Scientific Research Publishing

Journal of Biosciences and Medicines, 2020, 8, 69-79
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Evaluation of Antibacterial and Synergistic/Antagonistic Effect of Some Medicinal Plants Extracted by Microwave and Conventional Methods

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How to cite this paper: El Kahlout, K.E.M., El Borsh, W., Aksoy, A., El Kichaoi, A.Y., El Hindi, M.W. and El Ashgar, N.M. (2020) Evaluation of Antibacterial and Synergistic/Antagonistic Effect of Some Medicinal Plants Extracted by Microwave and Conventional Methods. *Journal of Biosciences and Medicines*, **8**, 69-79.
https://doi.org/10.4236/jbm.2020.89006

Received: July 11, 2020
Accepted: September 6, 2020
Published: September 9, 2020

Abstract

The purpose of the study was to evaluate the efficiency of the microwave (MW) extraction method by comparing it with a conventional method through evaluation of antimicrobial and synergism/antagonism activity of each aquatic and ethanolic extracts samples, which were extracted from the dried plants (*Ficus sycomorus* leaves, *Lawsonia inermis* leaves and *Glycerrhiza glabra* Linn.). Nine samples of each plant for both solvents were irradiated with MW at several power outputs (180 w, 360 w, and 540 w) in several interval times (1, 2 and 3 minutes). The antibacterial activities of extracts and the synergistic effect between plants and antibiotics were evaluated using disk diffusion method against clinical isolated *Staphylococcus aureus* and *Escherichia coli*. The result revealed that the inhibition zone for more than 50% using aquatic and ethanolic samples results (extracted in two minutes and MW power 180 w) had shown the optimum extract and better antibiotic activity for each plant. Also, the results of ethanolic extracts used against selected microorganisms showed antimicrobial and synergistic effect with most antibiotics better than aquatic extracts. Our results indicate the possibility of using MW apparatus as an extractor to obtain bioactive compounds from plants and thus used in the treatment of bacterial infections, and some results of this study were encouraging. However, the antagonistic reactions of some extracts with some antibiotics and their use in combination should be further studied for *in vitro* activities. It is clearly a need to be furthermore evaluated, to identify the effective components, the mode of action and the possible tox-

ic effect *in-vivo* of these ingredients.

Keywords

Microwave, Antibacterial, Synergistic, Antagonistic, Aquatic Extract & Ethanolic Extract

1. Introduction

Research in herbal medicine has increased in developing countries as a way to rescue ancient traditions as well as an alternative solution to health problems. Therefore, with the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important [1].

The emergence and spread of multidrug resistance as a phenomenon among bacterial pathogens has been a major problem confronting the field of antibacterial chemotherapy in modern years. However, it has been found that, in addition to the production of intrinsic antimicrobial compounds, some medicinal plants also produce multidrug resistance inhibitors which enhance the activities of antibiotics against multidrug resistant bacteria pathogens. It is this finding that prompted efforts in screening of crude extracts for synergistic interaction with standard antibiotics against resistant bacteria as this would have the way for possible isolation of multidrug resistance inhibitors of plant origin [2].

Nowadays, microwaves are used for extraction of bioactive compounds from plant materials because of tremendous research interest and potential [3] [4]. Conventional extraction techniques are time-consuming and require more solvent and most of them are not suitable for thermolabile constituents [5]. In MW, the process acceleration and high extraction yield may be the result of a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction. On the other hand, in conventional extractions, the mass transfer occurs from inside to the outside, although the heat transfer occurs from the outside to the inside of the substrate [6].

Fig, (*Ficus carica*), plant of the mulberry family (Moraceae), and its edible fruit. It is one of the old and historic plant species in the Palestine coastal valley and the study area as well. It is known and called in Palestine as Balami or Jummaze. The antibacterial activity of *F. sycomorus* could be related to the presence of bioactive compounds, such as tannins, saponins, flavonoids, steroids, anthraquinone glycosides and reducing sugars [7].

L. inermis belongs to family Lythraceae [8]. *L. inermis* is commonly known as Henna or Mhendi and abundantly available in tropical and subtropical areas. Henna leaves have been used traditionally in northern Nigeria as a remedy against diarrhea, dysentery and other related diseases. The main constituents of the plant are carbohydrates, glycosides, tannins, phenolic compounds and gums and mucilage [9].

