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ANTIMICROBIAL ACTIVITY OF INSTANT COFFEE AGAINST SOME MULTI-DRUG RESISTANCE BACTERIA AND PATHOGENIC FUNGI

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ABSTRACT: This paper presents the antimicrobial activity of instant coffee, a prevalent beverage utilized by millions of people worldwide every day. Several studies have shown that instant coffee contains many useful bioactive compounds, the most important: caffeine, trigonelline, chlorogenic acid, ferulic acid, caffeic acid, nicotinic acid, and phenolic compounds which are responsible for the antimicrobial activity. In this study, six strains of resistant bacteria were selected as follows (Methicillin-resistant *Staphylococcus aureus* MRSA, *S. aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, and *Acinetobacter baumannii*). The antibiotic sensitivity test revealed that the most tested bacterial strain was utterly resistant to the selected antibiotic discs. The antimicrobial activity of instant coffee was evaluated at different concentrations (20, 40, 60, and 80 mg/ml) against tested multi-drug resistant bacteria (MDR) and the fungi (*A. niger* and *A. flavus*). The results of antibacterial effect of instant coffee showed that the inhibition zone ranged between (7.6±0.5 and 19.6±0.5 mm). While the minimum inhibitory concentration (MIC) ranged between (62.5 and 32.25 mg/ml) against tested bacteria. Moreover, the instant coffee significantly inhibited the mycelial growth of *A. flavus* and the spore germination of *A. niger*. Based on the results obtained in this research, several future studies can propose to evaluate the biological activity of coffee, such as the molecular effect of coffee bioactive compounds against human microbiome. In addition to various medical and industrial applications.

INTRODUCTION: The widespread use of antibiotics against bacterial infections has led to developing resistance bacteria to the most commonly used antibiotics. Because bacteria are becoming more resistant to current antibiotics, new forms of antibiotics need to be created to manage and treat resistant pathogens [1]. Concerning treatments, plant metabolites with antimicrobial activity may be of great importance. Tea and coffee were studied previously for this purpose [2].

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The two crucial coffee classes are *Coffea arabica* and *Coffea robusta* [3]. Due to its pleasant taste and aroma add to the stimulating effect, coffee is one of the most popular and widely consumed beverages worldwide [4]. Also, coffee is linked to various beneficial health properties, including antioxidant, antimicrobial, and anti-inflammatory [5]. Moreover, coffee intake can protect against many non-infection diseases [6].

Coffee is a source of a high bioactive molecule, in particular caffeine, polyphenols, mostly chlorogenic acid, caffeic acid, and ferulic acid; these compounds help to total dietary polyphenol intake and are beneficial to consumer health [7, 8]. Caffeine is a natural alkaloid present in some plants such as coffee beans, tea leaves and is considered one of the most commonly used therapeutics vehicles in

the world [9]. Caffeine has several biological effects, including immune-modulatory, antioxidant, anti-cancer, and antimicrobial activity [2]. Also, it has a synergistic effect with different antibiotics such as carbenicillin, ceftizoxime, and gentamicin, which are effective against multi-drug resistant bacteria [10]. Antimicrobial activity of coffee is variant according to its chemical composition, type, and processing, including roasting and decaffeination [11, 12]. Moreover, early studies indicate that anti-microbial activity is restricted to roasted coffee, while it is not present in raw coffee [13]. Therefore, roasting coffee products were used to investigate antimicrobial activity.

Caffeine, which is about 2% in robusta coffee, reduce the growth rate of *Aspergillus versicolor*, *Penicillium spp*, and at specific concentrations, it can also prevent mycotoxin production, such as aflatoxin produced by *A. parasiticus* [14, 15]. Chlorogenic and caffeic acid, which are non-volatile organic acids found in coffee, has an antibacterial effect against Gram (+) and (-) bacteria. During roasting of coffee, volatile compounds formed, including ketones, aldehyde, and phenolic compounds, which are reported to have antimicrobial activities, and some phenolic compounds have an antibacterial effect [16]. On the other hand, roasted coffee has an antibacterial effect against a wide range of Gram (+) and (-) bacteria [3]. Moreover, *Coffea robusta*, which is the mostly instant coffee used, showed better antimicrobial activity than *Coffea arabica* [4].

