Short communication

The Effect of Tea Additives and Decoction Time on the Antioxidant Activity of Traditional Libyan Tea

ABSTRACT

Aims: This study is intended to determine the effect of tea additives and decoction time on the antioxidant activity of red tea.
Study Design: Department of Pharmacognosy, Faculty of Pharmacy, University of Tripoli
Methodology: Tea additives that have been selected in this study were geranium, spearmint, peanut, and table sugar. The extraction method (preparation of aqueous individual extract and poly-extract combination) was applied to mimic the Libyan folk method of tea preparation. Using the decoction method, each individual red tea was boiled for 10, 20, and 30 mins to study the effect of decoction time. An in-vitro analysis of antioxidant activity was applied using quantitative DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity.
Results: Compared to the red tea, each extract has exhibited a significant increase in antioxidant activity ($P < 0.05$) except spearmint and table sugar. Superior antioxidant activity was observed in the individual extracts of geranium and peanuts. In this work, the decoction time (20, 30 min) showed a decrease in the antioxidant activity in comparison to the decoction time (10 min).
Conclusion: The poly-extract combinations were statistically significantly superior in antioxidant activity than the individual red tea. Moreover, decoction time might influence the antioxidant activity of red tea.

Keywords: Tea; Libyan culture; Aromatic herbs; Nuts; DPPH.

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1. INTRODUCTION

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Tea is the chief attendant on many occasions in world countries. In Libya, tea is a daily drink at social events. It is believed that Libyans drink a lot of tea compared to other countries [1].

Libyan tea can be a heavily red or green tea, sweet like a syrup, and aromatic herbs refreshing hot drink. Traditional Libyan tea is prepared by steaming water in a teapot and adding a handful of green or red tea leaves. The mixture is left to boil for a long time. Once the tea is done, it is first poured into the steal mug, and the content is poured into the second steal mug and then returned into the first mug by raising the arm above the head. The process of pouring is continued 20-30 times until the froth or foam that Libyans call *raghwah* or *kashkushah* is produced. This process is considered a tea-time ritual in Libya. Tea is served after meals in rounds, and roasted peanuts or almonds are served at the end of the third round [1, 2].

Tea (*Camellia sinensis* L.) belongs to the family Theaceae. It is the most consumed drink after water [3]. According to the drying (fermentation) process, the produced tea can be many types like red, green, black, and white [4]. Tea inc

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variety of phytochemicals mainly polyphenols (catechins and tannins), purine alkaloids (caffeine), vitamins, and minerals. Depending on the fermentation process, catechins are main polyphenols of green tea, whereas red and black tea contains a high level of tannins [5].

Oxidative stress is a physiological trouble resulting from an imbalance between the pro-oxidants (free radicals or reactive oxygen species ROS) and the antioxidants, which neutralize the produced free radicals. This harmful process causes damage to cell components e.g. membrane, proteins, lipids, and DNA, and further, it leads to regressive diseases [6]. Polyphenols are powerful antioxidants, which play an integral role in the antioxidant activity of *C. sinensis* [7]. As a result of the antioxidant activity of tea, tea has various pharmacological activities such as anti-Alzheimer activity [8], anti-Parkinson activity [9], anti-aging activity [10], and prophylaxis against cardiovascular disease [11].
Each county has its special tea additives and distinctive preparation process. Libyan tea is flavored usually with aromatic herbs like peppermint, spearmint, geranium, rosemary, lemon blossom, and cinnamon. This study is intended to determine the effect of tea additives and decoction time on the antioxidant activity of red tea.

2. MATERIAL AND METHODS

2.1. Sample collection

Red tea (Zahra), roasted peanuts, and table sugar (Aljaied) were purchased from the Tripoli market. Geranium and spearmint were collected in April 2021 from Tripoli/ Libya. The plants have been identified by *Prof*. Mohamed Nuri Abuhadra, a plant taxonomist at the Department of Botany, Faculty of Science, University of Tripoli. Geranium was identified as *Pelargonium radula* Cav., and spearmint was identified as *Mentha spicata* L. The identified plants were washed by distilled water and then dried in shade for 1-2 weeks. After the completion of drying, the plants were grinded using an electric grinder and kept in sealed polyethylene bags in a refrigerator at 4^{o}C.

2.2. Extraction (*preparation of individual extract and poly-extract combination*)

The extraction method was applied to mimic the Libyan folk method of tea preparation. Using the decoction method, the individual red tea (0.5g in 25 ml distilled water) was boiled for 10, 20, and 30 mins to study the effect of decoction time. The individual extracts (geranium, spearmint, and peanut) were prepared by infusion method (0.5g in 25 ml distilled water). The poly-extract combinations were prepared by the same ratio of each component. For example, the poly-extract combination of (red tea: geranium: peanut 1:1:1) was prepared by the addition of the hot individual red tea (10 mins) to 0.5g of geranium and 0.5 g of peanuts. All the previous extracts were filtrated, and the filtrates were kept for the DPPH assay.

