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Antioxidative, Antibacterial and Antifungal Activities of Tea Infusions from Berry Leaves, Carob and Doum

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This study is carried out to evaluate the chemical composition and physicochemical properties of berry leaves, carob and doum tea infusions and their mixtures. Furthermore, the antioxidant activities of these extracts were investigated by scavenging of 1,1 diphenyl-2-picrylehydrozyl (DPPH) radicals. The proliferation inhibition activities on some types of bacteria and yeast were also measured for evaluating the antimicrobial activity of berry leaves, carob and doum tea infusions and their blends. The results showed that carob was characterized by its high protein content. The highest percentage of fiber was found in doum sample, followed by carob but ash was the highest in berry leaves samples. The levels of most elements were higher in carob sample, when compared with those of berry leaves and doum samples. The highest value of TSS was found in the tea infusions of doum and carob (22.5 Brix), meanwhile, the lowest was observed in the doum tea infusions (10.60 Brix). Acidity of berry leaves, carob and doum tea infusions was higher than that of the berry leaves +doum tea infusions, but lower than that of the berry leaves + carob + doum tea infusions. Total sugars formed the major components in doum and berry leaves + carob tea infusions. Doum and carob tea infusions had significantly higher non-reducing, but lower contents of reducing sugars than the other tested samples. The results indicated that berry leaves + carob + doum tea infusions sample showed higher quality attributes especially color, taste and overall acceptability. The berry leaves and berry leaves + carob tea infusions had a high level of vit. C (11.05 and 10.28 mg/100 g), while it was low in doum tea infusions. Blended samples showed a higher content of total phenol compounds compared to tea infusions of berry leaves, carob and doum. Data indicated that the berry leaves + carob + doum, berry leaves + carob, berry leaves + doum and doum + carob tea infusions samples were good antioxidants with strong DPPH radical-scavenging activity. The results confirmed that the investigated extracts had good antimicrobial activity, especially against bacteria.

INTRODUCTION

The development of food products or ingredients with specific health promoting benefits (nutraceuticals or functional foods) is currently the fastest growing and most consumer-driven segment of the food industry [Hardy, 2000]. Mulberry leaves, bark and branches have long been used in Chinese medicine to treat fever, protect the liver, improve eyesight, strengthen joints, facilitate discharge of urine and lower blood pressure [Zhisen *et al*., 1999]. In Japan, consumption of berry leaves as a tea or powdered juice has been increasing [Katsube *et al*., 2009]. Mulberry leaves contain an appreciable amount of proteins, carbohydrates, fats, fibers, mineral contents and some vitamins or precursors [Srivastava *et al.*, 2006; Ercisil & Orhan, 2007; Butta *et al*., 2008]. Carob was eaten in Ancient Egypt. Carob juice drinks are traditionally drunk especially during Ramadan. Carob (*Ceratonia siliqua* L.) contains about 8% protein and vitamins A, B, B2, B3 and D. It is also high in calcium, phosphorus, potassium and magnesium and contains iron, manganese, barium, copper and nickel. However, it should of course only be eaten in moderation alongside a balanced diet. It has no oxalic acid which prevents the body using calcium and zinc. The main constituents of carob are large carbohydrates (sugars) which make carob gummy and able to act as a thickener to absorb water and help bind together watery stools [Eissa *et al*., 2008]. The unripe kernels of doum are edible; the shoots of germinated seeds are also eaten as a vegetable. Herb tea of doum is popular in Egypt and believed good for hypertension. Research on the fruit pulp have shown that it contains nutritional trace minerals, proteins and fatty acids, particular the nutritionally essential linoleic acid [Cook *et al*., 2000]. A large number of plants have been screened as a viable source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds which are responsible for maintenance of health, to help the human body reduce oxidative damage and protection from coronary heart diseases and cancer [Yang *et al*., 2002; Kilani *et al*., 2008]. Therefore there is a growing interest in the substances exhibiting antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceuticals. Recently, natural antioxidants have become one of the major areas of scientific research [Demo

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et al., 1998; Sanchez-Mareno *et al*., 1999; Dawidowicz *et al*., 2006]. Several recent studies reported the antioxidant activity of mulberry leaves. Butanol extract of berry leaves scavenged the DPPH radical and inhibited the oxidative modification of rabbit and human LDL [Kris-Etherton, 2002; Harris *et al*., 2007]. Many plant extracts prepared from plants have been shown to exert biological activity *in vitro* and *in vivo*, which justified research on traditional medicine focused on the characterization of antimicrobial activity of these plants [Maertinez *et al*., 1996]. Plants are known to produce certain chemicals which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts, showing great potential especially against multidrug resistant bacteria [Pesewu *et al*., 2008; Tasdelen *et al*., 2009]. Detailed study of the photochemistry of fruits and vegetable provides insight about phenolic compounds [Giugliano, 2000; Katsube *et al*., 2004; Dimitrios, 2006]. These phenolic compounds often exhibit a wide range of physiological activities that include antioxidant, antimutagenic, anticarcinogenic, antimicrobial, and anti-inflammatory properties [Baliga & Katiyar, 2006; Heinonen, 2007]. Some information relate to the antinutritional factors such as of phytate, saponin and tannin. Is it possible that they can occur in tea infusions from doum [Umaru *et al*., 2007].

