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ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF *BOUGAINVILLEA SPECTABILIS* L. AND *MYRTUS COMMUNIS* L. LEAVES EXTRACTS

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ABSTRACT
This research aimed to investigate the effect of using two plants extracts on bacterial growth and test their antioxidant activity. *Bougainvillea spectabilis* and *Myrtus communis* ethanolic leaves extracts at (0, 15, 30, 45, 60 mg/ml) were used to study the antimicrobial activity on gram positive bacteria, *Bacillus sabtilis*, *Staphylococcus aureus* and gram negative bacteria *Escherichia coli, Klebsiella pneumonia.* Results showed that there was high sensitivity of the four bacterial strains to both plant extracts in different modes, *Staphylococcus aureus* was the most sensitive to *Bougainvillea spectabilis* extract, while *Escherichia coli* was the most sensitive to *Myrtus communis* extract. Both extracts were used to study their effects on biofilm formation, all bacterial strains showed high sensitivity, *Staphylococcus aureus* and *Klebsiella pneumonia* were most sensitive to *Bougainvillea spectabilis extract*, while *Bacillus sabtilis* and *Escherichia coli* were most sensitive to *Myrtus communis* extract. Antioxidant activity of *Bougainvillea spectabilis* and *Myrtus communis* leaves extracts was studied; results showed that *Myrtus communis* leaves extract had more antioxidant activity than *Bougainvillea spectabilis* leaves extracts. Phytochemical analysis of plants leaves extracts revealed the presence of many secondary metabolites in both plants extracts, that have in return the positive effect on retarding bacterial growth and biofilm formation at different levels and revealed the high antioxidant activity of two plant leaves extracts.

KEYWORDS: *Bougainvillea spectabilis* L., *Myrtus communis* L. antioxidant, antibacterial.

INTRODUCTION

Today, due to variation in the form of the resistance of pathogenic bacteria, using alternative medicines is an important goal in this field. Natural antimicrobials are effective chemotherapeutics that is present in many plant families. Secondary metabolites serve as plant defense mechanism against microorganisms[1] *Bougainvillea spectabilis* belong to family Nyctaginaceae, the genus have different common names according to the countries, in Arab world it is named jahanamya, growing from 1-2 meters, *Bougainvillea spectabilis* leaves extracts inhibited virus disease in tomato[2] and showed anti- inflammatory activities[3]. *Myrtus communis* (common name is myrtle) which belong to family Myrtaceae is an evergreen small tree growing to 5 meters, native to Mediterranean region and western Asia. The leaves, flowers and fruits have fragrant smell due to essential oils that it contains, so it was used in different industries, myrtle extracts have antibacterial activity[4] and antioxidant activity[5] organized bacterial communities that are embedded in an extra cellar matrix attacked to living or abiotic surface is called biofilms[6]. Bacteria within a biofilm exhibit altered physiology, including resistance to antibiotics and environmental stresses[7], therefore using the plants extracts was the alternative medicines. Plants are rich in secondary metabolites that were considered as antioxidants in addition to its role as antibiotics. The role of antioxidant is to remove the free radicals by donating hydrogen ions to free radicals and change its form to un-reactive form or reduce their damaging effects in the human body; addition hydrogen ions would remove an odd electron which is responsible for radical reactivity[8]. There are increasing interests in plants as a natural origin for antioxidants because of the carcinogenicity of synthetic antioxidants used for human[9].

The aim of this study was to investigate the efficacy of *Bougainvillea spectabilis* and *Myrtus communis* leaves extracts against four strains of bacteria through determine antimicrobial activity, inhibition of biofilm formation and antioxidant activity.

MATERIALS & METHODS

Plant materials

Leaves of *Bougainvillea spectabilis* and *Myrtus communis* plants were collected from University of Baghdad gardens and washed by tap water followed by distilled water, then left to dry in shade at 30^{o}C for three days. The dried leaves put in blender to be grind to fine powder.

Plant extract

Fine powder of each plant was extracted by tacking (20 g) of it with (100ml) absolute ethanol in conical flask and put in thermo shaker at 150 rpm for 20 hours at 30^{o}C then filtered through Whatman filter paper and concentrated by rotary evaporator. Stock solution for each plant was made of 15, 30, 45 and 60 mg/ml[10].

