

## The Addition of Herbal Additives Influences the Antioxidant Activity of Traditional Arabic Coffee

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**Abstract:** Traditional Arabic coffee is one of the most consumed beverages in the gulf region. In Saudi Arabia, people prefer to drink instant Arabic coffee with several herbal additives such as ginger, cloves, cardamom or a mixture of all of them. Therefore, this study was conducted to assess the effect of the most consumed additives including cloves, ginger and cardamom on the antioxidant properties of the Arabic coffee. In this research, different methods were performed to measure the antioxidant activity such as radical scavenging activity, metal ion chelating and reducing power activity. The results of the current study showed that the total polyphenol content, scavenging activity and the Fe<sup>2+</sup> chelating activity were significantly increased ( $p < 0.05-0.001$ ) in almost all Arabic coffee samples with herbal additives when compared to the plain Arabic coffee sample. On the other hand, total flavonoid contents were significantly decreased ( $p < 0.05$ ) only in Arabic coffee samples supplemented with either cardamom or ginger. In conclusion, herbal additives influence the antioxidant activity of Arabic coffee. This might be due to the presence of different phenolic compounds in ginger, cloves and cardamom.

**Key words:** Arabic coffee • Antioxidant activity • Ginger • Cloves • Cardamom

### INTRODUCTION

Coffee is one of the most consumed beverages worldwide after water [1]. It is a strong antioxidant because of the several phenolic and flavonoid compounds that are present in high concentrations in it [2, 3]. According to the International Coffee Organization (ICO), 1.4 billion cups of coffee are consumed a day worldwide. Coffee consumption has risen sharply in Saudi Arabia whereby 18, 000 tons at a total cost of \$54 million are imported [4]. In Saudi Arabia, people commonly use Coffea Arabica coffee from the Harar area of Ethiopia, commonly referred to as Harari. The beans from this type of coffee are yellow-green or golden-green in color. Arabic coffee is the general name that refers to the main way coffee is prepared in many Arab countries in the gulf region. In Saudi Arabia, Arabic coffee is mostly consumed with several herbal additives such as ginger, cloves, cardamom or a mixture of all of them. Previous researches have studied the pharmacological and medicinal properties of these herbs [5, 6]. Therefore, the antioxidants present in these herbs can be used to treat or

prevent the formation of complex diseases such stroke, diabetes, atherosclerosis, Alzheimer's and cancer [7].

Ginger is a member of the Zingiberaceae family and it has been used for over than 2000 years as food spices [8]. It has high concentrations of phenolic compounds such as beta-carotene, ascorbic acid, terpenoids, alkaloids and polyphenols such as flavonoids, flavones glycosides and rutin [8, 9]. On the other hand, cloves (*Syzygium aromaticum* (L.), *Eugenia aromaticum* (L.) or *Eugenia caryophyllata*), are considered as an important vegetal source of phenolic compounds such as flavonoids, hydroxycinnamic acid, hydroxyphenyl propene and hydroxybenzoic acid [10]. Cloves are strong antioxidant medicinal plants, which act as antimicrobial agents [11]. The queen of all spices is cardamom, also called (*Elettaria Cardamomum Maton*), is used by people all over the world as a public food additive, spices and flavoring agent [12]. It is an excellent source of natural antioxidants that can be used for indigestion and to stimulate the appetite in people with anorexia [6]. However, the seeds of the cardamom are also used in ayurvedic medicine for colds, bronchitis asthma, cough and indigestion [13].

From this point of view, the aim of this study was to measure the effect of the three major herbal additives, cloves, ginger and cardamom on the antioxidant properties of the Arabic coffee which is mostly consumed by Saudis.

## MATERIALS AND METHODS

**Coffee Preparation:** Harari coffee, cardamom, cloves and ginger were obtained from local organic market, Jeddah, Saudi Arabia. Coffee samples were prepared according to the traditional ways that are used by Saudis. The dried ginger, cloves and cardamom were crushed before adding them to coffee. The used coffee brand was Harari coffee, the most common brand based on a survey performed on 900 participants from Jeddah, Western region of Saudi Arabia. Coffee samples were prepared by mixing 30 mg of pure Arabic coffee with one L of boiling water, the mixture was heated for 15 minutes on a heater. After that, 2 mg/ml of each additive, cardamom, cloves, ginger or a mixture of equal concentrations of the three herbs, was added to the coffee and was left for 5 minutes on heater.

**Determination of Total Polyphenol and Flavonoid Contents:** Folin-Ciocalteu and aluminum chloride colorimetric assays were used to determine the total polyphenol and total flavonoid contents in the samples according to previous protocols [14, 15]. Each experiment was performed for three times and the final contents of polyphenol and flavonoid in all coffee samples were represented as gallic acid equivalent, for polyphenol contents, or as chatecine quivalent, for flavonoid contents.

