



## **Estimation of Total Phenolic Contents and *In vitro* Antioxidant and Antimicrobial Activities of the Most Common Coffee Brews Available in the Local Markets of the Northern Region of Saudi Arabia**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author OMN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MHES and AENAK managed the analyses of the study. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Coffee is the most preferred morning beverage throughout the world due to its pleasant flavor and stimulating properties. It contains a multipart combination of chemicals constituents, which associated with health benefits. In the Kingdom of Saudi Arabia there are many types of coffee in the local markets with different characteristics.

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**Aims:** The present study was designed to identify the total phenolic contents, antioxidant properties and antimicrobial activities for three of the most commonly consumed coffee brews; Intenso (Arabica), Reebass (Turkish) and Lavazza (Brazilian) purchased from some local markets at the Northern region in Kingdom of Saudi Arabia.

**Study Design:** It was an *in-vitro* study.

**Methodology:** The purchased coffee types were extracted using the boiling water method. Determination of total phenolic content of the obtained extracts was carried out using the Folin-Ciocalteu method. Antioxidant activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Measurement of antimicrobial activity was determined by disk diffusion method against number of microbial test strains. The obtained data were statistically analyzed and results with  $p < 0.05$  were considered statistically significant.

**Results:** The obtained results showed that, the estimated phenolic contents were arranged as  $745.50 \pm 10.5$ ,  $668.86 \pm 11.2$  &  $651.25 \pm 13.0$  mg/g of gallic acid/1 g of coffee for Lavazza (Brazilian), Reebass (Turkish) & Intenso (Arabica) respectively. DPPH radical scavenging activity was higher 78.83% for Turkish coffee than Arabica 71.59% and Brazilian 65.90% types. Regarding antimicrobial activity; Arabica coffee extract was the highest antimicrobial activity compared to the other two extracts of both Turkish and Brazilian types where the mean diameter of inhibition zone for three coffee types were ranged from  $11.0 \pm 0.15$  to  $16.0 \pm 0.40$ ;  $9.0 \pm 0.25$  to  $11.0 \pm 0.10$  and  $9.5 \pm 0.10$  to  $13.0 \pm 0.50$  mm for Arabica, Turkish and Brazilian coffee extracts respectively.

**Conclusion:** The present study concludes that coffee types under investigation showed high phenolic content and strong antioxidant activity as well as promising antimicrobial activity.

**Keywords:** Coffee brews; polyphenol; antioxidants; antibacterial; Saudi Arabia.

## 1. INTRODUCTION

Coffee (*Coffea L.*) is the world's beloved drink that is the most regularly consumed caffeine-containing beverage next to water and tea. It contains a multipart combination of chemicals constituents, which associated with health benefits, most consumers, begin their day with a minimum a cup of coffee after eating food, and end their workday with coffee. It is considered as a significant part of modern daily life because it has an alerting outcome on the human brain [1]. Interestingly, many recent studies have shown that coffee consumption has potentially inverse correlations with chronic diseases such as cancer, cardiovascular disease, obesity, and diabetes [2,3,4,5].

Coffee coming from the roasted seeds of several species of the genus *Coffea L.*, the popularity of coffee is due to its aroma, flavor and caffeine content which plays a major role in its popularity. Coffee is a complex mixture of more than a thousand different chemicals, many of which are reported to be biologically active compounds [6,7].

Coffee contains diverse phenolic and non-phenolic compounds such as caffeine, chlorogenic acid, trigonelline, kahweol, and cafestol. These constituents of coffee are known

to have similar effects to antioxidants in animals, which might be a reason for the disease-prevention benefit of coffee [8,9,10,11].

Polyphenols are very complex group of molecules present in plants [12,13]. Several compounds can be identified under the term "polyphenols"; these include mainly phenolic and flavonoids acids [14].

Beneficial health effects of coffee are usually attributed to its high antioxidant activity (ability to inhibit the process of oxidation). Many publications provide comparison of the antioxidant activity in such popular beverages as coffee, tea, and cocoa [15,16,17].