G. glabra Linn, commonly known as liquorice and sweet wood belongs to Leguminosae family. Reported antibacterial activity because of the presence of secondary metabolites such as; saponins, alkaloids, flavonoids [10] [11]. Thereby, this study tried to throw light on the importance of extraction step of bioactive compounds from *F. sycomorus*, *L. inermis* and *G. glabra* by using microwave irradiation through evaluation of antimicrobial and synergism/antagonism activity of each aquatic and ethanolic activity of the extracts.

2. Materials and Methods

2.1. Plant Sample Collection

The plant materials used in this study consisted of *F. sycomorus*, *L. inermis and G. glabra* which were collected from different areas in Gaza strip (Table 1).

2.2. Chemicals and Culture Media

Three types of media were used for carrying out this study, Nutrient broth, Nutrient agar and Muller Hinton agar. Distilled water and ethanol was used for extraction process. Cefotaxime, Ofloxacin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, ceftriaxone, chloramphenicol, and Amikacin used as reference antibiotics (Table 2). Dimethyl sulfoxide (DMSO) and ethanol 80%.

2.3. Bacteria

Clinical isolated species of *S. aureus*, and *E. coli* were obtained from Biology & Biotechnology Department at the Islamic University of Gaza (IUG), and were maintained on DMSO at −80˚C for further experiments.

Table 1. Plant materials used in this study.

Plant/Part used	Place	Time of collection
F. sycomorus/leaves	Jabalia-North of Gaza	March & April 2015
L. inermis/leaves	Market	April 2015
G. glabra/roots	Market	April 2015

Table 2. List of antibiotic potency.

Antibiotics	Symbol	Antibiotics potency	Manufactured by
Cefotaxime	CTX	30 µg	Bioanalyse, Turkey
Ofloxacin	OF	5 µg	Himedia, Indian
Ceftriaxone	CTR	30 µg	Himedia, Indian
Amikacin	AK	30 µg	Bioanalyse, Turkey
Chloramphenicol	C	30 µg	Bioanalyse, Turkey
Ciprofloxacin	CI	5 µg	Bioanalyse, Turkey
Levofloxacin	LEV	5 µg	Bioanalyse, Turkey
Nitrofurantoin	F	300 µg	Bioanalyse, Turkey

2.4. Microwave Apparatus

Commercial microwave oven (Panasonic) with ten power levels (80 to 800 W) was used NN-SE996S.

2.5. Preparation of Plant Extract

A total of plants extract were used in this study as shown in Table 3.

2.6. Preparation of Inoculums

According to (Mohammed *et al.*, 2015) stock cultures were maintained at 4˚C on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring 0.5 ml of culture to 5 ml of nutrient broth and incubated at 37˚C for 24 hours. The optical density of each active culture adjusted to 0.1 at 625 nm, using fresh broth to give standard inocula of 106 colony forming units (CFU) per ml [3] [14] [15].

2.7. Paper Disk Diffusion Assay

A modification procedure was followed to evaluate of antibacterial activity of plant extracts. Standardized inoculums of each bacterium, *i.e.*, 10^6 CFU (Colony Forming Units)/ml to 0.1 at 625 nm was introduced onto the surface of sterile Nutrient agar plates and a sterile cotton swab was used for even distribution of inoculums. After a few minutes, sterile filter paper discs of 5 mm diameter were placed on the surface of inoculated and labeled nutrient agar plates and impregnated with 20 µL of known concentration of extracts (200 mg/ml) for aquatic and ethanolic extracts. Sterile paper discs containing Dimethyl sulfoxide alone was served as negative control. The plates were placed at 4˚C for 2 h. and then subsequently incubated at 37˚C for 24 Hrs. After incubation, the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. For each test solution, three replicates were maintained [16].

2.8. Synergism/Antagonism between Plant Extract and Antibiotics

A modification procedure was followed to investigate the synergistic effect. The bacterial cultures were grown in sterile nutrient broth medium at 37˚C. After 4 h of growth, standardized inoculums of each bacterium, *i.e.*, 10^6 CFU/ml to 0.1 at

Table 3. Preparation of plant extract.