Most literature reviews have been performed on the raw and roasted coffee beans. Therefore, the present study aimed to determine the antimicrobial effect of instant coffee at the different concentration on the growth of some antibiotic-resistant bacteria (MRSA, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumonia*, and *A. baumannii*), and the pathogenic fungi (*A. niger* and *A. flavus*).

MATERIALS AND METHODS:

Microorganisms: The bacterial strains (MRSA, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumonia*, and *A. baumannii*), and the fungi (*Aspergillus niger* and *Aspergillus flavus*) were obtained from the microbiology laboratory of King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia. Standard methods have been used to identify the morphological and biochemical characterization of all isolates.

Antibiotic Sensitivity Test: Antibiotic efficiency against bacterial strains was tested, and the presence and measurement of inhibition have evaluated their performance [17]. The bacterial strain (MRSA, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumonia*, and *A. baumannii*) were tested against the antibiotics: Ciprofloxacin (CIP-5 µg), Ceftriaxone (CTR-30 µg), Meropenem (MRP-10 µg), Ceftazidime (CAZ-30 µg), Imipenem (IPM-10 µg), Ofloxacin (OF-5 µg), Cefoperazone (CFP-75 µg), Meropenem (MEM-10 µg), Gentamicin (GEN-10 µg), Amikacin (AKN-10 µg), Levofloxacin (LEV-5 µg), Piperacillin/Tazobactum (PIT100/10 mcg), Ampicillin/Sulbactam (A/S-10/10 mcg), Amoxicillin /Clavulanic acid, (AMC-30 µg), Ticarcillin/ Clavulanate (TCC-75/10 mcg) and Trimethoprim/ Sulfamethoxazole (SXT-25 µg).

Preparation of Instant Coffee Solutions: The instant coffee powder used in this study was obtained from the local Saudi market, which belongs to one of the most available and popular companies worldwide. Four concentrations (20, 40, 60, and 80 mg/ml) of instant coffee solutions were prepared freshly with distilled water and sterilized in the oven at 160 °C for one hour. Each concentration was kept in a shaking water bath at 50 °C for 15 min [18].

Antibacterial Activity of Instant Coffee Solutions: The bacterial suspensions of (MRSA, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumonia*, and *A. baumannii*) prepared at a concentration of 0.5 McFarland, then were cultured on Mueller-Hinton agar and allowed to dry for 1 h. Agar wells diffusion test was used through punched out the agar plate using sterilized cork borer (6mm), then 50 µL from each concentration of a prepared coffee solution were dissolved in 10% DMSO, and applied into each well [19]. DMSO is used as a negative control. The plates were incubated for 24 h at 37°C. After that, the inhibition zones were estimated in (mm).

Minimum Inhibitory Concentrations: The coffee solution, which yielded a positive result in the agar well diffusion test, were used as an antibacterial agent for measurement (MICs) using a micro-broth

dilution procedure [19]. Bacterial culture of 0.5 McFarland was prepared in Mueller-Hinton broth to obtain (1×10^8 bacterial/ml). 50 µl of bacterial suspension has been applied to each well, except for negative control. 100µlof coffee solution diluted in 10% DMSO was added to each well of the 96-well microtiter plate to obtain eight concentrations (500, 250, 125, 62.5, 31.25, 15.62, 7.6 and 3.9 mg/ml). The micro-titer plates were covered and incubated at 37 °C for 24 h. DMSO 10% were used as the negative controls. The coffee-free broth media is used as a positive control. The optical density (OD_{600}) of antibacterial activity was measured using a microtiter plate reader (BioTek Instruments, Inc., USA).The percent of bacterial growth inhibition estimated using the formula:

$$[(Ac–At)/Ac] \times 100$$

Where (Ac) is an average of three replicates of O.D. values at 600nm of the negative controls, and (At) is an average of three replicates of O.D. absorption values of the test samples. The (MIC) value was known as the lowest concentration that inhibited apparent growth.

