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PRELIMINARY SCREENING FOR ANTIBACTERIAL ACTIVITY OF *COFFEA ARABICA* BEANS (ROASTED AND UNROASTED) AGAINST DIFFERENT PATHOGENS

Research article

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ABSTRACT

Arabic Coffee (*Coffea arabica* L.) is one of the most famous beverages in the world. A Yemeni cultivar of *Coffea arabica* was screened for its antibacterial potential against 10 different referenced bacterial strains, using two methods; disc diffusion and cup-plate diffusion assays. The results revealed that ethanol extract (70% v/v) did not show any antibacterial activity using disc diffusion method, whereas weak antibacterial activity, varying between 6.5 to 8.5 mm inhibition zones, was observed with the un-roasted (green) beans against some bacterial strains, using cup-plate diffusion method, while the roasted coffee showed no antibacterial activity. The study concludes that roasting processes and long storage could reduce the potential antibacterial activity and recommend further future investigation using other different solvents since ethanol was not the best solvent to extract the presumable antibacterial molecules from *Coffea arabica* beans.

Keywords: *Coffea arabica*, antibacterial, disc-diffusion test, agar cup-plate method, pathogens

INTRODUCTION

Since more than 2000 years ago, Hippocrates said: "Let food be thy medicine and medicine be thy food" **(Smith, 2004).** Natural food and beverages are essential in the promotion of health and disease prevention. However, in this era, modern lifestyle has changed the food habits and led to numerous nutritional deficiency diseases **(Pandey *et al.*, 2010).**

Coffee is one of the most famous (non-alcoholic) drinks in the world. Coffee trees belong to the family *Rubiaceae* and there are more than 100 species of *Coffea* L.; however, only two species are commercially cultivated, which are *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee) **(Mishraand Slater, 2012).** Historically, Arabic coffee is an ancient plant product, believed that it is first exploited in Yemen since 575 AD; However, it could be known earlier in southwestern Ethiopia highlands since around 1500 years ago **(Anthony *et al.*, 2002).** It was found that *Coffea arabica* (*C. arabica*) contains many phytochemical compounds, such as alkaloids, particularly Caffeine which is the most important alkaloids present in high quantity in *C.arabica*, phenolic compounds, terpenoids that gives coffee its distinctive aroma, carotenoids and enzymes **(Patay *et al.*, 2016)** and many more. The reported biological activities of *C.arabica* include many bioactive properties such as antioxidant **(Mussatto *et al.*, 2011),** anti-tumor **(Huang *et al.*, 1988),** anti-cancer **(Ross, 2005),** hepatoprotective **(Lima *et al.*, 2013),** antibacterial **(Almeida *et al.*, 2006),** wound healing **(Affonso *et al.*, 2016)** and it is also used as stimulant on the central nervous system so it could probably reduce the incidences of Parkinson's disease **(Patay *et al.*, 2016).** The purpose of this study was to investigate the antibacterial properties of *C.arabica* beans against different pathogens, and also to compare between the green beans of coffee and the roasted beans, to see if this process affects on its potential antibacterial efficacy.

MATERIALS AND METHODS

Plant material and extraction

Arabic coffee beans (*C.arabica*) were purchased from a coffee store in Alrass town, Saudi Arabia. A Yemeni

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cultivar was chosen and authenticated. The Yemeni coffee beans were divided into two parts, green coffee beans (unroasted) and a roasted coffee beans (average roasting). 100 grams from each part was ground into fine powder and macerated in 500 mL of ethanol (70% v/v), for up to 3 days in a well tighten container inside an incubator at 40°C, with frequent shaking. Then, the macerates were filtered with muslin cloth and Whatman filter paper (No.1), the filtrates were evaporated to get a semi-solid crude, which was put in an incubator at 45°C for up to 3 days to getdryextracts. The ethanolic extracts (roasted and unroasted coffee beans) were reconstituted in 10% Dimethylsulfoxide (DMSO) to get 500 mg/mL of concentration, which was kept in a well tighten glass container s in a refrigerator until used.

Microorganisms

Ten referenced bacterial strains representing different Gram-positive and Gram-negative pathogens were used in this study (Table 1). Bacterial strains were sub-cultured in nutrient broth for 18 hours at 37°C to reach the exponential phase. Then, adjusted to 0.5 McFarland's standard to get bacterial density equivalent to around 1.0 × 10^8 CFU/mlL (CFU: Colony forming unit), which was directly used in the antibacterial activity test.

Table 1: Microorganisms used in the evaluation of antibacterial properties of *Coffeaarabica*beans.