However, according to the available literature, few scientific evaluations of the antioxidant and antimicrobial activities of berry leaves, carob and doum tea infusions have yet been done. Thus the objectives of the present study were focused on the antioxidant and antimicrobial activities of berry leaves, carob and doum tea infusions (drinks). Physicochemical and organoleptic properties of these extracts were evaluated as well.

MATERIALS AND METHODS

Materials

Berry leaves (*Sambucus nigra* L.) were collected from the garden of National Research Center (NRC). Carob fruit horny (*Ceratonia siliqua* L.), and doum fruit (*Hyphaene thebaica*) were purchased from a local herbal shop in a form of small pieces (Dokki, Egypt). Tryptic soy broth (TSB), Tryptic soy agar, (TSA), Muller Hinton agar, (MHA), Malt extract broth (MEB) and Malt extract agar (MEA) were obtained from Oxide.

Preparation of material samples desired for tea infusion

The materials were washed carefully, dried to a constant weight in a hot air oven at 40ºC for 6 h. Then, 100 g from each material were ground in a Braun cutting mill to obtain particle size in the range of 0.8–1.0 mm. Exactly weighted portions of these samples were subsequently packed in tea bags, put in carton box, then stored at room temperature (25ºC) until used.

Characterization of chemical composition and mineral contents of dried berry leaves, carob and doum

Moisture, protein, fat, ash, total sugar, reducing sugars, total solids, total acidity and vitamin C contents were determined according to the A.O.A.C. [2000]. Potassium, magnesium, sodium, calcium, iron, manganese, zinc and copper were determined using Perkin Elmer 2380, Atomic Absorption Spectrophotometer according to the method of A.O.A.C. [2000].

Preparation of tea infusions

In order to prepare infusions, 15 g of ground berry leaves, carob and doum were boiled for 5 min in about 100 mL of distilled water, 5 g of sucrose was dissolved and each tea infusion (aqueous extract of all materials) was rapidly filtrated through a Buchner funnel. The formula used in this study was as follows:

Infusion 1: 15 g of berry leaves
Infusion 2: 15 g of carob
Infusion 3: 15 g of doum,
Infusion 4: 7.5 g of berry leaves + 7.5 g of carob,
Infusion 5: 7.5 g of berry leaves + 7.5 g of doum,
Infusion 6: 7.5 g of carob + 7.5 g of doum
Infusion 7: 5 g of berry leaves +5 g of carob +5 g of doum.

Characterization of physico-chemical and sensorial properties of tea infusions

The content of total soluble solids (TSS) expressed as Brix (0–32) was determined using a Hand refractometer (ATAGO, Japan). Non reducing sugars were determined by the difference between the total sugars and reducing sugars. Brix / acid ratio was calculated by dividing the value of total soluble solids on the total acidity value for each sample. The pH values of tea infusions samples were measured using a digital pH–meter (HANNA, HI 902 m Germany). Three measurements were taken for each sample. The viscosity measurements were carried out using HAAKE viscometers (HAAKE, Mess-Technik Gmbhu. Co., Germany) with thermostatic bath to control the working temperature within the temperature of 25°C. Results of viscosity were expressed in centipoises (cP) according to the method of Ibarz *et al*. [1994]. Non reducing sugars were determined by the difference between the total sugars and reducing sugars.

Hunter color values of tea infusions from berry leaves, carob, doum and their mixture

The color of different samples was measured using a spectro-colorimeter (Tristimulus Color Machine) with CIE lab color scale (Hunter, Lab Scan XE, Germany) calibrated with a white standard tile of Hunter Lab Color standard (Lx No. 16369): X=77.26, Y=81.94 and Z=88.14 (Lx=92.35; ax=- 0.85; bx=-0.14). Color difference (Δ E) was calculated from a, b and L parameters. Using Hunter-Scotfields equation [Hunter, 1975].

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

where $a = a - a_0$; $b = b - b_0$ and $L = L - L_0$.

Subscript (0) indicates color of control. Hue angle (tg^{-1} b/a) and saturation index [$a^2 + b^2$] were also calculated.

Sensory evaluation of tea infusions from berry leaves, carob, doum and their mixture

A fifteen-member trained panel experienced in discrimination and descriptive analysis on different food products performed assessments. Quantitative descriptive analysis (QDA) was used to determine differences in the sensory characteristics of the tea infusions from berry leaves, carob

and doum and their mixture. For evaluation, approximately 100 mL of each tea was presented to assessors in random order. The panelists evaluated the appearance, color, taste and flavor on unstructured 10 cm line scales verbally anchored at each end. The results from the linear scale were subsequently converted to numerical values (from 0 to 10 units) by a computer. The panelists were also asked to evaluate the overall acceptability of the tea infusions on the basis of overall appearance, color, taste and flavour. An unstructured graphical scale was anchored on both ends: not accept (0) – fully accept (10) [Meligaard *et al*., 1991].