Soxhlet extraction		Microwave extraction	
Aqueous extraction	Ethanol extraction	Aqueous extraction	Ethanol extraction
Air dried powder (20 g) was added to 150 ml of distilled water, (100˚C), as a solvent for 8 hours using soxhlet equipment. Then the extract was filtered and allowed to evaporate in oven (45˚C) through 48 hr [12].	Air dried powder (20 g) was added to 150 ml of 80 % ethanol, (78.5˚C), as a solvent for 8 hours, using soxhlet equipment. Then the extract was filtered and allowed to evaporate in oven (45˚C) through 48 hr [12].	4 g of the powder was mixed with 100 ml distilled water. Then the mixture was irradiated with microwave at several power output (180, 360 and 540) in several interval times (1, 2 and 3) minuets to obtain nine samples [13].	4 g of the powder was mixed with 100 ml (80%) ethanol. Then the mixture was irradiated with microwave at several power (180, 360 and 540) in several interval times (1, 2 and 3) minuets to obtain nine samples [13].

625 nm was introduced onto the surface of sterile Nutrient agar plates and a sterile cotton swab was used for even distribution of inoculums. After a few minutes, the antibiotic filter paper disk of 5 mm in diameter placed on the surface of inoculated and labeled Nutrient agar plates and impregnated with 20 µL of known concentration of extracts (200 mg/ml) for aquatic and ethanolic extracts. The plates were incubated at 37˚C for 24 h. The diameters of cleared zones were measured and compared with that of the antibiotic alone. For each test solution, three replicates were maintained [7].

3. Results and Discussion

3.1. Evaluation of Antibacterial Activity of Plant Extracts and the Synergistic/Antagonistic Effect

The results in Tables 4-9 showed that ethanolic extracts used against selected microorganisms offered antimicrobial and synergistic effect with most antibiotics better than aquatic extracts. In case of aquatic extracts; *G. glabra* had the best antibitic activity against *E. coli*. In case of ethanolic extracts, the best activity was observed with *G. glabra* against *E. coli*. Also, synergistic activity of the plant extracts, in case of aquatic extracts; *F. sycomorus* had the best synergism against *S. aureus* and *E. coli*. In case of ethanolic extracts, the best synergism was observed with *L. innermis* against *E. coli*, and with *F. sycomorus* against *S. aureus*. High levels of antagonism reaction exhibited by all aquatic plant extracts of both methods when combined with antibiotics which showed sensitivity when tested as alone against *S. aureus*. Also all ethanolic extracts of *L. innermis* and *G. glabra* of both methods exhibited antagonism reactions when combined with antibiotics which showed sensitivity when tested as alone against *S. aureu.* In addition, antagonism reaction occurred against *E. coli* for antibiotics which were resistant against *E. coli* when combined with aquatic and ethanolic extracts for both methods of *G. glabra* which showed sensitivity when tested as alone against *E. coli*. In addition, dilution reactions against *S. aureus* occurred with some antibiotics combined with ethanolic extracts of *F. sycomorus* & *L. innermis*.

Table 4. Antibacterial activity of antibiotics against *E. coli* and *S. aureus* by disc diffusion method.

Microorganism / Antibiotics	*S. aureus*	*E. coli*
	Inhibition zone (mm)	
Ofloxacin	R	25 ± 1.0*
Ceftriaxone	R	R
Ciprofloxacin	R	R
Cefotaxime	R	22 ± 1.58*
Amikacin	R	R
Chloramphenicol	R	R
Levofloxacin	R	25 ± 0.7*
Nitrofurantoin	R	25 ± 2.0*

mm = millimeter, *Mean ± Standard Deviation, R = Resistance.