Interestingly, the compound, such as chlorogenic acid and polyphenols, which contributed to the antioxidant activity in coffee, is geographically related [18]. The coffee fruit was found to have more chlorogenic acids (CGA) in Arabica coffee fruit planted in Mexico and India compared to the coffee fruit grew in China. In addition, evidence indicates that extraction procedures could affect the antioxidants contents in coffee fruit as well as the caffeine content [19]. It has been shown that the antioxidant activity was high in coffee fruit extract with low caffeine concentration in comparison with coffee fruit powder.

Antimicrobial agents have been extensively used to control microbial contamination effectively, although the broad use of chemicals has led to many ecological and medical difficulties due to continuing toxicity, hormonal imbalance, teratogenicity, carcinogenicity, etc. Indeed, the safety aspects of chemical preservatives are still controversial and, consequently, the increasing need in the use of natural antimicrobial substances for managing pathogens and/or toxins. Coffee beverages intended for use as antioxidants may also present biological effects on bacteria or fungi, including antimicrobial activity against a range of Gram-positive and Gram-negative bacteria [20]. There are many types of coffee (local and exotic) commonly consumed in Saudi Arabia. Most of these types are traded and consumed without knowing their positive health benefits. So, comparing these types with its chemical and biological activities is greatly required. Thus, the purpose of our research was to compare the total phenolic contents, antioxidant capacities and antimicrobial activities of three of the most popular coffee types purchased from some local Saudi markets by using several different measurement methods. According to our knowledge, this is the first study to compare these characteristics of the most common coffee brews available in the local markets of the Northern region of Saudi Arabia country.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) used as the source of free radicals and Folin-Ciocalteu's phenol reagent used for estimation of TPC were purchased from Sigma-Aldrich Chemical Co. (Pool, UK).

### 2.2 Coffee Samples

Three coffee samples, Intenso (Arabica), Reebass (Turkish) and Lavazza (Brazilian) purchased from some supermarkets at the Northern Border region in Kingdom of Saudi Arabia were used in this study. The coffee samples were stored in a cool dry place before analysis.

### 2.3 Microbial Strains

Antimicrobial activity was assayed against a panel of microorganisms certified by American Type Culture Collection (ATCC) and National

Collection of Pathogenic Fungi (NCPF), including three Gram-positive bacteria *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538, and against Gram-negative *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 7839, a fungus (yeast) - *Candida albicans* (NCPF-stock laboratory strain). Cultures were inoculated into specific broths and incubated at 37°C for 24 h.

### 2.4 Sample Preparation

Coffee samples were extracted using the boiling water method [21]. Coffees (2 g) were extracted with 250 ml of hot water three times, with continuous swirling at 120 rpm in an orbital shaker, for 1 h each time. The boiling water was allowed to cool throughout the extraction process to mimic coffee brewing. After filtration under suction through Whatman No. 1 filter paper, the residues were re-extracted again with 250 ml of hot water. The water in the extracts was removed using a freeze dryer. Dried extracts were kept at -20°C in a freezer for further analysis.

### 2.5 Determination of Total Phenolic Content

Total phenolic content (TPC) of extracts was determined using the Folin-Ciocalteu method [22,23]. Samples (300 µl, in triplicate) were introduced test tubes wrapped in aluminum foil followed by addition of 1.5 ml of FC reagent (10 times dilution) and 1.2 ml of sodium carbonate solution (7.5% w/v). The tubes were allowed to stand in the dark for 30 min before absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalent (GAE) in mg/g of coffee sample. The calibration equation for gallic acid was  $y = 0.0111x + 0.0148$  ( $R^2 = 0.9998$ ).

