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Assessment of Ultrasound-Assisted Extraction of Caffeine and its Bioactivity

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ABSTRACT

This research focused on using spent coffee grounds as a source of caffeine by ultrasound-assisted extraction technique. Two types of ultrasound techniques (ultrasonic bath and ultrasonic probe) were studied to extract caffeine. The effect of the sonication type sonication power, extraction time, and extraction temperature on the extraction efficiency was investigated. The results demonstrated that extraction by an ultrasonic probe is superior to ultrasonic bath extraction. The highest caffeine recovery was obtained at 55 °C by using an ultrasound bath where caffeine concentration was 795.74 mg/L after 60 min. The bioactivity of extracted caffeine was also studied and compared with the bioactive of standard caffeine against *candida albicance* yeast. The results showed that the minimum inhibition concentration for natural caffeine was 100 mg/L which is half of the minimum inhibition concentration of standard caffeine.

Keywords: sonication, spent coffee grounds, caffeine, c*andida. Albicans.*

INTRODUCTION

Coffee has been consumed as a beverage for a long time (Blinová et al., 2017). Coffee beans are the feedstock for producing coffee beverages by soluble coffee industries (Campos-Vega et al., 2015). The making of coffee beverages can take place in houses, cafeterias, restaurants, and cafes. Caffeine (1, 3, 7-trimethyl-3, 7-dihydro-1H-purin-2, 6-dion) is a methyl xanthine alkaloid of chemical formula $C_8H_{10}N_4O_2$. It is essentially obtained from coffee beans and leaves (Musa Ali et al., 2012), and also it can be found in tea leaves (Soni, 2019), cocoa beans (Grassia et al., 2019), and kola-nut (Umeda et al., 2020). This alkaloid is the most valuable, and its value comes from its importance to humans, where it works as a stimulant drug that improves attention (van Dam et al. 2020) (Alsamarrai, 2022). However, the importance of caffeine as a pharmaceutical compound goes beyond just being a stimulant drug (Monteiro et al., 2019), where its effectiveness as a drug was studied for many medical cases to reduce their intensity or their possibility of accruing, such as consuming coffee may stimulate the gallbladder, which will reduce the risk of gallstones (Grosso et al., 2017). Caffeine could also reduce the development of some diseases like Parkinson's risks by protecting the brain cells (Aaseth et al., 2018), and it can relieve asthma attacks (Wolde, T. 2014, Platritis et al., 2013). Although caffeine has health benefits, some adverse health effects may come along with high consumption rates, such as high incorporation with decreasing bone density, which leads to osteoporosis (Bijelic et al., 2017). It can cause dehydration (Erickson-Levendoski et al., 2011) and affect sleep quality (O'callaghan et al., 2018), it is related to causing strokes seizures when consumed in a certain dosage (Ali, 2019). The bioactivity of caffeine as an inhibitor for pathogenic microorganisms is also important to explore and use in pharmaceutical industries (Han et al., 2016, Raut et al., 2013). Caffeine extraction has been carried out with several extraction techniques, such as Soxhlet extraction (Torres-Valenzuela et al., 2019), solid-liquid, and liquid-liquid extraction (Andrade et al., 2012). These methods are commonly used to extract caffeine from spent coffee grounds. However, these technologies have some disadvantages,

such as solvent usage, which may be toxic and have a negative effect on the environment. In addition, they have a high capital cost (Pavlović et al., 2013). Non-traditional methods have been developed to extract caffeine with less negative environmental impact, such as ionic liquid-assisted extraction (Zhang et al., 2021), and microwave-assisted extraction (Lopes et al., 2020). These methods provide a great caffeine recovery with less negative environmental impact by reducing solvent usage, yet they require expensive materials and a substantial energy supply (Ferreira et al., 2021). The ultrasound extraction method is one of the modern "green and innovative" methods (Hasan et al., 2018) (Mohammed et al., 2016) that are considered an efficient alternative to conventional solid-liquid extraction, methods as it is relatively cost-effective, simple, versatile, and it is possible to scale up (Chemat et al., 2017). The mechanism of this method depends on the effect of ultrasound when traveling through a liquid-solid media, which involves the formation of microbubbles. The bubbles form cavitation within the liquid media part (Al-Yaqoobi et al., 2021), providing a high shear force, and when the implosion of the bubbles takes place, it causes micro mixing and macro turbulent, offering a higher level of contact between the solid and the liquid. That causes several effects, such as erosion, surface peeling, and particle breakdown of the solid in contact. When these mechanisms take place, an ultrasound extraction process is achieved (Chemat et al., 2017).