Antifungal Activity of Instant Coffee: Fungal strains were grown in potato dextrose agar (PDA) for five days at 26 °C. Then coffee agar plate method was conducted to examine the antifungal activity of instant coffee through mixing PDA medium with different concentrations of instant coffee (20, 40, 60, 80 mg/ml). Coffee agar plate inoculated with sterile (6mm) fungal disk of *A. niger* and *A. flavus*, then incubated for sex days at 26 °C. The antifungal effect of instant coffee against tested fungi was done by estimating the amount of fungal growth by observing the formation of mycelium and spore germination at different coffee concentrations during and after incubation. Control fungal plates cultured on PDA agar were included in each experiment under the same conditions as a positive control. All experiments were performed in triplicate.

Statistical Analysis: All experiments were performed three times, and mean ± standard deviation values determined using SPSS statistics. The level of significance for all measurements was determined at $P<0.05$.

RESULTS AND DISCUSSION: Most of the bacterial strain in the current study were resistant to several antibiotics, whereas the sensitivity profile of tested strains (MRSA, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumonia*, and *A. baumannii*) revealed that *K. pneumoniae* was sensitive to Imipenem (IPM-10 µg) and Gentamicin/Amikacin, while *S. aureus* was sensitive to Levofloxacin (LEV-5 µg) and Amikacin (AKN-10 µg). The other bacterial strains showed complete resistance to all types of tested antibiotics **Fig. 1**. Accordingly, all tested bacterial strains are considered Multi-drug resistant bacteria.

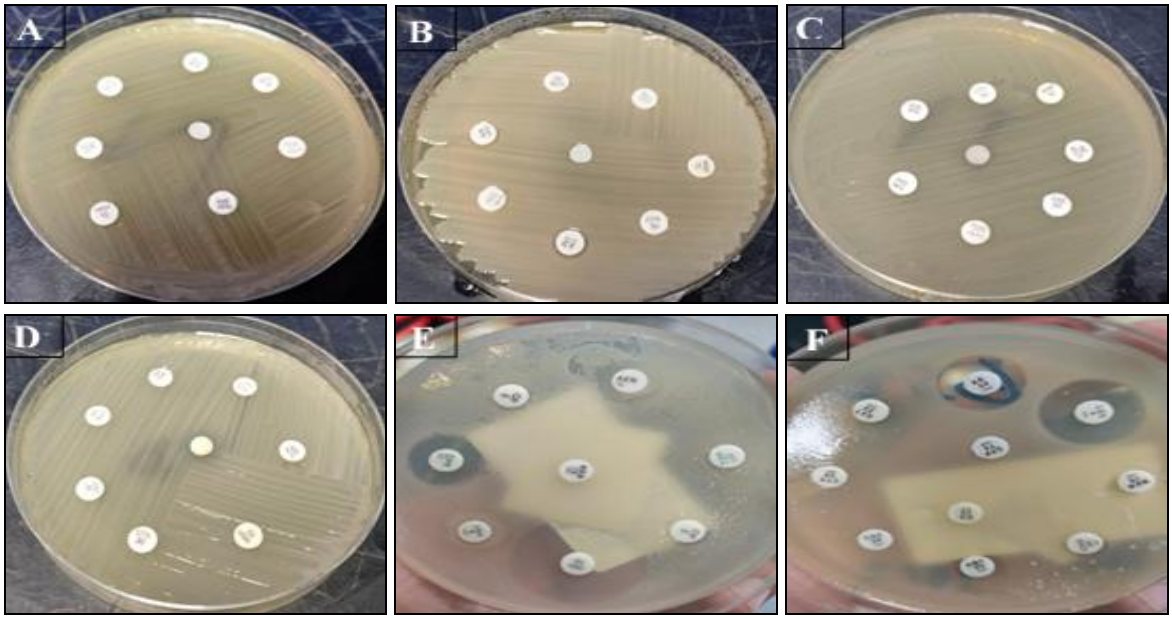

FIG. 1: ANTIBIOTICS SENSITIVITY TEST AGAINST TESTED BACTERIA. (A) MRSA (B) *E. COLI* (C) *A. BAUMANNII* (D) *E. FAECALIS* (E) *K. PNEUMONIAE* (F) *S. AUREUS*