Table 5. Antibacterial effect of *G. glabra* extracts against *S. aureus* and *E. coli.*

Plant extract	Extraction method	Solvent	Sample symbol	*Power* (*W*)	*Time* (*min*)	*S. aureus*	*E. coli*
						Inhibition zone in (mm)	
G. glabra	Soxhlet	D.W.	C	-	8 hr.	R	15±0.7*
		Ethanol	C	-	8 hr.	R	17±1.0*
	Microwave	D.W.	a	180	1	R	R
			b	360		R	R
			c	540		R	15 ± 0.7*
			d	180	2	R	15 ± 0.7*
			e	360		R	15 ± 0.7*
			f	540		R	15 ± 1.0*
			g	180	3	R	15 ± 0.0*
			h	360		R	15 ± 0.0*
			i	540		R	15 ± 1.5*
		Ethanol	a	180	1	R	15 ± 1.0*
			b	360		R	15 ± 1.0*
			c	540		R	15 ± 0.7*
			d	180	2	R	17 ± 0.7*
			e	360		R	17 ± 0.7*
			f	540		R	17 ± 2.0*
			g	180	3	R	17 ± 1.0*
			h	360		R	17 ± 0.0*
			i	540		R	17 ± .0.0*

mm = millimeter, *Mean ± Standard Deviation, n = 3, R = Resistance.

Table 6. Synergistic/antagonistic effect of *F. sycomorus* extracts and antibiotics against *S. aureus.*

P.E	Extraction method	Solvent	S.S	*Power* (*W*)	*Time* (*min*)	*S. aureus*					
						Inhibition zone in (mm)					
		Synergism with				CTR	CTX	OFX	LEV	AK	F
F. sycomorus	Soxhlet	D.W.	A	-	8 hr.	12 ± 0.7*	R	R	R	R	R
		Ethanol	A	-	8 hr.	R	20 ± 0.7*	R	R	R	17 ± 2.0*
	Microwave	D.W.	a	180	1	R	R	R	R	R	R
			b	360		R	R	R	R	R	R
			c	540		R	R	R	R	R	R
			d	180	2	15 ± 1.0*	R	R	R	R	R
			e	360		15 ± 1.5*	R	R	R	R	R
			f	540		15 ± 1.5*	R	R	R	R	R
			g	180	3	15 ± 0.0*	R	R	R	R	R
			h	360		15 ± 1.0*	R	R	R	R	R
			i	540		15 ± 2.0*	R	R	R	R	R
		Ethanol	a	180	1	R	R	R	R	R	R
			b	360		R	R	7 ± 1.0*	R	R	R
			c	540		R	17 ± 0.7*	21 ± 1.5*	R	R	15 ± 1.0*
			d	180	2	R	21 ± 2.0*	21 ± 0.0*	R	21 ± 0.7*	R
			e	360		R	21 ± 0.0*	21 ± 1.0*	R	21 ± 0.7*	R
			f	540		R	21 ± 0.0*	21 ± 2.0*	R	21 ± 1.0*	R
			g	180	3	R	21 ± 1.0*	21 ± 0.7*	R	21 ± 1.5*	17 ± 1.0*
			h	360		R	21 ± 1.5*	21 ± 0.7*	R	21 ± 1.5*	R
			i	540		R	21 ± 1.0*	21 ± 0.0*	R	21 ± 0.0*	R

mm = millimeter, *Mean ± Standard Deviation, n = 3, R = Resistance, D.W.: Distilled water. P.E.: Plant Extract, S.S.: Sample Symbol, F: Nitrofurantoin; OFX: Ofloxacin; LEV: Levofloxacin; AK: Amikacin; CTX: Cefotaxim.

Table 7. Synergistic/antagonistic effect of *F. sycomorus* extracts and antibiotics against *E. coli.*

P.E	Extraction method	Solvent	S.S	*Power* (*W*)	*Time* (*min*)	*E. coli* Inhibition zone in (mm)					
		Synergism with				CTR	CTX	OFX	LEV	AK	F
F. sycomorus	Soxhlet	D.W.	A	-	8 hr.	16 ± 0.0*	R	R	R	R	R
		Ethanol	A	-	8 hr.	R	R	R	R	R	R
	Microwave	D.W.	a	180	1	R	R	R	R	R	R
			b	360		R	R	R	R	R	R
			c	540		R	R	R	R	R	R
			d	180	2	17 ± 1.5*	R	R	R	R	R
			e	360		17 ± 0.7*	R	R	R	R	R
			f	540		17 ± 0.7*	R	R	R	R	R
			g	180	3	17 ± 0.0*	R	R	R	R	R
			h	360		17 ± 2.0*	R	R	R	R	R
			i	540		17 ± 1.5*	R	R	R	R	R
		Ethanol	a	180	1	R	R	R	R	R	R
			b	360		R	R	R	R	R	R
			c	540		R	R	R	R	R	R
			d	180	2	R	R	R	R	R	R
			e	360		R	R	R	R	R	R
			f	540		R	R	R	R	R	R
			g	180	3	R	R	R	R	R	R
			h	360		R	R	R	R	R	R
			i	540		R	R	R	R	R	R

mm = millimeter, *Mean ± Standard Deviation, n = 3, R = Resistance, D.W.: Distilled water. P.E.: Plant Extract, S.S.: Sample Symbol, F: Nitrofurantoin; OFX: Ofloxacin; LEV: Levofloxacin; AK: Amikacin; CTX: Cefotaxim.