Determination of vitamin C, total phenolics content and antioxidant activity of tea infusions

Vitamin C contents were determined according to the A.O.A.C. [2000]. Total phenolic compounds in the tea infusions from berry leaves (*Sambucus nigra* L.), carob (*Ceratonia siliqua* L.), and doum (*Hyphaene thebaica*) were determined according to the method of Taga *et al*. [1984]. Tea infusions (100 μL) were added to 2 mL of 2% Na_2CO_3. After 2 min, 50% Folin–Ciocaulteu reagent (100 μL) was added to the mixture which was then left to stand for 30 min. Absorbance was measured at 750 nm on a spectrophotometer and compared to gallic acid calibration curves. All analyses were run in triplicate and mean values were calculated. Antioxidant activity was also determined by scavenging the radical 1,1-diphenyl-2-picryhydrazyl (DPPH) as described by Tadolini *et al*. [2000]. The stock solution was prepared by stirring 75 mg of DPPH in 1 L of methanol overnight. In the assay, 0.75 mL of the extract, blank (methanol) and 1.5 mL of DPPH solution were mixed. The absorbance of samples, standards, and blanks at 517 nm (T80+UV/VIS Spectrometer PG Instrument ltd, United Kingdom) was determined after 5 min. For each tea infusion, a blank with 1.5 mL methanol, instead of the DPPH reagent, was included to correct for any sample absorbance at 517 nm.

Determination of antibacterial and antifungal activity of tea infusions from berry leaves, carob, doum and their mixture

Microorganisms

The strains of bacteria used included: *Bacillus subtilis* and *Bacillus megaterium* from Department of Microbiology, Faculty of Agriculture, Cairo University, Giza; *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* from Department of Microbiology, International Centre for Irradiation Research and Technology; and yeast (*Debaryomyces hansenii*, *Zygosaccharo ycesrouxii*, *Rhodotorula rubra, Candida shehatae, Candida tropicalis)* from Department of Microbiology, Faculty of Agriculture, Cairo University, Giza. All bacterial and yeast strains were maintained at 4°C on TSA and MEA, respectively and transferred monthly. Before use, bacterial and yeast strains were twice activated in TSB at 35° C and in MEB at 28°C, respectively for 24 h.

Antimicrobial activity assay

The antibacterial activity (ABA) and antifungal activity (AFA) of berry leaves, carob, and doum tea infusions were assessed by disc diffusion method [Bazaraa *et al*., 2005]. All bacterial and yeast strains were activated (24 h) in TSB and MEB, respectively and then inoculated onto the surface of MHA for bacteria and MEA for yeast using sterile cotton swabs. The inoculum was allowed to dry for about 15 min and filter paper discs loaded with different concentrations of beery leaves, doum and carob tea infusions and their mixture (10 μL) were aseptically placed on the surface of the inoculated agar (3–4 discs per plate). The plates were then incubated at 37°C for 24 h (bacteria) and at 28°C for 24 h (yeast). The diameter of the resulted inhibition zone of each concentration was then measured in millimeters (mm) and recorded.

Statistical analysis

Results of the physico-chemical analyses are given as mean values and the standard deviation of three independent measurements. Results of sensory evaluation of tea infusions were subjected to statistical analysis using of variance and least significant differences (LSD) as described by Rao & Blane [1985].

RESULTS AND DISCUSSION

Chemical composition and minerals content of berry leaves, carob, and doum

Table 1 shows the chemical composition of berry leaves, carob and doum. Berry leaves were characterized by a high protein content and the content of fat was on the same level. Data in the same table indicated that the total carbohydrates in berry leaves, carob and doum samples were 42.00, 73.14, and 72.89%, respectively. From the obtained results it can be observed that the highest percentages of fiber and ash were found in the berry leaves, followed by doum and carob samples. Total sugars constitute the major components in doum. Doum and carob had a significantly higher content of non-reducing sugars reached to 10.67 and 8.89%, respectively, but berry had higher reducing sugars (3.02%) than doum (1.99%) or carob (0.68%). These results are in agreement with those found by Eissa *et al*. [2008].

The results presented in Table 1 indicated the LSD at 0.05 level to range between 0.112 and 1.19.

The elemental composition of berry leaves, carob and doum is shown in Table 1 as well. Calcium seems to be a predominant element in berry leaves and doum samples. Meanwhile sodium, potassium and iron are the major elemental components in carob samples. Other less abundant elements turned out to be Mg, Fe, Cu, Zn and Mn in the all investigated samples.

The results in Table indicated the LSD at 0.05 level to range between 0.001 and 1.96 of raw materials.

Physico-chemical properties of berry leaves, carob, doum tea infusion and their blends

Total soluble solids (TSS) of berry leaves, carob, doum tea infusions and their blends are shown in Table 2. The highest value of TSS was found in the tea infusion of carob+doum (22.5 Brix), while the lowest TSS value was observed in the doum tea infusion (10.60 Brix). The in-

TABLE 1. Chemical composition and mineral contents of dried berry leaves, carob and doum.