Type of microorganisms	Scientific name	Strain code
Gram-positive Bacteria	*Bacillus cereus*	ATCC® 10876™
	Staphylococcus epidermidis	ATCC® 12228™
	Staphylococcus aureus	ATCC® 29213™
	Staphylococcus saprophyticus	ATCC® 43867™
	Streptococcus pneumonia	ATCC® 49619™.
Gram-negative Bacteria	*Escherichia coli*	ATCC® 25922™
	Proteus vulgaris	ATCC® 6380™
	Klebsiella pneumonia	ATCC® 27736™
	Pseudomonas aeruginosa	ATCC® 9027™
	Shigellaflexsneri	ATCC® 12022™

Testing for antibacterial activity

Disc diffusion test

Paper discs (6 mm in diameter) were cut from Whatman No.1 filter paper, put into well-tighten bottle and sterilized in an autoclave. About 20mL of the autoclaved Mueller Hinton agar were poured onto sterile disposable Petri dishes and left at room temperature until solidified. Petri dishes were then turned upside down and kept in the refrigerator for a while to get rid of any water droplets. 100µL of the previously adjusted bacterial suspensions were gently spread on the surface of the solidified Mueller-Hinton Petri-dishes, using sterile cotton swabs. Some sterile blank discs were saturated with the re-constituted extract at a concentration 500 mg/mL and put over the inoculated Petri dishes. Another group of sterile blank discs were saturated with 5 mg/mL chloramphenicol were also loaded, to serve as positive control. The solvent, 10% DMSO did not show any inhibition effect on bacteria. The seeded Petri dishes were incubatedat 37°C for 24 hrs. The susceptibility of the bacteria towards the extract was expressed as the mean zone of growth inhibition in millimeter (mm) **(Abdallah, 2016).**

Cup-plate diffusion test

Bijou Bottles containing 20mL of autoclaved Mueller-Hinton agar were poured on sterile Petri dishes (90 mm in diameter) and left to solidify at room temperature. Then, the previously adjusted bacterial stains were spread over the agar plates using sterile cotton swabs. 4 wells were punched on the agar surface of each Petri-dish using flamed cork borer (6 mm in diameter). 50µL from the reconstituted extracts (500 mg/mL) were taken with Eppendorf pipette and loaded into respective wells, also

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50µL of chloramphenicol (2.5 mg/mL) was loaded to a separate well and served as positive control. The pre-experimental testing showed that 10% DMSO has no effect on bacterial growth. The prepared plates were incubated for 24 hours at 37°C. After incubation, only clear zones of inhibition around the cups were measured in millimeters (mm) with a ruler and the mean of two zones were calculated **(Mekonnen *et al.*, 2016).**

Statistical analysis

Some quantitative data were expressed as a mean ± standard deviation; Paired-Samples T-test was employed to determine possible significant antibacterial differences between the ethanol extract of *C. arabica* and the antibiotic; chloramphenicol, at $P < 0.05$. The program used in tabulation and graphing was SPSS-Statistical Package, version 11.

RESULTS AND DISCUSSION

The antibacterial potential of the hydro-alcoholic (70%) ethanol extracts (Roasted and unroasted) of*C. arabica* beans, were determined by the disk diffusion and the cup-plate diffusion methods. Concentrations of 500 mg/mL were used for assaying the crude extracts against all test bacteria, which were 10 different ATCC bacterial strains (Table 1). The results of disc diffusion test showed no antibacterial activity (Table 2), while only weak or no antibacterial activity was recorded with the cup-plate diffusion method (Table 3). Moreover, as shown in (Table 3), the unroasted extract of *C. arabica* beans exhibited weak antibacterial activity, which was ranging between 6.5 to 8.5 mm inhibition zones. Whereas, the roasted extract of *C. arabica* beans showed approximately no antibacterial effects, which were ranging between 6 to 6.25 mm inhibition zones. These results were non-significant ($P<0.05$) when compared with the reference antibiotic, the chloramphenicol (Figure 1). Moreover, it was observed that, the cup-plate diffusion test was better in the antibacterial evaluation than the disc-diffusion test (Figure 2), it could be related to the quantity of the extract, in the disc diffusion method, the 6 mm blank disc absorb only 15µL of the extract solution, while in the cup-plate diffusion method the quantity of the extract solution that loaded in the 6mm cup was 50µL.