### 2.6 Determination of Antioxidant Activity

Antioxidant activity was measured using the DPPH radical scavenging assay [23,24]. Different dilutions of the extracts (1 ml) were added to 2 ml of DPPH (5.9 mg/100 ml methanol) in test tubes wrapped in aluminum foil. Absorbance (A) was measured at 517 nm after 30 min incubation in the dark. All measurements were made with distilled water as a blank. The scavenging ability (%) of the samples was calculated as  $(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$  and calculated as  $IC_{50}$ , the concentration of sample needed to scavenge DPPH free radicals by 50%.  $IC_{50}$  was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) using the equation:

AEAC (mg AA/g sample) =  $IC_{50}(AA)/IC_{50}(sample) \times 10^5$ . The  $IC_{50}$  of AA used for calculation of AEAC was 0.00387 mg/ml.

## 2.7 Assay for Antimicrobial Activity

Antimicrobial activity was determined by the disk diffusion method [25], using bacterial cell suspensions with concentrations equilibrated to a 0.5 McFarland standard. Each bacterial suspension ( $1.5 \times 10^8$  cfu/ml) was spread on Mueller-Hinton agar plates. Six wells were cut on each plate using a cork borer, 6 mm diameter, and filled with cold and hot coffee extract. After incubation for 24 h at 37°C, the presence of inhibition zones was observed.

## 2.8 Statistical Analysis

The data were statistically analyzed using Microsoft Excel 2016. Results with  $p < 0.05$  were considered statistically significant. All experiments were performed in triplicate and the values were expressed as mean  $\pm$  SD. The differences between the samples were assessed using single factor analysis of variance (ANOVA).

## 3. RESULTS AND DISCUSSION

### 3.1 Total Phenolic Content (TPC)

Coffee is one of the most widely consumed beverages worldwide, lauded for its pleasant flavor and aroma, its pharmacological

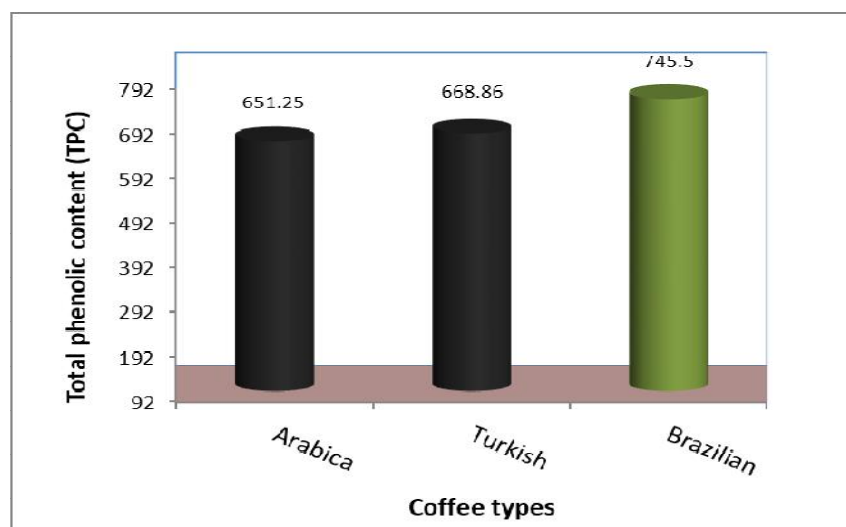
characteristics and, most importantly, its role as a stimulant on mental and physical activity. Recently, the scientific and popular interest concerning its significance on health has increased due to the beneficial pharmacological properties established in clinical and epidemiological studies.

TPC of coffee types under study were expressed as gallic acid equivalent. The color of Folin-Ciocalteu reagent changes from yellow to blue upon the detection of phenolics in the extracts which is normally due to the chemical reduction of tungsten and molybdenum oxide mixture in the reagent. In the present study, TPC of coffee types under study were expressed as gallic acid equivalent and represented in Fig. 1. The results showed that the content of polyphenols were arranged as  $745.50 \pm 10.5$ ,  $668.86 \pm 11.2$  &  $651.25 \pm 13.0$  mg/g for Lavazza (Brazilian), Reebass (Turkish) & Intenso (Arabica) respectively (Table 1).