The results in **Table 1** revealed that instant coffee solution showed a significant antibacterial effect against tested bacterial strains (MRSA, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumonia*, and *A. baumannii*). The inhibition zones ranged between (7.6±0.5 and 19.6±0.5 mm). The maximum inhibition zone was observed against *A. baumannii* and *E. faecalis* (19.6±0.5 and 19.0 mm), and the minimum inhibition zone reported was against *S. aureus* (7.6±0.5). The effectiveness of instant coffee was evaluated by comparing the respective diameters obtained in agar well diffusion assay against tested bacterial isolates.

Instant coffee solutions at the concentrations (40, 60, and 80 mg/ml) gave (13±0.5, 16.6±0.5 mm) an inhibition zone against MRSA. The same concentrations reported (7.0±0.5, 17.3±0.4, and 17.6±0.5 mm) against *S. aureus*, and (9.6±0.2, 19±0.0 mm) against *E. faecales*. However, it gave (9.0±0.5, 14.3±0.5, and 15±0.0 mm) against *E. coli*. The diameter of inhibition zones against *K. pneumoniae* and *A. baumannii* were ranged between (17.0±0.0 and 19.6±0.5 mm) at the concentrations (60 and 80 mg/ml), respectively.

Supposition that the inhibition zone's diameter might increase with an increase in the concentration and time of exposure of instant coffee, the bacterial isolates monitored for more than 48 h. However, there was no increase in the diameter of the inhibition zones. Our results agreed with that obtained by Ribeiro *et al*., [20] who reported that instant coffee has antibacterial activity against *S. aureus* and *E. coli*. Moreover, Almeida *et al*.,[21] found that the inhibition zone of arabica coffee was much smaller than the current study's value, whereas the antibacterial effect of instant coffee in the present study was higher, it could attribute to having a higher percentage of antibacterial compounds in instant coffee than that of Arabica coffee [22]. Another study reported by Muslim and Dephinto [23] showed antibacterial activity of Robusta coffee water extract against *S. aureus* and *E. coli*. On the other hand, Ullah *et al*., [24] reported that instant coffee, showed suitable antibacterial activities against *P. aeruginosa*, *S. aureus*, and *E. coli*, with maximum inhibition zone (18mm) against *S. aureus*, which is very close to our results. Over and above Duangjai *et al*., [25] demonstrated that coffee pulp extracts had antibacterial activity against both Gram (+) (*S. aureus* and *S. epidermidis*) and the Gram (-) bacteria (*P. aeruginosa* and *E. coli*).

The results illustrated in **Table 2** explain the value of (MIC), which is used to determine the effectiveness of instant coffee accurately. (MIC) values exhibited antibacterial activity ranged between (31.25 and 62.5mg/ml). The instant coffee solution gave (MICs) values of (62.5mg/ml) against *E. coli, S. aureus, E. faecalis*, and MRSA, while it was (31.25mg/ml) against *A. baumannii* and *K. pneumonia* for both of them. Our results were similar to those obtained by Akhlaghi *et al*., [26] who found that (MIC) of coffee achieved at (62.5mg/ml) against *S. mutans*.

TABLE 1: INHIBITION ZONE OF INSTANT COFFEE SOLUTION AT DIFFERENT CONCENTRATION AGAINST TESTED BACTERIA

Bacterial strains	Inhibition zone (mm) at different coffee con. (mg/ml)				
	(-) control	20	40	60	80
MRSA	-	-	13±0.5	16.6±0.5	16.6±0.5
S. aureus	-	-	7.0±0.5	17.3±0.5	17.6±0.5
E. faecalis	-	-	9.6±0.2	19±0.0	19±0.0
E. coli	-	-	9.0±0.5	14.3±0.5	15±0.0
K. pneumonia	-	-	-	17.6±0.5	17±0.0
A. baumannii	-	-	-	17.4±0.0	19.6±0.0

(-) Control: Negative control (10% DMSO). (–) No antibacterial activity. Data are expressed as Mean ± SD of triplicate experiments.