Components (%)	Berry leaves	Carob	Doum	LSD at 0.05
Moisture	$8.2^{c}\pm0.73$	$10.1^{b}\pm0.73$	$11.0^{a}\pm0.20$	0.199
Protein	$18.2^{a}\pm0.03$	$8.95^{b}\pm0.02$	$6.45^{c}\pm0.44$	1.01
Fat	$5.5^{a}\pm0.22$	$5.48^{a}\pm0.06$	$4.89^{b}\pm0.17$	0.117
Fiber	$23.5^{a}\pm0.15$	$8.91^{c}\pm0.43$	$11.55^{b}\pm0.23$	0.115
Ash	$10.8^{a}\pm0.43$	$3.52^{c}\pm0.21$	$4.22^{b}\pm0.22$	0.112
Total carbohydrate	$42.00^{c}\pm0.23$	$73.14^{a}\pm0.55$	$72.89^{b}\pm0.55$	1.19
Total Sugars	$6.82^{c}\pm 0.08$	$9.17^{b}\pm0.13$	$12.66^{a}\pm0.19$	1.12
Reducing Sugars	$3.02^{a}\pm0.002$	$0.68^{c}\pm0.001$	$1.99^{b}\pm0.013$	0.86
None Reducing Sugars	$3.80^{c}\pm0.001$	$8.89^{b}\pm0.09$	$10.67^{a}\pm0.13$	1.14
Elements (mg/100 g)				
Potassium	$3.68^{c}\pm0.13$	$28.30^{a}\pm0.23$	$5.99^{b}\pm0.06$	1.96
Calcium	$271.81^{a}\pm0.23$	$26.04^{c}\pm0.32$	$92.24^{b}\pm0.12$	1.92
Magnesium	$2.57^{b}\pm0.03$	$3.17^{a}\pm0.02$	$1.31^{c}\pm0.18$	0.13
Iron	$2.42^{b}\pm0.05$	$3.18^{a}\pm0.13$	$1.95^{c}\pm0.23$	0.17
Copper	$1.75^{b}\pm0.02$	$2.136^{a}\pm0.03$	$1.82^{b}\pm0.01$	0.19
Zinc	$0.08^{b}\pm0.003$	$0.35^{a}\pm0.01$	$0.04^{c}\pm0.003$	0.001
Manganese	$0.57^{a}\pm0.02$	$0.25^{b}\pm0.023$	$0.09^{c}\pm0.001$	0.003

a, b, c, d, e, f, g: Mean values in each row followed by a different letter are significantly different ($p\leq0.05$).

TABLE 2. Physico-chemical properties of drinks from berry leaves, doum, carob and their mixture.

Parameter	Berry leaves	Carob	Doum	Berry leaves + Carob	Berry leaves + Doum	Carob + Doum	Berry leaves + Carob + Doum	LSD at 0.5
TSS ^{o} Brix	$14.20^{e}\pm0.29$	$13.0^{e}\pm0.13$	$10.60^{g}\pm0.09$	$13.50^{e}\pm0.28$	$15.25^{b}\pm0.44$	$22.50^{a}\pm0.35$	$12.80^{f}\pm0.16$	0.349
Acidity (%)	$0.42^{d}\pm0.01$	$0.35^{e}\pm0.003$	$0.55^{b}\pm0.01$	$0.50^{c}\pm0.002$	$0.30^{f}\pm0.0$	$0.40^{d}\pm0.03$	$0.70^{a}\pm0.011$	0.01
Ratio TSS/ acidity	$33.80^{d}\pm0.6$	$37.14^{c}\pm0.18$	$19.27^{f}\pm0.02$	$27.00^{e}\pm0.29$	$50.83^{b}\pm0.44$	$56.25^{a}\pm0.36$	$18.28^{f}\pm0.16$	1.16
pH	$8.95^{a}\pm0.42$	$6.83^{e}\pm0.35$	$6.45^{e}\pm0.25$	$8.06^{b}\pm0.52$	$7.50^{c}\pm0.36$	$7.35^{c}\pm0.38$	$7.13^{d}\pm0.43$	1.02
Viscosity	$0.27^{a}\pm0.009$	$0.27^{a}\pm0.004$	$0.29^{a}\pm0.001$	$0.27^{a}\pm0.002$	$0.24^{b}\pm0.006$	$0.22^{b}\pm0.03$	$0.29^{a}\pm0.001$	0.02

a, b, c, d, e, f, g: Mean values in each row followed by a different letter are significantly different ($p\leq0.05$).

Viscosity = flow time/sec

crease of TSS was obvious with the increasing of tea infusion concentration. This increasing of TSS was attributed to the greater degree of tissue breakdown, releasing more components that contribute to soluble solids [Eissa & Salama, 2002].

Titratable acidity of berry leaves + carob +doum tea infusion was higher than the other tested drinks, which may be due to enzymatic desertification and increased pH resulting in an increased content of total acids.

The TSS/ acid ratio is the major analytical measurement for quality of natural extracts. The TSS/ acid ratios of carob + doum and berry leaves + doum tea infusion were higher than these of the other samples, as shown in Table 2. The TSS/ acid ratio was shown to be correlated with sweetness [Daniel *et al*., 1993]. The TSS/ acid ratio of traditional hibiscus drink was increased by increasing the sugar concentration [Eissa *et al*., 2008].