**Table 1. Total phenolic content of extracts of coffee types**

No	Type	Brand (County)	TPCa (mg GAE/g)
1	Intenso	Arabica	$651.25 \pm 13.0$
2	Reebass	Turkish	$668.86 \pm 11.2$
3	Lavazza	Brazilian	$745.50 \pm 10.5$

Note: <sup>a</sup>TPC (total phenolic content) represented by means  $\pm$  SD (n = 3)



**Fig. 1. Total phenolic content of extracts of coffee types**

The value expressed as means  $\pm$  SD (n = 3). Comparison of means was made using unpaired t-test ( $P > 0.05$ )

It was clear that, Lavazza (Brazilian) coffee extract showed the highest amount of total phenolics content ( $745.50 \pm 10.5$  mg GAE/g, whereas the Intenso (Arabica), Reebass (Turkish) Coffee varieties had almost similar TPC. The deviation in the total phenolic contents might be attributed to the geographical factors as well as the different cultivation methods [26]. There were no statistical significant differences ( $p > 0.05$ ) in TPC values observed among the tested coffee varieties. A previous study had reported that phenolic content was influenced by the origin of the coffee beans and extracting solvents [27,28].

### 3.2 DPPH Radical Scavenging Activity

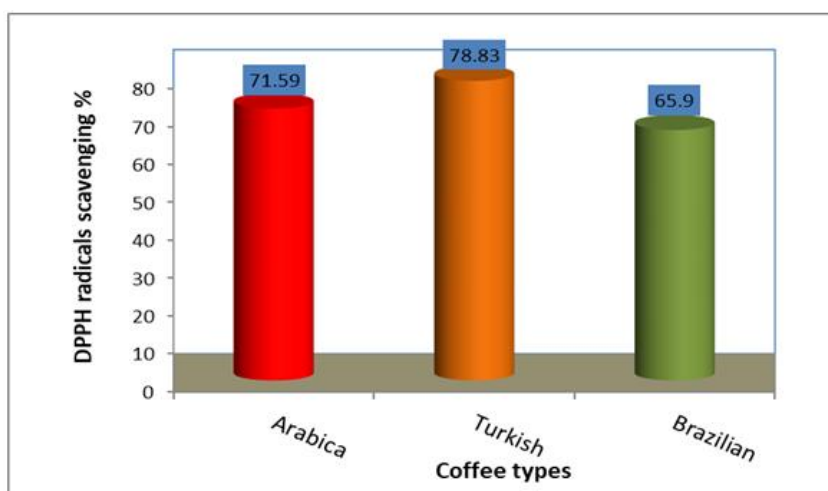
DPPH assay is used to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts. DPPH is a stable free radical with a dark violet color. This method is based on the principle that DPPH accepts a hydrogen atom from the antioxidant, resulting in the reduction of DPPH to DPPH<sub>2</sub>, the violet color changes to yellow with a consequent decrease in absorbance at 517 nm. The efficiency of the antioxidant compound is measured by its ability to change color. The percentage of inhibition was higher 78.83% for Turkish coffee followed by Arabica 71.59% and Brazilian 65.90%. The results showed that there were significant decreases ( $p < 0.01$ ) in scavenging activity in Brazilian and Arabica coffee compared to Turkish coffee (Fig. 2).

The proton radical scavenging action is known to be one of the various mechanisms for measuring

antioxidant activity. DPPH is one of the compounds that possess a proton free radical and shows a maximum absorption at 517 nm. When DPPH encounters proton radical scavengers, its purple color fades rapidly. This assay determines the scavenging of stable radical species of DPPH by antioxidants. As can be seen in Fig. 2, all the extracts were capable of scavenging DPPH free radicals. Among different coffee extracts, Turkish showed the maximum inhibition activity (78.83%) followed by Arabica (71.59%) and Brazilian (65.90%). This difference is strongly related to the phenolic content and also to the type of the active compound present in each variety [26].