In this research, the extraction of caffeine by ultrasound was performed by ultrasound bath extraction and ultrasound probe extraction to study the efficiency of ultrasound extraction on the concentration of caffeine extracted from spent coffee grounds. Furthermore, ultrasonic power, extraction time, and temperature of the process were investigated, as they are the most functional parameters affecting the extraction processes. The second part of this work highlighted the bioactivity of extracted caffeine as an inhibitor for pathogenic yeast (*Candida albicans).*

MATERIALS

Chemicals

Plant materials and reagent

The spent coffee ground was obtained from a local bakery, where coffee drinks are prepared in high-pressure professional machines using the percolation method. Spent coffee grounds were generated at the rate of (0.5-1 kg) per day, and the samples were dried at 110 °C in a drier for four hours to avoid any molding possibility and to reduce the water content to 43.9% w/w. The pure standard caffeine was purchased from (wasserfrei) (100%) purity.

Experimental work

Caffeine extraction process:

- ultrasound bath extraction – a sample of the suspension was prepared with 5g of spent coffee grounds suspended in 100 ml of distilled water. The suspension was prepared in a 200 ml Duran, which was placed in an ultrasound sonication bath device (model: Cole-Parmer SS) (Chicago, United States) with a power of 350 W. The extraction process was performed for one hour at a bath temperature of 25 °C (no heating) and at 55 °C (with heating) to study the temperature effect. Five-milliliter samples were taken periodically. Each sample was filtered in a 0.22 μm filter and placed in a labeled tube.
- ultrasound probe extraction – a sample of the suspension was prepared with 5 g of spent coffee grounds suspended in 100 ml of distilled water. The suspension was prepared in a 200 ml beaker. An ultrasound probe device (Branson Digital Sonifier 250) (Park Lawn Dr, United States) with a maximum power supply of 450 W with adjustable amplitude was used to provide the sonication waives. The probe was placed in the beaker, and two amplitudes of the device power (20%, 40%) were introduced into the suspension. Five millimeter samples were taken periodically, filtered by a 0.22 μm filter, and placed in a labeled tube.

HPLC analysis

HPLC analysis was conducted with HPLC (model 1514, Shimadzu Corporation). Zorbax Eclipse Plus, 18 C Column was used, characterized by pore size of 5μ, the internal diameter of 4.6nm, and a length of 150 mm. Reverse phase – ODS and Flow rate of 1 ml/min (constant), The column temperature was at 40 °C, and the UV detector was set at 275 nm. The mobile phase was Water: Methanol (60:40), which is HPLC grade. The injection sample volume was 10 μ.

The use of caffeine as an antifungal agent against *Candida albicans*

Sample collection

The isolate of the diseased *Candida albicans* was obtained from the Food Contamination Research Center (Iraq-Baghdad). The genus and species of the isolate were confirmed by growing it on the chromogen medium of *Candida albicans*.

Test the inhibitory activity of caffeine

To examine the susceptibility of *C. albicans* to caffeine extraction from waste coffee by sonication method, five different concentrations of caffeine were prepared (25, 50, 100, 150, 200) (mg/L) with RPMI medium 1640 (Gibco). The pre-cultured *C. albicans* of 103 CFU ml /l was inoculated into 1mL of the media at various concentrations of caffeine and then incubated at 37 °C in a shaking incubator at 120 rpm for 48 hours.