The pH values of berry leaves and + carob tea infusion were higher than the pH of the other samples. The pH values of the above mentioned tea infusion drinks were 8.95 and 8.06, respectively.

Viscosity (cp) was selected as a measure of tea infusions quality. The viscosity of all tested samples ranged from 0.27 to 0.29 (cp).

The results in Table 2 indicated the LSD at 0.05 level to range between 0.01 and 1.16 of samples.

Color characteristics of berry leaves, carob, doum tea infusion and their blends

Color of doum, carob and berry tea infusions represents the major quality factor. So, color parameters (L*, a* & b*) were measured directly in tea infusion samples and presented in Table 3. Data in this table showed that the doum tea infusion had the high values of lightness (L) and yellowness (b), which were 14.28 and 15.68, respectively. Higher values of redness (a) and a/b were found in berry leaves+ carob + doum tea infusion than in the other investigated drink samples. Berry leaves tea infusions had the highest Hunter hue angle. Meanwhile the lowest one was found in berry leaves+ carob + doum, tea infusions sample. Berry leaves and carob tea infusion samples were characterized by its saturation index values (chroma). The A/B ratio indicated

TABLE 3. Hunter color values of drinks from berry leaves, carob, doum, and their mixture.

Samples	L	a	b	a/b	Δ E	Hue	Saturation
Berry leaves	$3.87^{f}\pm0.02$	$0.88^{g}\pm0.0$	$4.86^{e}\pm0.11$	$0.18^{f}\pm0.0$	$8.27^{f}\pm0.13$	$79.74^{a}\pm1.03$	$4.93^{f}\pm0.015$
Carob	$2.21^{g}\pm0.01$	$1.16^{f}\pm0.001$	$3.66^{f}\pm0.09$	$0.32^{d}\pm0.001$	$4.42^{g}\pm0.019$	$72.41^{b}\pm1.16$	$3.83^{g}\pm0.011$
Doum	$14.28^{a}\pm0.11$	$5.00^{d}\pm0.13$	$15.68^{a}\pm0.25$	$0.32^{d}\pm0.001$	$21.79^{a}\pm0.15$	$72.31^{b}\pm0.92$	$16.45^{b}\pm0.16$
Berry leaves +Carob	$10.02^{e}\pm0.09$	$5.30^{e}\pm0.16$	$12.05^{d}\pm0.19$	$0.43^{c}\pm0.003$	$16.50^{e}\pm0.18$	$66.25^{c}\pm0.75$	$13.16^{e}\pm0.13$
Berry leaves +Doum	$11.70^{c}\pm0.07$	$4.33^{e}\pm0.07$	$14.04^{c}\pm0.23$	$0.31^{e}\pm0.002$	$18.78^{d}\pm0.12$	$72.86^{b}\pm0.88$	$14.69^{d}\pm0.08$
Carob + Doum	$11.27^{d}\pm0.03$	$6.62^{b}\pm0.19$	$13.85^{c}\pm0.18$	$0.47^{b}\pm0.001$	$19.04^{c}\pm0.16$	$64.45^{d}\pm0.56$	$15.35^{c}\pm0.19$
Berry leaves + Carob + Doum	$13.36^{b}\pm0.22$	$7.72^{a}\pm0.11$	$14.85^{b}\pm0.22$	$0.52^{a}\pm0.003$	$20.16^{b}\pm0.19$	$62.53^{e}\pm0.62$	$16.73^{a}\pm0.17$
LSD (0.05%)	0.08	0.09	0.49	0.012	0.068	1.16	0.018

a, b, c, d, e, f, g: Mean values in each row followed by a different letter are significantly different ($p\leq0.05$).

TABLE 4. Sensory evaluation of tea infusions from berry leaves, carob, doum and their mixture.

Samples	Color	Odor	Taste	Appearance	Overall acceptability
Berry leaves	$7.0^{ab}\pm0.61$	$6.6^{a}\pm0.16$	$7.5^{a}\pm0.06$	$6.9^{ab}\pm0.02$	$7.6^{ab}\pm0.42$
Carob	$6.5^{b}\pm0.30$	$7.2^{a}\pm0.39$	$5.5^{abc}\pm0.26$	$6.7^{ab}\pm0.21$	$7.0^{ab}\pm0.39$
Doum	$7.5^{ab}\pm0.45$	$6.8^{a}\pm0.73$	$5.7^{abc}\pm0.56$	$6.6^{ab}\pm0.38$	$7.3^{ab}\pm0.18$
Berry leaves +Carob	$7.2^{ab}\pm0.53$	$6.4^{a}\pm0.55$	$6.1^{ab}\pm0.82$	$7.8^{a}\pm0.17$	$7.2^{ab}\pm0.28$
Berry leaves +Doum	$6.9^{ab}\pm0.72$	$6.2^{a}\pm0.26$	$6.25^{ab}\pm0.36$	$7.2^{a}\pm0.66$	$7.5^{ab}\pm0.22$
Carob + Doum	$7.4^{ab}\pm0.33$	$6.5^{a}\pm0.22$	$6.5^{ab}\pm0.11$	$7.6^{a}\pm0.24$	$7.6^{ab}\pm0.31$
Berry leaves + Carob+ Doum	$8.1^{a}\pm0.68$	$6.6^{a}\pm0.18$	$7.8^{a}\pm0.16$	$7.4^{a}\pm0.81$	$8.3^{a}\pm0.30$
LSD (0.05%)	1.598	NS	0.76	0.46	1.461

a, b: Mean values in each row followed by a different letter are significantly different ($p\leq0.05$). NS= Non significant.

the intensity of red (+a) characteristics [Francis & Clydesdale, 1975]. From the results in the same table it can be observed that the highest value of delta E was found in doum tea infusion sample, followed by berry leaves+ carob + doum, tea infusions, which changed due to particle precipitation as seen in Table 4. These results are in a good agreement with those of Eissa *et al*. [2008] as well as De Rosso & Mercadante [2007].