**Determination of Scavenging Activity:** The ability of coffee samples to scavenge hydrogen peroxide and DPPH was measured following the procedure of Gülçin *et al.* [16] and Ohnishi *et al.* [17], respectively. The final product concentration from each assay was measured on a spectrophotometer at either 230 nm (for H<sub>2</sub>O<sub>2</sub> radical scavenging activity) or 517 nm (for DPPH-free radical scavenging activity). The scavenging activity percentage was calculated by following the equation:

$\% \text{ inhibition} = [(AB-AA)/AB] \times 100$ , where (AB) = absorbance of blank sample and (AA) = absorbance of sample.

**Determination of Ferrous Ion Chelating Activity:** The ferrous ion chelating activity was determined according to the protocol of Konèiæ and his collegeous [18] with some modification. First, 5µl of sample was added to 50µl of 2 mM ferrous chloride (Sigma-Aldrich Chemical Co., Poole, UK) then 1.5 ml distilled water was added and then the mixture was incubated for 30 seconds. After that, 100µl of (5 mM) ferrozine (Sigma-Aldrich Chemical Co., Poole, UK) was added to the mixture and was incubated for 10 minutes at room temperature.

The final product was assessed by spectrophotometer at 562 nm using this equation: Chelating effect % = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100

**Determination of Reducing Power Activity:** The activity of samples to act as a reducing agent was determined according to the method of Karawita *et al.* [19] with some modification. First, 1 ml of each sample was added to 2.5 ml of phosphate buffer pH 6.6 (Fisher Scientific, Loughborough, UK) and 2.5 ml of (1%) potassium ferricyanide (Sigma-Aldrich Chemical Co., Poole, UK) were mixed then incubated for 30 minutes in water bath at 50°C. After that, 2.5 ml of (10%) Trichloroacetic acid (TCA) (BDH, Poole, UK) was added to the previous mixture and centrifuged at 6000 rpm for 10 minutes. Then 2.5 ml of deionized water and 0.5 ml of (1%) ferric chloride (BDH, Poole, UK) were added to 2.5 ml of the supernatant. The final product absorbance was detected by spectrophotometer at 700 nm.

**Statistical Analysis:** All data were analyzed by GraphPad Prism version 7. The differences between groups were calculated by one-way analysis of variance (ANOVA) followed by Bonferroni's test correction. The results were represented as mean ± SD and the cut-off level of statistical significance was p<0.05.

## RESULTS

**Total Polyphenol and Flavonoid Contents:** Total polyphenol content was estimated by Folin-Ciocalteu's method and Gallic acid was used as a standard. The total polyphenol content for pure Arabic coffee, Arabic coffee with cardamom, Arabic coffee with ginger, Arabic coffee with cloves and Arabic coffee mixed with cardamom, ginger and cloves were 564, 513, 654, 611 and 553 µg of Gallic acid, respectively (Fig. 1A). Regarding the total

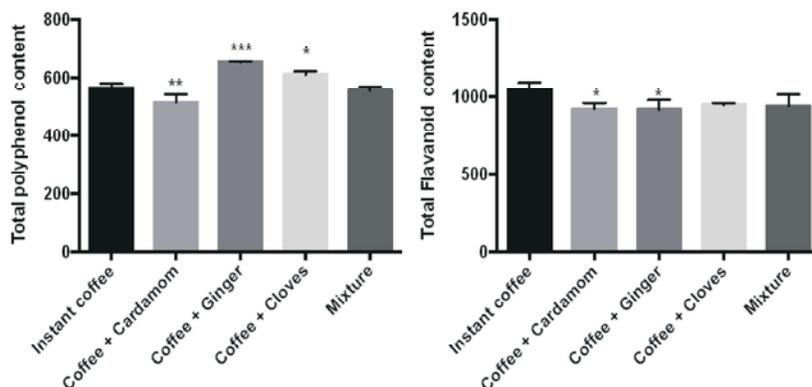


Fig. 1: Total polyphenol and flavanoid contents: (A) The total polyphenol content of Arabic coffee with and without herbal additives; (B) Total flavanoid contents of Arabic coffee with and without herbal additives. For each assay, three independent experiments were performed (n=3) and data were represented as (mean±SD). The differences were calculated with one-way ANOVA and the errors were corrected with Bonferroni's test (\* $p$ <0.05, \*\* $p$ =0.01, \*\*\* $p$ =0.001).

flavanoid content, aluminum chloride method was performed and quersetin was used as a standard. Fig. 1B shows the total flavanoid contents of pure Arabic coffee, Arabic coffee with cardamom, Arabic coffee with ginger, Arabic coffee with clove and Arabic coffee mixed with cardamom, ginger and clove were 1046, 923, 919, 947 and 938  $\mu$ g of chatecin, respectively.