2.3. *In-vitro* analysis of antioxidant activity

The quantitative DPPH scavenging activity was determined in accordance with (Kumarasamy *et al*., 2007; Kumar *et al*., 2014) [12, 13] protocols with little modifications.
The methanolic solution of DPPH with a concentration (of 80µg/ml) was prepared. 1ml of each diluted solution was mixed with 1ml of DPPH solution and left for 30mins in a dark place for complete reaction. After 30mins the UV absorbance was recorded by spectrophotometer at 517 nm. The mixture of 1ml DPPH and 1ml distilled water was utilized as a control. Distilled water was employed to zero the spectrophotometer. The experiment for each extract was measured in triplicate.
The results were expressed as a percentage of radical scavenging activity and were calculated by the following formula:

$$\% \text{ Inhibition of DPPH} = \frac{\text{Absorbance (control) - Absorbance (sample)}}{\text{Absorbance (control)}} \text{ X100}$$

2.4. Statistical analysis

The results were expressed as mean ± S.D with n= 3 using (IBM SPSS statistics 20). The data were statistically analyzed by one-way ANOVA with Tukey's post hoc test to determine which means were different. The *P* values less than 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

The results of the antioxidant activity of individual extract and poly-extract combinations are presented in Table 1 and Figure 1. Among the individual extracts, geranium showed the highest total antioxidant capacity followed by peanut, spearmint, and red tea. While in poly-extract combinations, red tea: geranium (1:1) showed the highest % Inhibition of DPPH followed by red tea: spearmint (1:1), red tea: geranium: peanut (1:1:1) and red tea: table sugar (1:2) combination.

Table 1: The antioxidant activity of individual extract and poly-extract combinations

Individual extract	% Inhibition of DPPH
Red tea	22.04 ±2.17[a]
Spearmint	33.28 ±2.33[a]
Geranium	78.78 ±2.36[b]
Peanut	75.91 ±1.85[b]
Poly-extract combinations	
Red tea : spearmint (1:1)	74.48 ±5.15[b]
Red tea : geranium (1:1)	89.96 ±7.38[b]
Red tea : geranium : peanut (1:1:1)	65.80 ±11.65[b]
Red tea : table sugar (1:2)	31.16 ±18.42[a]

Values are mean ± S.D (n=3).

Values with different superscript letters are significantly different (P <0.05) by Tukey's post hoc test.

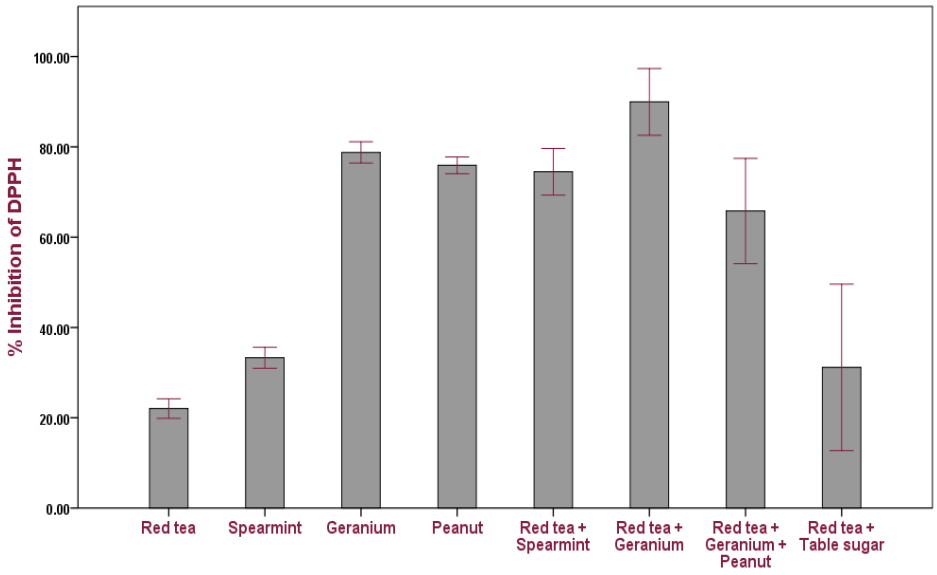

Figure 1: The antioxidant activity of individual extract and poly-extract combinations. Error bars represent the S.D (n=3).