Table 8. Synergistic/antagonistic effect of *L. inermis* extracts and antibiotics against *S. aureus.*

P.E	Extraction method	Solvent	S.S	*Power* (*W*)	*Time* (*min*)	*S. aureus* Inhibition zone in (mm)					
		Synergism with				CTR	CTX	OFX	LEV	AK	F
L. inermis	Soxhlet	D.W.	B	-	8 hr.	R	R	R	R	R	R
		Ethanol	B	-	8 hr.	R	R	R	R	R	17 ± 0.7*
	Microwave	D.W.	a	180	1	R	R	R	R	R	R
			b	360		R	R	R	R	R	R
			c	540		R	R	R	R	R	R
			d	180	2	R	R	R	R	R	R
			e	360		R	R	R	R	R	R
			f	540		R	R	R	R	R	R
			g	180	3	R	R	R	R	R	R
			h	360		R	R	R	R	R	R
			i	540		R	R	R	R	R	R
		Ethanol	a	180	1	R	R	R	R	R	R
			b	360		R	R	R	R	R	R
			c	540		R	R	R	R	R	15 ± 1.5*
			d	180	2	R	R	R	R	R	R
			e	360		R	R	R	R	R	R
			f	540		R	R	R	R	R	R
			g	180	3	R	R	R	R	R	17 ± 0.7*
			h	360		R	R	R	R	R	R
			i	540		R	R	R	R	R	R

mm = millimeter, *Mean ± Standard Deviation, n = 3, R = Resistance, D.W.: Distilled water. P.E.: Plant Extract, S.S.: Sample Symbol, F: Nitrofurantoin; OFX: Ofloxacin; LEV: Levofloxacin; AK: Amikacin; CTX: Cefotaxim.

Table 9. Synergistic/antagonistic effect of *L. inermis* extracts and antibiotics against *E. coli.*

P.E	Extraction method	Solvent	S.S	*Power* (*W*)	*Time* (*min*)	*E. coli* Inhibition zone in (mm)					
		Synergism with				CTR	CTX	OFX	LEV	AK	F
L. inermis	Soxhlet	D.W.	B	-	8 hr.	R	R	R	R	R	R
		Ethanol	B	-	8 hr.	R	11 ± 1.0*	13 ± 0.7*	14 ± 0.7*	20 ± 1.5*	R
	Microwave	D.W.	a	180		R	R	R	R	R	R
			b	360	1	R	R	R	R	R	R
			c	540		R	R	R	R	R	R
			d	180		R	R	R	R	R	R
			e	360	2	R	R	R	R	R	R
			f	540		R	R	R	R	R	R
			g	180		R	R	R	R	R	R
			h	360	3	R	R	R	R	R	R
			i	540		R	R	R	R	R	R
		Ethanol	a	180		R	R	R	R	R	R
			b	360	1	R	R	R	R	R	R
			c	540		R	9 ± 0.7*	11 ± 2.0*	14 ± 1.5*	20 ± 0.0*	R
			d	180		R	11 ± 0.7*	13 ± 1.5*	14 ± 1.5*	20 ± 1.0*	R
			e	360	2	R	11 ± 1.0*	13 ± 0.0*	16 ± 1.5*	20 ± 1.5*	R
			f	540		R	11 ± 0.0*	13 ± 0.7*	16 ± 0.7*	20 ± 0.7*	R
			g	180		R	11 ± 0.0*	13 ± 0.7*	16 ± 0.7*	20 ± 0.7*	R
			h	360	3	R	11 ± 1.5*	13 ± 1.0*	16 ± 1.0*	20 ± 0.7*	R
			i	540		R	11 ± 2.0*	13 ± 1.0*	16 ± 1.0*	20 ± 1.5*	R

mm = millimeter, *Mean ± Standard Deviation, n = 3, R = Resistance, D.W.: Distilled water. P.E.: Plant Extract, S.S.: Sample Symbol, F: Nitrofurantoin; OFX: Ofloxacin; LEV: Levofloxacin; AK: Amikacin; CTX: Cefotaxim.