**Scavenging Activity:** The scavenging activity for coffee samples with and without herbal additives to remove hydrogen peroxide and DPPH were determined in this study. The percent of inhibition activity of  $H_2O_2$  for pure Arabic coffee, Arabic coffee with cardamom, Arabic coffee with ginger, Arabic coffee with clove and Arabic coffee mixed with cardamom, ginger and clove were

32.3%±5, 75.73%±2.5, 44.1%±5, 94.17%±4 and 80.83%±9, respectively. Fig. 2A showed that Arabic coffee with herbal additives, especially coffee with cloves, had higher significant scavenging activity than pure Arabic coffee only (\*\* $p$ =0.01).

Regarding DPPH-free radical scavenging activity, the percent of inhibition caused by pure Arabic coffee, Arabic coffee with cardamom, Arabic coffee with ginger, Arabic coffee with clove and Arabic coffee mixed with cardamom, ginger and clove were 46.17%±4.3, 49.88%±4.4, 45.67%±4.1, 32.75%±3.8 and 38.12%±1.2, respectively. In this experiment, a significant reduction in the scavenging activity of Arabic coffee with clove was observed (\* $p$ <0.05) compared to the other samples (Fig. 2B).

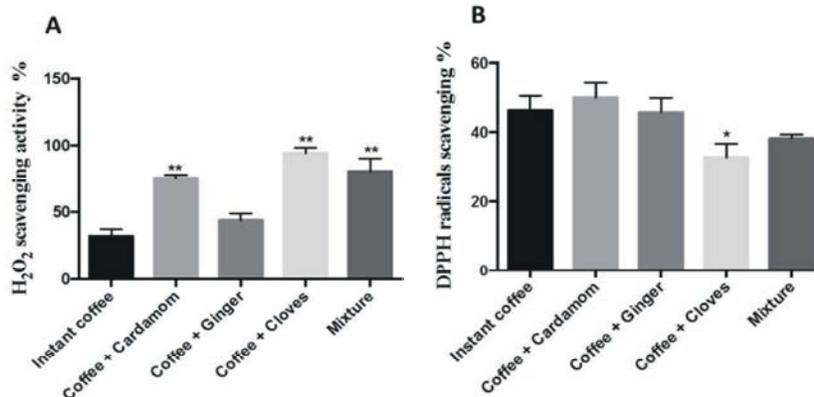


Fig. 2: Scavenging activity for Arabic coffee with and without herbal additives: (A) The  $H_2O_2$  scavenging activity assay for Arabic coffee with and without herbal additives; (B) The DPPH-free radical scavenging activity assay for Arabic coffee with and without herbal additives. For each assay, three independent experiments were performed (n=3) and data were represented as (mean±SD). The differences were calculated with one-way ANOVA and the errors were corrected with Bonferroni's test (\* $p$ <0.05, \*\* $p$ =0.01).

**Ferrous Ion Chelating Activity:** This assay measures the ability of antioxidant compound to inhibit the formation of ferrous-ferrozine complex. Chelating ability can be an indicator of an antioxidant activity [20]. The ferrous chelating activity of pure Arabic coffee, Arabic coffee with cardamom, Arabic coffee with ginger, Arabic coffee with clove and Arabic coffee mixed with cardamom, ginger and clove were 44.81%±2.4, 81.78%±7.7, 73.06%±7.8, 62.1%±5.2 and 94.11%±0.79, respectively. As shown in Fig. 3, mixing the Arabic coffee with any of the three herbals increase the ferrous-chelating activity more significantly compared with the pure Arabic coffee only (\*\**p*=0.001).

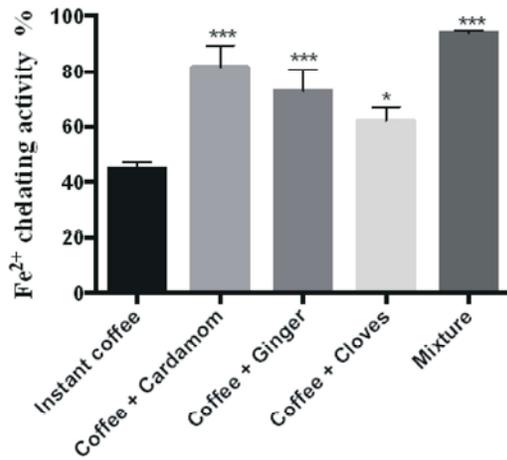


Fig. 3: The Fe<sup>2+</sup> chelation activity for Arabic coffee with and without herbal additives. Data are obtained from three independent experiments (n=3) and were represented as (mean±SD). The differences were calculated with one-way ANOVA and the errors were corrected with Bonferroni's test (\**p*<0.05, \*\*\**p*=0.001)