The effect of decoction time effect on the percentage inhibition of DPPH was studied by boiling samples individuals and combinations for 10-, 20-, and 30-mins Table 2 and Figure 2 as shown below, 10 min decoction time had the highest percentage of inhibition of DPPH.

Table 2: The effect of decoction time on the antioxidant activity of red tea.

Decoction time (min)	% Inhibition of DPPH
10	22.04 ±2.17[a]

20	13.07 ±5.21[a]
30	15.34 ±0.48[a]

Values are mean ± S.D (n=3).

Values with different superscript letters are significantly different (P <0.05) by Tukey's post hoc test.

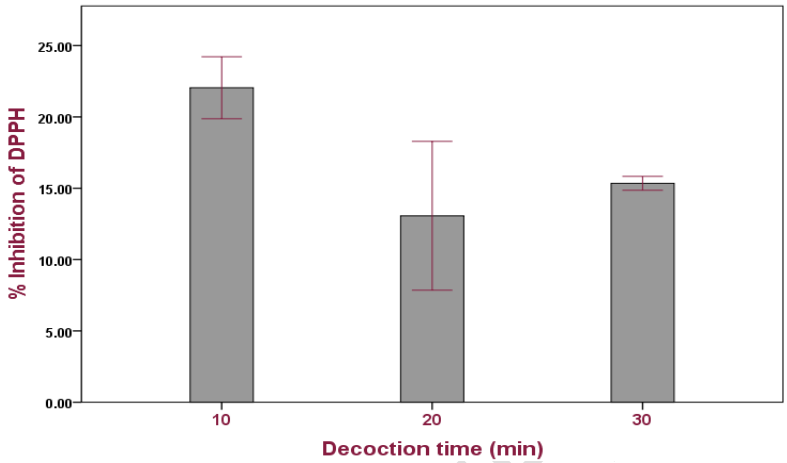

Figure 2: The effect of decoction time on the antioxidant activity of red tea. Error bars represent the S.D (n=3).

In Libyan culture, it is rare to drink tea individually. Additives like nuts and aromatic herbs are more desirable to be added to the cup of tea. In this study, the antioxidant activity is expressed as the percentage inhibition of DPPH. In comparison to the red tea, each individual extract has exhibited a significant increase in the antioxidant activity (P < 0.05) except spearmint and table sugar.

In this work, superior antioxidant activity was observed in the individual extracts of geranium and peanuts. The high phenolic content such as (gallic acid and its derivatives and flavonoids in geranium [14]) and (the flavonoids, coumarins, and anthocyanins in peanuts [15]), is the main responsible for their superior antioxidant activity [16, 17] compared to red tea alone. For poly-extract combinations, either the double or the triple combinations have revealed a significant enhancement in the percentage inhibition of DPPH (P <0.05) than the individual red tea. The higher percentage inhibition of DPPH for the combination (red tea: spearmint) in relation to the individual extracts of red tea and spearmint, this synergistic effect in the antioxidant activity might be due to the combination of the phenolic compounds in the red tea (catechins) and in the spearmint (carvone) [18, 19]. In the poly-extract combinations, the higher activity against oxidation was a result of the combination of the phenolic compound in each herb [20, 21]. In this study, the poly-extract combinations were statistically significantly superior in antioxidant activity than the individual red tea.

Libyan tea is syrupy. Table sugar (sucrose) is a very important additive to Libyan tea. Individual sucrose has not an antioxidant activity, but in accordance with (Peinado *et al*., 2010) [22] study, sugars can increase the stability of phenolic compounds and then increase the antioxidant activity of beverages. The results of this study exhibited a little increase in the percentage inhibition of DPPH (P >0.05) of (red tea: table sugar) in relative to the individual red tea.

Although, the difference in the antioxidant activity among the time of decoction (10, 20, 30min) was not statistically significant (P >0.05), the decoction time (20, 30 min) showed a decrease in the antioxidant activity in comparison to the decoction time (10 min). The decoction is the preparation method of Libyan tea; however, the time of decoction is subject to folk use variations. (Abdul Rahim *et al*., 2010) [23] have confirmed that the stability of phenolic compounds has been affected by long-time boiling; therefore, the antioxidant activity will decrease as the boiling time increase. Consequently, the decoction time might influence the antioxidant activity of red tea.

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4. CONCLUSION

From this work, the poly-extract combinations were statistically significantly superior in antioxidant activity than the individual red tea. Moreover, the decoction time might influence the antioxidant activity of red tea. It is recommended to drink tea with some additives like aromatic herbs rather than individual tea. These additives improve flavor and antioxidant activity. A long time of decoction might be destroying the phenolic compounds; therefore, it is recommended to boil the tea for a short time or use the infusion method as an alternative for tea preparation.

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