RESULTS AND DISCUSSION

Caffeine extraction

Effect of sonication type

The sonication technique was performed by using two modes of operation, bath and probe ultrasonicator. Figure 1 demonstrates the effect of ultrasonic power on the extracted caffeine concentration. The experiments were performed at room temperature. The sonication process conducted by the probe shows a higher caffeine concentration of (160 mg/L) for an ultrasound power of 90 W after 15 min of the extraction process and (164 mg /L) for an ultrasound power of 180 W. On the other hand, with the ultrasound bath method, the concentration of caffeine extracted obtained after 15 min was only (60.75 mg/L). This can be explained by the fact that in the process conducted with the probe, the ultrasound-generating tool is in direct contact with the sample that the effect of the waves traveling through the sample is direct and only delivered through a smaller medium which is the tip of the probe, unlike in the ultrasonic bath where the waves are delivered through water and the glassware then to the sample.

It is also can be observed from Figure 1 that the concentration of caffeine increases along with the extraction time. By increasing the extraction time to 30 minutes, the caffeine concentration increased to 192 mg/l, 179 mg/l, and 42 mg/l with sonication power of 90 W, 180 W, and with bath sonication, respectively. Furthermore, it can be indicated from Figure 2 that for the ultrasonic bath extraction technique, caffeine concentration increased when the time of extraction increased, whereas after a time of 15 min, the concentration extracted at (T = 25 °C) was 60.75 mg/L and after 1 hr the concentration of caffeine was 92.91 mg/L.

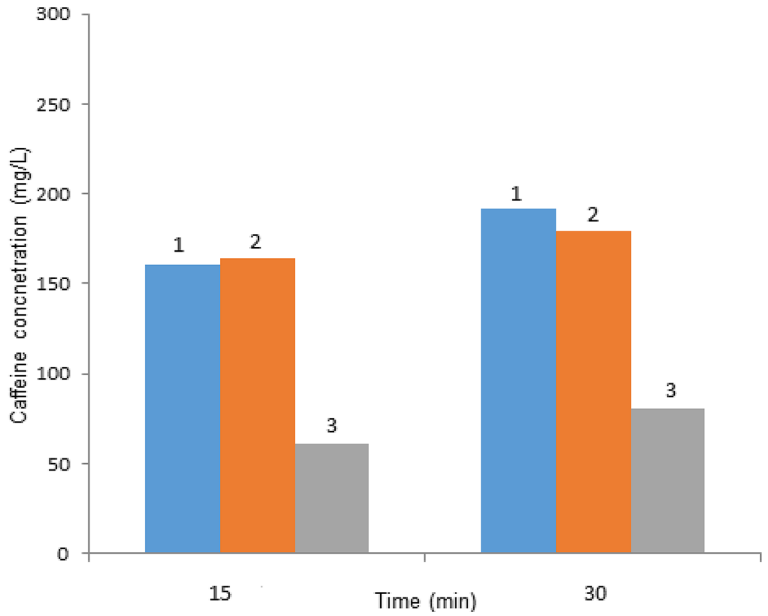

Figure 1. Demonstration of the effect of sonication type of probe power 90 W (block 1) probe 180 W (block 2) and ultrasound bath power 350 W (block 3) on the caffeine concentration with time