Although, each method has its own advantages. In general, studies on *C. arabica* in Arab countries are scant and no data found regarding the antibacterial activity of the Yemeni cultivar of *C. arabica*. However, the results of the current investigation are in contradiction with the previous studies on the antibacterial potential of *C. arabica* from other different localities; **Daglia *et al.*, (1994)** cited that the aqueous extract of the roasted beans of *C. arabica* has an antibacterial activity. **Duangjai *et al.*, (2016)** stated that the aqueous extract of *C. arabica* showed varied antibacterial activities against *Staphylococccus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Although, **Wijaya *et al.*, (2016)** metioned that beans of Robusta coffee ethanolic extract recorded antibacterial activity greater than the Arabica coffee extract. In our study, the ethanol extract of the roasted beans of *C. arabica* did not show any antibacterial effects compared with the green (unroasted) beans, which may be due to lost or denaturation of some bioactive molecules during roasting. Our results could be supported by the findings of **(Patay *et al.*, 2016)** who reported that, in Coffee, some phytochemical contents such as alkaloids (Caffeine) is reduced during the roasting process. Accordingly, the weak activity showed from the current study is related to two main reasons; firstly, the nature of the antibacterial molecules present in *C. arabica* are tending to be more polar, as most of the previous studies tested mostly the aqueous extracts. Secondly, the antibacterial nature of *C. arabica* affected greatly by long storage. The author tends to the second interpretation, **Selmar *et al.*, (2008)** metioned that Storage of Coffee beans for a prolonged time might be the cause of the decrease in potential aroma precursors. Yet, the compound(s) responsible of the claimed antibacterial efficacy of *C. arabica* remains unknown; **Almeida *et al.*, (2012)** considered that the antibacterial efficacy of *C. arabica* is attributed to the presence of Caffeine. **Runti (2015)** claimed that the decaffeinated aqueous extract showed good antibacterial activity and this activity is independent from caffeine content. In conclusion, since the ethanolic extract of the un-roasted beans of *C. arabica* showed weak antibacterial activity against different pathogens, so the antibacterial molecules are presumably present in low quantity, meaning that 70% ethanol is not the best solvent for this plant product. It was reported that the extraction parameters affected the yield of the bioactive constituents of the plant products **(Baldosano *et al.*, 2015).** Accordingly, a further future study using different solvents is recommended in order to isolate these antibacterial molecules, if present. On the other side, based on the findings of the current study, the roasting process could decrease the potential antibacterial activity.

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Table 2: The antibacterial activity of the ethanol extracts of *Coffea arabica* beans using disc diffusion test

Tested compounds	Mean zone of inhibition (mm)									
	Gram-positive bacteria					Gram-negative bacteria				
	Sa	Se	Ss	Sp	Bc	Ec	Pa	Pv	Kp	Sf
Roasted (500 mg/mL)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Unroasted(500 mg/mL)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Chloramphenicol (5 mg/mL)	36	35	33	29	36	30	22	28	27	29

*6.0= No inhibition (the disc diameter), Sa=*Staphylococcus aureus* ATCC29213, Se=*Staphylococcus epidermidis* ATCC 12228, Ss=*Staphylococcus saprophyticus* ATCC 43867, Sp=*Streptococcus pneumonia* ATCC 49619, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 25922, Pa=*Pseudomonas aeruginosa* ATCC 9027Pv=*Proteus vulgaris* ATCC 6380, Kp=*Klebsiella pneumonia* ATCC 27736, Sf=*Shigella flexsneri* ATCC 12022.

Table 3: The antibacterial activity of the ethanol extract of *Coffea arabica* beans using cup-plate diffusion test

Tested compounds	Mean zone of inhibition±Std.Deviation									
	Gram-positive bacteria					Gram-negative bacteria				
	Sa	Se	Ss	Sp	Bc	Ec	Pa	Pv	Kp	Sf
Roasted(500 mg/mL)	6.25 ±0.35	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.25 ±0.35	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.25 ±0.35
Unroasted(500 mg/mL)	7.0 ±0.0	8.0 ±0.0	7.0 ±0.0	6. 5 ±0.0	8.0 ±0.0	7.25 ±0.35	9.0 ±0.7	6.5 ±0.0	7.5 ±0.7	8.5 ±0.7
Chloramphenicol (2.5mg/mL)	36.0	35.0	33.0	33.0	36.0	30.0	22.0	28.0	27.0	26.0

*6.0= No inhibition (the disc diameter), Sa=*Staphylococcus aureus* ATCC29213, Se=*Staphylococcus epidermidis* ATCC 12228, Ss=*Staphylococcus saprophyticus* ATCC 43867, Sp=*Streptococcus pneumonia* ATCC 49619, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 25922, Pa=*Pseudomonas aeruginosa* ATCC 9027Pv=*Proteus vulgaris* ATCC 6380, Kp=*Klebsiella pneumonia* ATCC 27736, Sf=*Shigella flexsneri* ATCC 12022.