**Reducing Power Assay:** This experiment is the most widely used assay to determine the total antioxidant capacity. This method measures the ability of antioxidant compound to reduce ferric ion in the ferric chloride (Fe<sup>+3</sup>) into ferrous (Fe<sup>+2</sup>) ions by donating electron. The results in Fig. 4 represent the absorbance of the reducing power activity at 700 nm for all samples. The ferric-reducing power activity for pure Arabic coffee, Arabic coffee with cardamom, Arabic coffee with ginger, Arabic coffee with clove and Arabic coffee mixed with cardamom, ginger and clove were 0.5513, 0.4558, 0.714, 0.658 and 0.6938, respectively. As shown in Fig. 4, coffee mixed with ginger or mixed with the three herbals has significant increase in reducing power activity (\*\**p*=0.01).

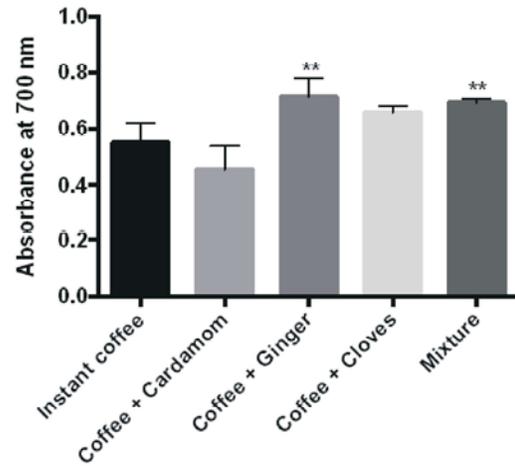


Fig. 4: The reducing power activity for Arabic coffee with and without herbal additives. Data are obtained from three independent experiments (n=3) and were represented as (mean±SD). The differences were calculated with one-way ANOVA and the errors were corrected with Bonferroni's test (\*\**p*=0.01)

## DISCUSSION

Polyphenols are the most abundant antioxidants in our diet. They are present naturally in herbs, seeds and fruits [7, 21]. The main groups of polyphenols are flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans [21]. Consequently, to estimate the antioxidant activity, it is important to determine the amount of phenolic contents and flavonoids. The results of the current study showed that the Arabic coffee cloves or ginger has the highest total polyphenol content among all samples. These results may be referred to the high antioxidant activity of bioactive components that are mainly found in ginger such [6]-gingerol and [6]-shogaol [22] and to the presence of volatiles and non-volatiles constituents such tannins, triterpenes, sterols and flavonoids that are present in cloves [10, 23]. In contrast to our results, a study conducted by Denre [24], found that the total polyphenol contents in cloves and cardamom are more than ginger extract. Regarding the flavonoids content, our results showed that pure Arabic coffee and Arabic coffee with cloves have higher flavonoid contents than Arabic coffee with ginger or cardamom. The reason for the high proportion of total flavonoid contents in pure Arabic coffee can be explained by the presence of high percentage of chlorogenic acid in coffee [24].

In addition, the scavenging activity, results in this study showed that Arabic coffee with cloves has high ability to scavenge free radicals such as ( $H_2O_2$  and DPPH). A study conducted by Khalaf and his team [7], indicated that the scavenging activity of DPPH free radicals by cloves was more than ginger and cardamom extract. The results obtained from total flavonoid contents assay are compatible with the results obtained from hydrogen peroxide scavenging assay (Arabic coffee with cloves had high scavenging activity hydrogen peroxide). Many studies showed that flavonoids can scavenge molecular species such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical ( $\cdot OH$ ), singlet oxygen ( $^1O_2$ ), or peroxy radical [25].

Ferrous ion chelating activity assay can be used as an indicator of the antioxidant activity of a compound [18]. The results of iron chelating activity assay showed that Arabic coffee with either cardamom or ginger as well as Arabic coffee with cardamom, ginger and cloves have higher metal chelating activity compared to Arabic coffee alone. This might be due to the existence of some compounds that have chelating abilities such as  $\alpha$ -terpineol in cardamom, gingerol and shogaol in ginger and eugenol in cloves [26-29].

### CONCLUSIONS

In conclusion, the results revealed that Arabic coffee samples with herbal additives have a slightly higher content of polyphenols compared to the plain Arabic coffee. This might be due to the presence of additional phenolic compound in ginger, cloves and cardamom. However, the antioxidant activity was improved when Arabic coffee was mixed with these herbs. Further researches are needed to determine the active components of each herbal additive used in this study, in order to reveal the mechanisms by which these herbs can influence the antioxidant activity of coffee.

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**Author's Contributions:** H.A. designed the experiments, analyzed the data and revised the manuscript; E.A. performed the experiments and wrote the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

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