TABLE 2: (MIC) OF INSTANT COFFEE SOLUTION AGAINST TESTED BACTERIA

Bacterial strains	MIC (mg/ml)
MRSA	62.5±0.8
S. aureus	62.0±0.8
E. faecalis	62.5±0.8
E.coli	62.5±0.8
K. pneumonia	31.25±0.4
A. baumannii	31.25±0.4

The antifungal activity of instant coffee was studied against *A. niger* and *A. flavus* at different concentrations (20, 40, 60, and 80 mg/ml) by monitoring the fungal mycelium growth and the spore germination using coffee agar plate test **Table 3**. As a result, there is significant growth inhibition of *A. flavus* **Fig. 2B**, while it was

inhibited only the spore germination of *A. niger*, compared to the control **Fig. 2A**. The inhibitory effect of instant coffee was ranged between 60 to 80 mg/ml with the pronounced growth inhibition of *A. flavus* while it was unclear for *A. niger*. The spore germination of *A. niger* inhibited with increasing coffee concentration gradually. In comparison, it has no effect on the spore germination of *A. flavus* concentrations. Overall, instant coffee had a significantly high inhibitory effect on both *A. niger* and *A. flavus*. Similar results were obtained by Nonthakaew *et al*., [9] who's observed antifungal effect of coffee extract against *Aspergillus niger, Aspergillus flavus, Penicillum chrysogenum,* and *Penicillum citrinum*.

TABLE 3: EFFECT OF INSTANT COFFEE ON FUNGI BY USING COFFEE AGAR PLATE AT DIFFERENT CONCENTRATION AFTER 6 DAYS AT 26 °C

Coffee Con. mg/ml	*A. niger*		*A. flavus*	
	Mycelium	Spores	Mycelium	Spores
Control	+ + +	+ + +	+ + +	+ + +
20	+ + +	+ + +	+ + +	+ + +
40	+ + +	+ +	+ +	+ +
60	+ + +	+ +	+	+
80	+ + +	+	+	+

(+): Weak growth, (++): Moderate growth, (+++): Heavy growth. Control: PDA medium without coffee