The results in Table 3 indicated the LSD at 0.05 level to range between 0.012 and 1.16 of samples.

Sensory evaluation of tea infusions

Sensory evaluation data of berry leaves, doum, carob tea infusions and their blends were analyzed statistically. Comparison of the mean values achieved for the following parameters: color, odor, taste, appearance and overall acceptability, used to evaluate the above mentioned drinks is shown in Table 4. In general, the results indicated that samples of berry leaves, doum, carob tea infusions showed higher quality attributes especially in terms of color, taste and overall acceptability, compared with the other investigated tea infusion samples. From the same table it could be observed that no significant differences between the suggested drink samples for odor were found. The obtained results showed also that the carob drink sample received lower scores of color, taste and overall acceptability than all other drink samples. Meanwhile, the berry leaves + doum tea infusions sample received a lower score for odor. In turn, the berry leaves + carob and carob tea infusions samples were highly accepted for appearance and odor, respectively.

Content of vitamin C, total phenolics, and antioxidant activity of tea infusions

In recent years, researchers have paid particular attention to the biologically active ingredients, especially vitamin C, alkaloids and polyphenols in food and tea infusions due to their positive effects on human health. The berry leaves and berry leaves + carob tea infusion had a high level of vitamin C (11.05 and 10.28 mg/100 g). While, vitamin C content was low in doum tea infusion. The high content of vitamin C in berry leaves tea infusion may suggest them to be a good source of this vitamin. Blended tea infusions showed a higher content of total phenol compounds compared to tea infusions of berry leaves, carob and doum. The tea infusion of berry leaves+ carob +doum had total phenols content of 1405.98 mg/100 g, followed by berry leaves + carob with total phenols content of 1118.89 mg/100 g. Meanwhile, the lowest total phenolic content was found in berry leaves tea infusion (696.41 mg/100 g). These results are in accordance with findings by Heinonen [2007] and Eissa *et al*. [2008].

The antioxidant activity of a plant extract is of particular interest both because of beneficial physiological activity on human cells and the potential they have to replace synthetic antioxidants used in foodstuffs [Amarowicz *et al*., 1999]. In addition to their activity as antioxidants, these compounds often display biological activity of various kinds against bacteria [Rauha *et al*., 2000; Dykes *et al*., 2003]. DPPH (1,1-diphenyl-2picrylhydrazyl) radical is commonly used as a substrate to evaluate antioxidant activity, it is a stable free radical that can gain acceptance

TABLE 5. The content of vitamin C, total phenolics, and antioxidant activity of tea infusions.

Samples	Vitamin C (mg/100 mL)	Total phenolic compounds (mg/100 mL)	Antioxidant activity (%)
Berry leaves	$11.05^{a}\pm0.15$	$696.41^{g}\pm1.16$	$42.70^{f}\pm0.12$
Carob	$9.5^{c}\pm0.12$	$993.09^{e}\pm2.32$	$58.29^{d}\pm0.22$
Doum	$7.8^{e}\pm0.07$	$846.31^{f}\pm2.09$	$49.62^{e}\pm0.29$
Berry leaves +Carob	$10.28^{b}\pm0.21$	$1118.89^{b}\pm3.17$	$81.12^{b}\pm0.17$
Berry leaves +Doum	$9.44^{c}\pm0.08$	$1055.67^{c}\pm2.36$	$75.90^{c}\pm0.56$
Carob + Doum	$9.05^{d}\pm0.11$	$1005.38^{d}\pm1.75$	$71.58^{d}\pm0.48$
Berry leaves + Carob+ Doum	$9.45^{c}\pm0.13$	$1405.98^{a}\pm3.26$	$89.72^{a}\pm0.92$
LSD (0.05%)	0.094	1.75	3.83

a, b, c, d, e, f, g: Mean values in each row followed by a different letter are significantly different ($p\leq0.05$).

TABLE 6. The antibacterial activity of tea infusions from berry leaves, carob, doum and their mixtures.