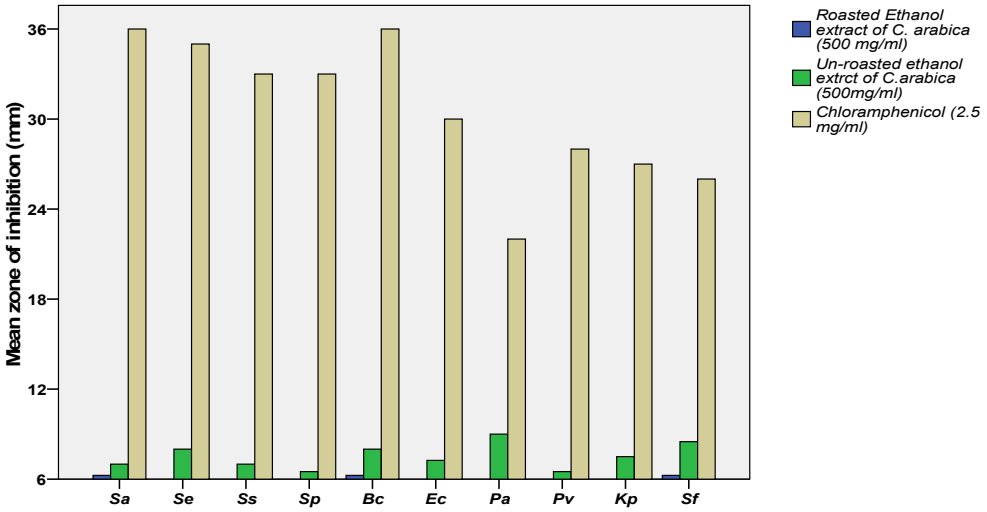

Figure 1: Weak antibacterial effects compared with the antibiotic, using cup-plate diffusion method

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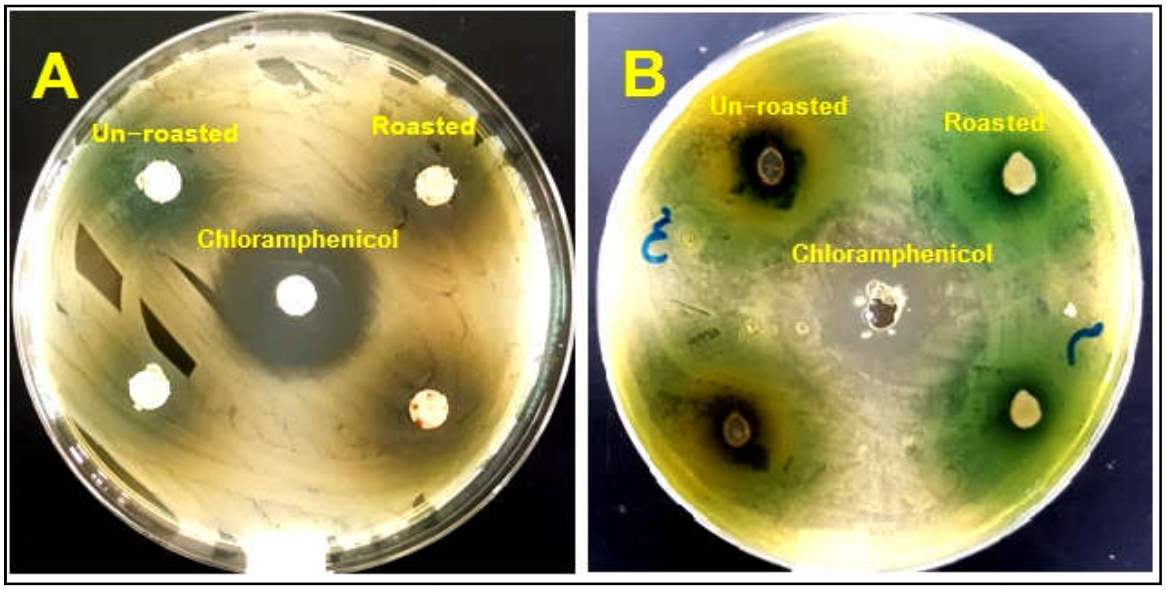

Figure 2: Representative photo showing a comparison between (A) disc diffusion and (B) cup-plate diffusion methods

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