### 3.3 Antimicrobial Properties

The inhibitory effect of the coffee extracts on some microbial strains is indicated in Table 2. The results obtained showed that Arabica coffee extract was the highest antimicrobial activity compared to the other two extracts of both Turkish and Brazilian types. The mean diameter of inhibition zone for three coffee types were ranged from  $11.0 \pm 0.15$  to  $16.0 \pm 0.40$ ;  $9.0 \pm 0.25$  to  $11.0 \pm 0.10$  and  $9.5 \pm 0.10$  to  $13.0 \pm 0.50$  mm for Arabica, Turkish and Brazilian coffee respectively. Also it was noticed that all coffee types exhibited a promising antimicrobial activity against all tested microbial strains especially against Gram positive bacteria and *Candida albicans*, but weak activity was recorded against Gram negative bacteria especially against *P. aeruginosa* strain.



**Fig. 2. Radical scavenging activity of extracts of coffee types**

The value expressed as means  $\pm$  SD ( $n = 3$ ). Comparison of means was made using unpaired t-test ( $P > 0.05$ )

**Table 2. Antimicrobial activity of extracts of coffee types using the disc-diffusion method**

No	Type	Brand (county)	Inhibition zone diameter (mm)				
			Gram positive bacteria		Gram negative bacteria		Unicellular fungi
			<i>B. subtilis</i> ATCC 6633	<i>S. aureus</i> ATCC 6538	<i>P. aeruginosa</i> ATCC 9027	<i>E. coli</i> ATCC 7839	<i>Candida albicans</i>
1	Intenso	Arabica	14.0 ± 0.35	16.0 ± 0.40	11.0 ± 0.15	12.0 ± 0.22	13.0 ± 0.24
2	Reebass	Turkish	10.0 ± 0.15	9.0 ± 0.22	9.0 ± 0.25	11.0 ± 0.10	10.0 ± 0.15
3	Lavazza	Brazilian	13.0 ± 0.50	11.5 ± 0.20	9.5 ± 0.10	10.0 ± 0.48	12.0 ± 0.30

The *in vitro* antimicrobial activity of commercial coffee extracts was investigated by the disc diffusion method on five microbial strains included Gram positive and Gram negative bacteria in addition to unicellular fungi. Coffee beverages intended for use as antioxidants may also present biological effects on bacteria or fungi, including antimicrobial activity against a range of Gram-positive and Gram-negative bacteria [20].

Our results indicated that all coffee extracts exhibited promising antimicrobial activity against all tested microbial strains with different degrees. On the other hand, when comparing the susceptibility of the different strains to the coffee extracts, it was found that there is no significant differences were observed. Larger diameters of the inhibition zones were observed for Gram positive bacteria *B. subtilis* and *S. aureus* indicating the higher sensitivity of this species to the coffee extracts. Smaller diameters were observed for Gram negative bacteria *P. aeruginosa* and *E. coli* which indicated that it was less sensitive to the coffee extracts. The other strains of unicellular fungi showed intermediate sensitivity to these extracts. Research has shown that coffee possesses antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* [20,29]. The results obtained were confirmed by literature reports. The antimicrobial activity of caffeic acid was described against *Pseudomonas*, *E. coli*, *S. aureus*, and *Bacillus cereus* [30,31,32]. According to these studies, caffeic acid had an appreciable antibacterial activity against these microorganisms.

#### 4. CONCLUSION

In the present work three of the most common coffee brews available in the local markets at the Northern Region of Saudi Arabia; Intenso (Arabica), Reebass (Turkish) and Lavazza (Brazilian) were investigated for their total

phenolic contents, antioxidant and antimicrobial activities. The results indicated that, there were no significant differences in TPC values observed among the tested coffee varieties as well as all the extracts were capable of scavenging DPPH free radicals with similar percentages which may due to the phenolic content. Furthermore, we recorded that all coffee extracts exhibited promising antimicrobial activities against all tested microbial strains.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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