	Inhibition zone (mm) ± SD 10μL						
Bacteria	Berry leaves	Carob	Doum	Berry leaves + Carob	Berry leaves + Doum	Carob + Dom	Berry leaves + Carob + Doum
Staphylococcus aureus	$1.7^{e}_{B}\pm0.08$	$3.0^{c}_{A}\pm0.02$	$2.3^{d}_{B}\pm0.01$	$4.0^{b}_{B}\pm0.12$	$3.7^{b}_{B}\pm0.05$	$4.0^{b}_{B}\pm0.01$	$4.7^{a}_{B}\pm0.05$
Bacillus subtilis	$1.3^{d}_{B}\pm0.08$	$2.0^{c}_{B}\pm0.02$	$0.0^{e}_{D}\pm0.05$	$2.7^{b}_{D}\pm0.07$	$1.7^{c}_{D}\pm0.06$	$2.6^{b}_{D}\pm0.02$	$3.3^{a}_{D}\pm0.09$
Bacillus megaterium	$1.7^{c}_{B}\pm0.03$	$3.0^{b}_{A}\pm0.02$	$3.0^{b}_{B}\pm0.04$	$3.3^{b}_{C}\pm0.11$	$3.3^{b}_{B}\pm0.11$	$3.7^{a}_{C}\pm0.07$	$4.0^{a}_{C}\pm0.05$
Escherichia coli	$2.7^{e}_{A}\pm0.04$	3.0^{e}A±0.01	$3.6^{d}_{A}\pm0.02$	$6.0^{b}_{A}\pm0.05$	$4.4^{c}_{A}\pm0.01$	$5.7^{b}_{A}\pm0.05$	$7.0^{a}_{A}\pm0.06$
Enterococcus faecalies	$0.0^{d}_{C}\pm0.02$	$1.6^{c}_{B}\pm0.01$	$1.3^{c}_{C}\pm0.02$	$2.0^{b}_{E}\pm0.04$	$2.3^{b}_{C}\pm0.04$	$2.0^{b}_{E}\pm0.04$	$3.0^{a}_{D}\pm0.01$

Values are means ± SD of three measurements. Mean values in each row followed by a different small letter are significantly different ($p\leq0.05$). Values in each column followed by a different capital letter are significantly different ($p\leq0.05$).

TABLE 7. The antifungal activity of tea infusions from berry leaves, carob, doum, and their mixtures.

	Inhibition zone (mm) ± SD 10μL						
Yeast	Berry leaves	Carob	Doum	Berry leaves + Carob	Berry leaves + Doum	Carob + Dom	Berry leaves + Carob + Doum
Debaryomyces hansenii	$1.0^{d}_{B}\pm0.03$	$2.0^{c}_{B}\pm0.01$	$*1.1^{d}_{C}\pm0.05$	$2.3^{b}_{B}\pm0.11$	$1.7^{c}_{C}\pm0.01$	$2.4^{b}_{B}\pm0.01$	$3.0^{a}_{B}\pm0.01$
Zygosaccharo mycesrouxii	$1.3^{d}_{B}\pm0.03$	$2.0^{c}_{B}\pm0.01$	$2.0^{c}_{B}\pm0.01$	$2.3^{b}_{B}\pm0.02$	$2.4^{b}_{B}\pm0.05$	$2.8^{b}_{B}\pm0.01$	$3.4^{a}_{B}\pm0.02$
Rhodotorula rubra	$2.7^{c}_{A}\pm0.01$	$3.4^{c}_{A}\pm0.01$	$3.0^{c}_{A}\pm0.02$	$4.3^{b}_{A}\pm0.03$	$3.8^{b}_{A}\pm0.01$	$4.0^{b}_{A}\pm0.05$	$5.0^{a}_{A}\pm0.05$
Candida shehatae	$1.0^{c}_{B}\pm0.01$	$1.3^{c}_{C}\pm0.01$	$0.0^{d}_{D}\pm0.02$	$2.0^{b}_{B}\pm0.04$	$1.8^{b}_{C}\pm0.02$	$2.0^{b}_{C}\pm0.01$	$3.0^{a}_{B}\pm0.01$
Candida tropicalis	$1.4^{c}_{B}\pm0.03$	$1.0^{d}_{C}\pm0.01$	$1.0^{d}_{C}\pm0.01$	$2.0^{b}_{B}\pm0.02$	$2.0^{b}_{C}\pm0.05$	$1.7^{b}_{C}\pm0.03$	$2.7^{a}_{C}\pm0.02$

Values are means ± SD of three measurements. Mean values in each row followed by a different small letter are significantly different ($p\leq0.05$). Values in each column followed by a different capital letter are significantly different ($p\leq0.05$).

electron or hydrogen radical to become a stable molecule. Table 5 shows the scavenging effects of investigated tea infusion samples on DPPH free radicals. Berry leaves + carob + doum tea infusion exhibited a strong ability to quench DPPH radical, followed by berry leaves +carob tea infusion. Meanwhile, the weakest one was observed in berry leaves tea infusion. Data in the same table indicated that the berry leaves + carob+ doum (89.72%), berry leaves + carob (81.12%), berry leaves + doum (75.90%) and carob + doum (71.52%) tea infusion samples were good antioxidants with a strong DPPH radical-scavenging activity. These tea infusions are rich in polyphenolic compounds which possess various bioactivities. The accepted mechanism is that free radical-scavenging activity of polyphenols contributes to the reduction in the oxidative stress and to prevention of diseases development [Huang *et al*., 2001]. The results in Table 5 indicated the LSD at 0.05 level was 0.094 for vitamin C, 1.75 for total phenol compounds and 3.83 for antioxidant activity of the samples.