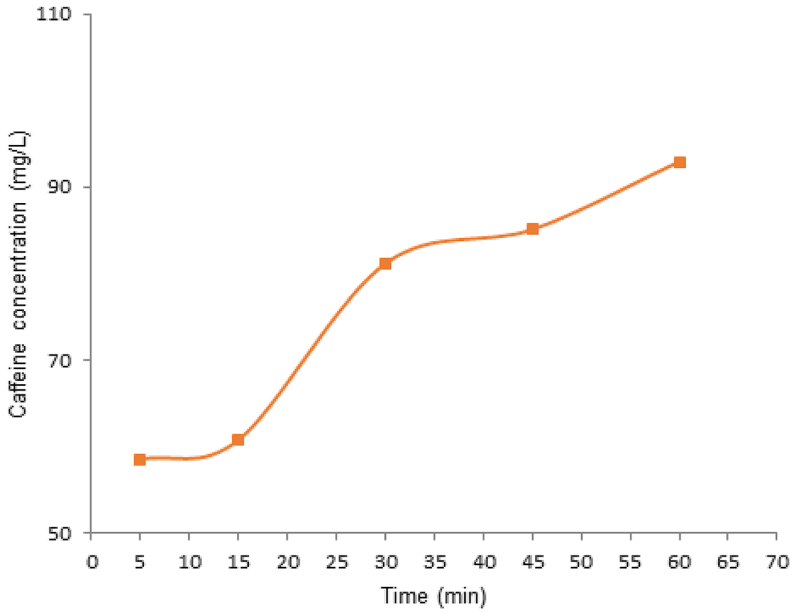

Figure 2. Demonstration of the effect of extraction time on caffeine concentration in ultrasound bath

In addition, Figure 3 demonstrated that for the probe extraction method, the extraction process might start instantly only after five minutes of experiment time, the extracted caffeine concentration was 160 mg/L with ultrasound power of 90 W. It was 164 mg/L with an ultrasound power of 180 W. It also could be noticed from Figure 3 that after 30 min. the caffeine concentration decreased from 192 mg/L at a power of 180 W to 179 mg/L at a power of 180W. The longer time of treatment under the effect of an ultrasound probe device may cause some physical modification to the extracted organic compound, which can be destructive due to its intensity and strength, which affects the concentration of the extracted caffeine and results in reducing of the measured concentration, which is known as Detexturation (Chemat et al., 2017).

Temperature effect

Temperature is one of the most important parameters that affect the extraction process. The results listed in Figure 4 show that for the

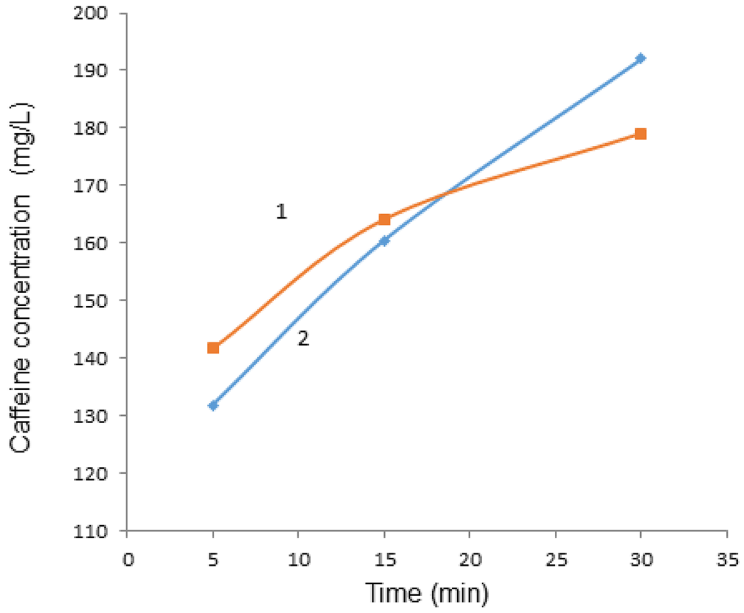

Figure 3. Demonstration of the effect of extraction time and high intensity ultrasound on caffeine concentration power 180 w (curve 1) power 90 w (curve 2)