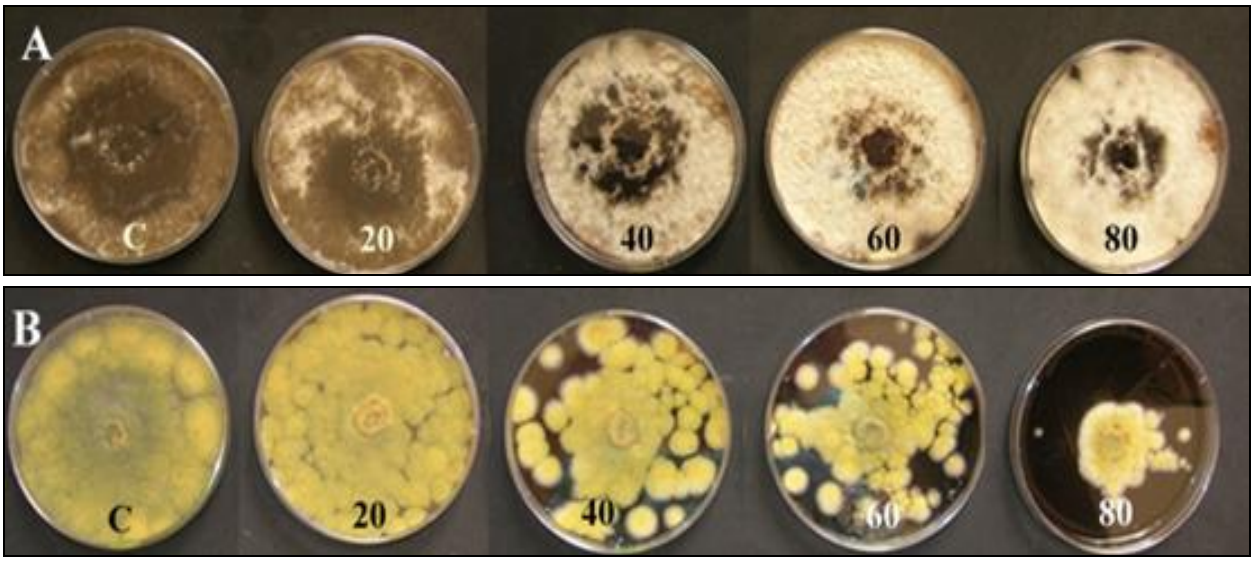

FIG. 2: ANTIFUNGAL ACTIVITY OF INSTANT COFFEE AT (20, 40. 60 and 80mg/ml) AGAINST (A) *A. NIGER* AND (B) *A. FLAVUS* AFTER 6 DAYS AT 26 °C

To identify the bioactive components that exhibit antimicrobial activity in instant coffee, previous studies reported that caffeine, trigonelline, chlorogenic acid, ferulic acid, caffeic acid, nicotinic acid and phenolic compounds are the primary chemical compounds responsible for the antimicrobial activity [27]. Therefore, the antimicrobial activity is not related to a distinct chemical or particular pathway, but many compound and cell target [28]. Moreover, Aromatic and phenolic substances demonstrate antibacterial effect by modifying the plasma membrane's composition and function, disrupting active transport, electron transfer, and proton motive force [29]. The process responsible for phenolic toxicity to microorganisms can also involve inhibition of the enzyme by oxidized molecules, probably by interactions with sulfidrylic groups or many nonspecific protein interactions [30].

While caffeine is one of the main components in coffee, several derivatives of caffeine, such as xanthin, have also been reported[31]. Furthermore, different amounts of caffeine compounds have been reported in green and roasted beans for both Arabica and Robusta coffee [32]. Therefore, the literature studies have indicated caffeine's antimicrobial activity as the primary compound and its minor composition. It has been observed that caffeine will move through the bacterial cell wall, then, continue inhibiting the DNA synthesis. Lower

DNA contributes to reducing development in all bacterial cells. Therefore, enzyme and protein synthesis also decreased [33].

In fungi, caffeine can prevent the germination of spores. Therefore, the lag time is then prolonged and observed. Moreover, Kumar *et al*., [34] concluded that caffeine was shown to inhibit the growth of two strains of *C. albicans*, and inhibiting both mycelium development and spore germination of *Monacrosporium ambrosium* [34]. This finding agreed with our results and can explain the inhibitory effect that occurred with tested fungi. Furthermore, caffeine can inhibit aflatoxins production by preventing some critical sugar synthesis such as glucose, fructose, and maltose [35, 36, 37].

CONCLUSION: Our finding proves the antimicrobial activity of instant coffee against the tested multi-drug resistant bacteria (MRSA, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumonia*, and *A. baumannii*), as well as, an antifungal effect against (*A. flavus* and *A. niger*). Among the tested bacteria, *A. baumannii* was the most sensitive to instant coffee according to the inhibition zone (19.6±0.5 mm). Moreover, the sensitivity of different bacteria and fungi can vary depending on coffee concentration. Furthermore, fungi are more sensitive to coffee in comparison to bacteria. The antimicrobial activity in coffee is attributable to many bioactive compounds such as caffeine, trigonelline, chlorogenic acid, ferulic acid, caffeic acid, nicotinic acid, and phenolic compounds. Consequently, instant coffee can be used as a potential alternative antimicrobial substance due to its antimicrobial properties. It will be interesting to study the bioactivity and action mode of different coffee types and components for crucial medical and industrial application, concerning its antimicrobial, antioxidant, anticancer, and immune-modulatory activity.

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