3.2. Effect of Solvent System

Through evaluation of antimicrobial activity of plant extracts and in synergisms/Antagonism, our results which obtained from ethanolic extracts (ethanol as a solvent) had shown better results than that obtained from aquatic extracts (water as a solvent). In addition most of aquatic extracts results had shown no inhibition zone. The same solvent system of samples used in extraction process in both methods MW and soxhlet in this study had shown mostly the same inhibition zone results, and some tests of MW samples had shown better than soxhlet samples. So, because of high capacity with high dielectric constant, ethanol is the best solvent in our experiments of extraction process. This evaluation is in agreement with previous studies [6] [13] [17] [18] [19] [20].

3.3. Effect of Irradiation Time

Through evaluation of antimicrobial activity of plant extracts and in synergisms/Antagonism, our results showed that, the inhibition zone is increased by increasing the time of extraction till reaching in a steady state values of inhibition zone. This may due to increasing of the time of extraction which lead to increasing of the yield of bioactive compounds till to reach a saturation although of increasing time. The samples of our plants in this study which extracted in one minute were in mostly had shown no inhibition zone except samples extracted by using a power 540 w which had shown a weak inhibition zone. But in

general the samples extracted in tow and three minutes had shown a maximum value and the same results of inhibition zone. So we can considered that the best with optimum time of extraction is a sample-d which extracted in tow minutes and had shown a maximum inhibition zone with a lowest power 180 w. Our results showed that, the MW method gave better results or at least same outputs as soxhlet method. We recommended MW method since it provides bioactive compounds in a very short time, mostly few minutes and a very low power. Other conventional methods may attribute to a degradation of bioactive compounds in long time of extraction process of conventional methods. This evaluation is in agreement with previous studies [6] [13] [17] [18] [19] [20].

3.4. Effect of Microwave Power and Temperature

Through evaluation of antimicrobial activity of plant extracts and synergisms/Antagonism effect, our results showed that, the inhibition zone is increased by increasing the MW power of extraction process till reaching in a steady state result of inhibition zone. This may due to increasing of the MW power of extraction which lead to increasing of the yield of bioactive compounds till reaching a saturation although of increasing MW power. The samples of our plants in this study which extracted in one minute were in mostly showed no inhibition zone, although of increasing of MW power except some samples extracted by using a power 540 w which showed a weak of inhibition zone. But in most, they didn't show any effect by increasing MW power for samples extracted in two and three minutes which showed a maximum and the same results of inhibition zone. So we can consider that the best with optimum MW power of extraction is a sample-d which extracted at lowest power 180 w in tow minutes and showed a maximum inhibition zone. According to the temperature, the results of ethanolic samples were better than aquatic samples. Due to high dielectric properties of ethanol, it leads to increasing of temperature of the medium solvent-solute, and thus increasing of the extraction of the bioactive compound, then had shown more of antimicrobial activity of inhibition zone. This evaluation is in agreement with previous studies [6] [13] [17] [18] [19].

4. Conclusion

In conclusion, the results from these studies were encouraging to find new antimicrobial agents or new ways that are effective for the treatment of infectious diseases caused by test pathogenic microorganism especially drug-resistant bacteria, after optimizing three parameters (solvent nature, irradiation time, microwave power) and evaluation of antimicrobial activity of plants extracts and in combination with antibiotics. In general, the positive inhibitory zone results of MW aquatic and ethanolic extracts samples were the best comparing with samples extracted by conventional method (soxhlet method in this study and some of the previous studies), especially more than 50% of aquatic and ethanolic sample results (extracted two minutes and MW power 180 w) had shown a maxi-

mum magnitude of inhibition zone, though it consumed less time and power.

Acknowledgements

The authors thank **Department of Biology and Biotechnology & Department of Chemistry**, Islamic University for providing excellent research facilities.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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