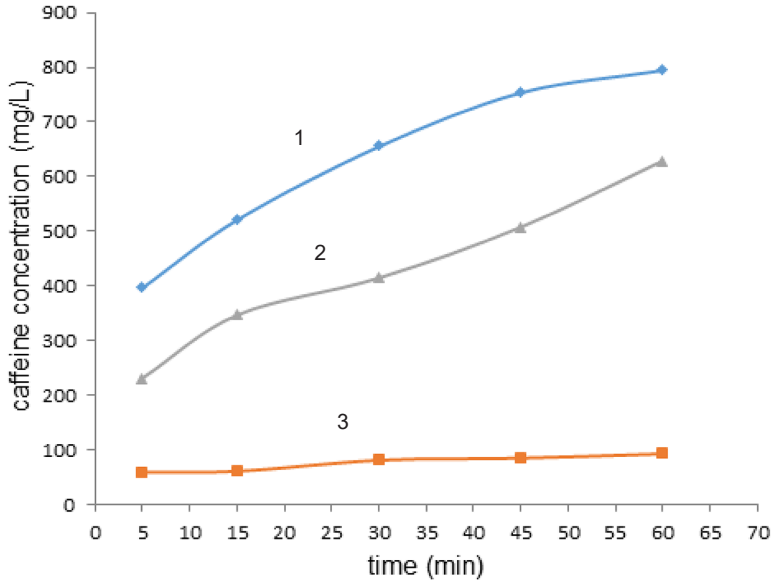

Figure 4. Demonstration of the effect of temperature on the concentration of caffeine extracted by the ultrasound bath method with time 55 °C (curve 1) 35 °C (curve 2) 25 °C (curve 3)

ultrasound bath extraction method, the extracted caffeine concentration increased along with temperature. The probe extraction was carried out under controlled temperature conditions, and for the bath, the highest caffeine concentration of 796 mg/L was obtained at T= 55 °C after extraction time of 30 min., which was obviously higher than that the caffeine concentration obtained at a temperature of 35 °C and 25 °C which were 629 mg/L and 92 mg/L, respectively By increasing the experimental time, the effect of temperature was augmented dramatically. With the process performed at a temperature of 55 °C, the caffeine extracted was 397 mg/L and increased to 521.44 mg/L at extraction time of (15 min), and it rose to its highest value of 796 mg/L after 30 min., while the concentration of extracted caffeine increased from 59 mg/L at time of 5 min. to 92 mg/L at an extraction time of 30 min. This effect can be explained by the effect of temperature on the solid-liquid extraction process, which increases the kinetic energy of suspension molecules as well as decreases viscosity and surface tension. However, it has been reported that the increase of the temperature to a very high value can be intersected with the effect of ultrasound bubbles where increasing the temperature raises the vapour pressure, which results in more solvent vaporization to fill the bubbles cavity, reducing the effect of implosion and collapsing of the bubbles. That inhibits the sonication effect by reducing the intensity of bubbles collapse (Santos et al., 2009).

Caffeine bioactivity

According to the chromogen media test for *Candida* species, each type of Candida appears in a special colour that distinguishes it, as shown in Figure 5. The results showed colonies of *Candida albicans* in green, as presented in Figure 6.

Figure 5. *Candida* species appearance in the chromogen media

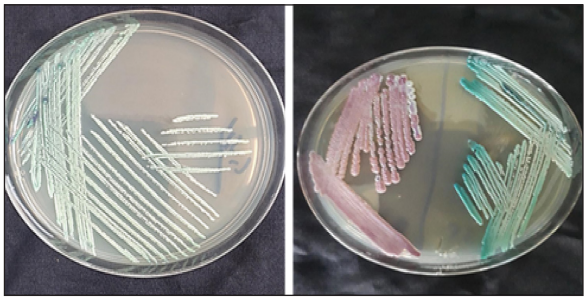

Figure 6. *Candida albicans* appearing as green colonies in chromogen media

Table 1. The minimum fungicidal and minimum inhibited concentration of caffeine against *C. albicans*

Media	Caffeine extract		Caffeine	
	No. of colony	Inhibition %	No. of colony	Inhibition %
25	37	81.87	70	65.7
50	29	86.4	60	70.58
100	10	94.1	55	73
150	6	97.5	45	77.9
200	1	99.5	2	99

