis Beverages and an-Line Evaluation of Individual Antioxidant Activity, Molecules, 18, 2013, 3859-3871.

Pramai P, Abdul Hamid N, Mediani A, Maulidiani M, Abas F, Jiamyangyuen S, Metabolite profiling, antioxidant, and a-glucosidase inhibitory activities of germinated rice, nuclear-magnetic-resonance-based metabolomics study, Journal of food and drug analysis, 26, 2018, 47-57.

Rafehi H, Ververis K, Karagiannis T, Mechanisms of Action of Phenolic Compounds in Olive, Journal of Dietary Supplements, 9 (2), 2012, 96–109.

Ulusoy S, Bos G, Tınaz G, Sec¸ Ilmis, Canbay H, Tocopherol, Carotene, Phenolic Contents and Antibacterial Properties of Rose Essential Oil, Hydrosol and Absolute, Current Microbiol, 59, 2009, 554–558.

Vermerris W, Nicholson R, Phenolic compounds biochemistry, Springer, 2006.