Antibacterial and antifungal activity of tea infusions from berry leaves, carob, doum and their mixture

In the present study the antimicrobial activities of the berry leaves, carob, doum tea infusions and their mixture against some pathogenic bacteria (*Escherichia coli Staphylococcus auraus, Enterococcus feacalis, Bacillus subtilis* and *B. megate-*

rium) and some yeast *(Debaryomyces hansenii, Zygosaccharo mycesrouxii, Rhodotorula rubra, Candida shehatae* and *Candida tropicalis*) have been established. The antibacterial activity (ABA) and antifungal activity (AFA) of the berry leaves, carob and doum tea infusions and their mixture are displayed in Tables 6 and 7, respectively. Data in Table 6 revealed that the addition of each tea infusion and their mixture inhibited the growth of all tested bacteria. Tassou *et al.* [1997] found that various concentrations of the phenolic tea infusion of carob beans inhibited to a different degree, the growth of most bacteria (*Listeria monocytogenes, Salmonella enteritidis, Staphylococcus aureus S-6* and 722 *Pseudomonas fragi, Shewanella putrefaciens* and *Brochothrix thermosphacta*). The ability to inhibit food-borne pathogens such as *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus,* and *Campylobacter jejuni,* has been reported for different varieties of tea or tea extracts, including Oolong, Jasmine and Black [Si *et al*., 2006]. Tuber extracts of Cyperus showed a remarkable activity against the Gram-positive bacteria: *Staphylococcus aureus* and *Enterococcus faecalis* [Kilani *et al*., 2008]. The combination of three-component tea infusions of berry leaves, carob and doum was the most effective inhibitor of bacterial strains, followed by two-component mixtures of berry leaves and doum, berry leaves and carob and carob and doum, and individual tea infusions had a lesser effect. This finding shows that various combinations of herb tea infusions have a higher inhibitory effect towards the tested bacteria than the individual tea infusions. Synergistic effects were reported by Lee *et al*. [2007]. The combinations of herb extracts (Chinese medicinal plant extracts) showed higher inhibitory effect towards tested bacteria and fungi than the individual tea infusion. Data generally revealed that the largest inhibition zones (7.0, 4.7 and 4.0 mm) were obtained with *Escherichia coli, Staphylococcus aureus* and *Bacillus megaterium*, respectively. This indicates that these bacteria were the most sensitive among the tested bacterial species. On the other hand, *Enterococcus faecalies* and *Bacillus subtilis* were considered as the least sensitive one against the berry leaves, carob and doum. While the doum tea infusion did not show any inhibition against *Bacillus subtilis*. Extracts of karkade and tamarind showed different inhibitory effects on the growth of the pathogenic strains, varying from high, moderate, slight to a negative effect [Abdel-Karem *et al*., 2002].

The results of screening the antifungal activity (AFA) of the berry leaves, carob and doum tea infusions are given in Table 7. Data in this table revealed that the mixture of berry leaves, carob and doum tea infusions exhibited a significant inhibitory effect on the growth of the all tested fungal strains, suggesting that the studied tea infusions alone or in a mixture are potentially a safe and natural source of antifungal agents. The mixtures of tea infusions showed additively or synergistic antimicrobial effect on food borne microbes [Dufour *et al*., 2003]. Results revealed that *Rhodotorula rubra* showed the maximal inhibition zones (5.0 mm) at the three mix herbal extracts followed by *Zygosaccharo mycesrouxii* (3.4 mm) then *Debaryomyces hansenii* (3.0 mm). On the other hand, *Candida shehatae* and *Candida tropicalis* were considered as the least sensitive ones against the all herbal tea infusions tested, which indicates that these yeast were the most resistant among the tested yeast species. Finally, the doum herbal tea infusions did not show any inhibition against *Candida shehatae*.

Results in Tables 6 and 7 indicated that extracts studied were generally more effective against bacteria than yeast. The mixture tea infusion is expected for incorporating in various food products where a naturally antimicrobial additive is desired. However, further studies are needed to examine how the combinations of herbal tea infusions exhibit their antimicrobial activity in a practical food system. Among those antimicrobial compounds, phenolic compounds and alkaloids are very important components in antimicrobial or antioxidant effects, and epidemiologists have observed that a diet rich in those compounds may result in a positive health effect [Fernandez *et al*., 1996; Ríos & Recio, 2005]. From the forgoing results, it appeared that herbal extracts exhibited antimicrobial activities against food spoilage as well as food pathogenic microorganisms.

CONCLUSION

In conclusion our results indicated that the berry leaves, carob and doum tea infusions and their mixtures, specially berry leaves+ carob+ doum sample, were rich in phenolic compounds which posses various bioactivities. This sample holds a good natural antioxidant with strong DPPH radical scavenging activity. The results confirmed also that berry leaves+ carob+ doum tea infusions mixture exhibited antimicrobial activities against spoilage as well as food pathogenic microorganisms. From our results it appears important to develop natural antioxidants and bacterial inhibitors from these tea infusions, and this may be a good way to extensively utilize the berry leaves, carob and doum resource.

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