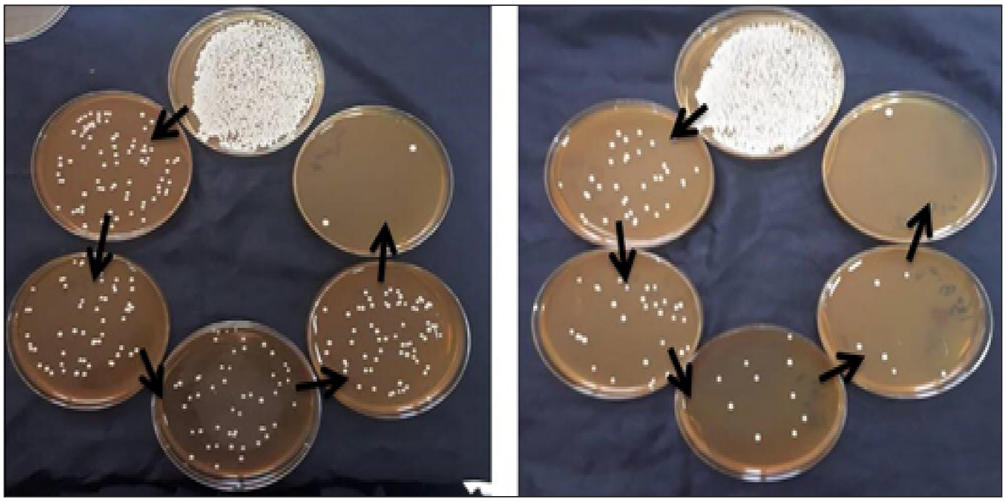

Figure 7. *C. albicans* susceptibility to different concentration of caffeine;
A: Control, B: 25, C: 50 D: 100, E: 150, F: 200 mg /L

Different concentrations of caffeine were prepared (25, 50, 100, 150,200 mg/l) from stock solution (600 mg /l) with RPMI medium1640 (Gibco) to determine the minimum concentration that inhibited the growth of 90% of *C. albicans* and minimum fungicidal concentration for the lowest concentration resulting 99.9% of *C. albicans*, compared with the caffeine-free plates. The rustles in Table 1 showed that the fungicidal effect on *candida* could be achieved at a minimum concentration of 200 mg/L, and the growth inhibition effect can be achieved at a concentration of 100 mg/L. The results shown in Figure 7. agreed with what (Mathur, I., Shruthi, S., et al 2021) found when comparing green tea extract with green coffee extract where the green coffee showed a 50% reduction of *C. albicans* at 160 mg/mL and a 90% reduction at 200 mg/ml. On the other hand, green tea showed a 50% reduction at 200 mg/ml. The antifungal activity of caffeine against *Candida albicans* is due to the ability of caffeine to cause damage to the cytoplasmic membrane of the budding yeasts (Mathur et al., 2021).

CONCLUSIONS

This research covered the process to extract caffeine from the spent coffee grounds in a cost effective modern extraction technology furthermore, the effect of a number of parameters which are time, extraction technique and power, as well as temperature was studied on the amount of the caffeine extracted via ultrasound assistant extraction. For the parameter of time, the concentration kept increasing with time reaching the highest concentration at a time of 60 min and a room temperature of 25 °C with 181.6 mg/L for probe extraction and 92.91 mg/ L for bath extraction,when studying the type of extraction technique and comparing between probe and ultrasound bath extraction the probe extraction gave higher concentration at a less time and for less power levels where at a power of 90 watt the amount of caffeine extracted was 160 mg/L and at a power of 180 watt the caffeine extracted was 164.2 mg/L which is significantly higher compared with the ultrasound bath extraction method where after the same time and for a power level of 350W

however the direct contact between the probe and the sample can cause detexturation which can reduce the levels of caffeine measured where caffeine extracted under the power of 180 w decrease from 186.2 mg/L to 174.2 mg/L, for the parameter of temperature the caffeine concentration increased along with temperature, reaching the highest extraction recovery at temperature of 55 °C with 795.7 mg/L after 60 min,the caffeine bioactivity against *C. albicans* the results showed that compared to the commercial caffeine, natural caffeine showed a better effectivity were the minimum inhibitory concentration in pure natural caffeine which was able to inhibit the candida concentration in 94.1% with only 10 colonies at the concentration of 100 mg/L, where growth of 55 colonies was detected at the same concentration in the commercial caffeine. The inhibitory effect of natural caffeine was higher than the commercial, where at a concentration of 150 mg/L, only six colonies were detected with an inhibition percent of 97.4% in natural caffeine, and 44 colonies with an inhibition percent of 77.9 % were detected for the commercial caffeine.

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