Phytochemical screening

Qualitative analysis of secondary metabolites in plant leaves was carried out according[11, 12].

Bacterial strains

Bacterial strains were *Bacillus sabtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia.*

Microbial activity

Bacterial isolates were cultured on the media Muller-Hinton Agar that was prepared and poured on petri dishes previously. Wells of 6 mm diameters were punched into the medium and filled with gradient concentrations (0, 15, 30, 45 and 60 mg/ml) of each plant extract. The dishes were incubated at 37^{o}C for 24 hours[13].

Biofilm formation

The bacterial strains that isolated from fresh brain heart infusion agar were incubated in 10 ml trypton soya broth tube then incubated overnight at 37^{o}C. After that the tubes were diluted 1:100 with fresh broth, the wells of sterile flat bottom microtiter plate were inoculated with 200 µl cultured broth as positive control. The negative control was made of sterile broth without culture, then the effect of plant extracts were showed on biofilm.

The other wells were filled with 100 µl of cultured broth and 100 µl of plant extracts at 6mg/ml instead of the concentrations that was used, this was due to high false reading resulted by interference of the pigments of each extract with the stain. The plate incubated at 37^{o}C for 24 hours, the floating bacteria were removed by gentle tabbing and washed with distilled water then stained by 0.1% crystal violate at 25^{o}C for 10 minutes, then the plate was washed with 200 µl of 96% ethanol for 10 minutes, the plate was red with micro plate reader (ELISA) at 630 nm [14].

Inhibition mediated reduction of biofilm formation was calculated by the formula [15]:

$$\% \text{ biofilm inhibition} = \frac{\text{O.D. of control} \_ \text{O.D. of treatment}}{\text{O.D. of control}} \times 100$$

Free radical scaving activity (Antioxidant activity):

The extracts of *Bougainvillea spectabilis* and *Myrtus communis* was measured for the free radical scaving activity by DPPH assay, this conclude equal volumes of DDPH (60µM) and each plant extracts at (15, 30, 45 and 60 mg/ml), that were mixed in a cuvette and allowed to stand for 30 minute at room temperature, then the absorbance was red at 517nm using UV spectrophotometer, the control reading was DPPH solution, the percentage of DPPH discoloration of the sample was according to the formula[16] :

$$\% \text{ Decolouration} = \frac{\text{Abs. of control} \_ \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

RESULTS & DISCUSSION:

Results of qualitative phytochemical analysis presented in (table 1). Alkaloids, phenols, flavonoids, terpens, tanins and glycosides were found in *Bougainvillea spectabilis* leaves, while volatile oils and saponins were absent; these phytochemicals improved the antioxidant and antimicrobial property of this plant [17].

The phytochemical analysis of *Myrtus communis* leaves revealed the presence of alkaloids, flavonoids, terpens, volatile oils, saponins, phenols, tanins and glycosids that considered as antioxidants and antibacterials [18].

TABLE 1- Qualitative phytochemical analysis of *Bougainvillea spectabilis* and *Myrtus communis* leaves extract (+ : present , - : absent).

Phytochemicals	*Bougainvillea spectabilis* leaves extract	*Myrtus communis* leaves extract
Alkaloids	+	+
Phenols	+	+
Terpenoids	+	+
Flavonoids	+	+
Tannins	+	+
Glycosides	+	+
Saponins	-	+
Volatile oils	-	+

The results in (table 2, figure 1 and 2) revealed that all plant extracts at all concentrations had inhibited the growth of gram positive and negative bacteria, *Bougainvillea spectabilis* extract inhibited the growth of *Staphylococcus aureus* more than *Klebsiella pneumoniae* that was more than *Escherichia coli* which was more than *Bacillus sabtilis*. The antibacterial effect of this plant maybe due to its content of phenols, alkaloids, terpenoids, flavonoids, tannins and glycosides that considered as antimicrobials [19].

TABLE 2- Inhibition zones (mm) caused by *Bougainvillea spectabilis* leaves extract

Bacterial strains	*Bougainvillea spectabilis* L. ethanolic extract concentrations (mg/ml)				
	0	15	30	45	60
Staphyloccocus aureus	0	36	36	36	36
Bacillus sabtilis	0	20	21	22	23
Escherichia coli	0	21	22	23	23
Klebsiella pneumonia	0	30	31	32	33

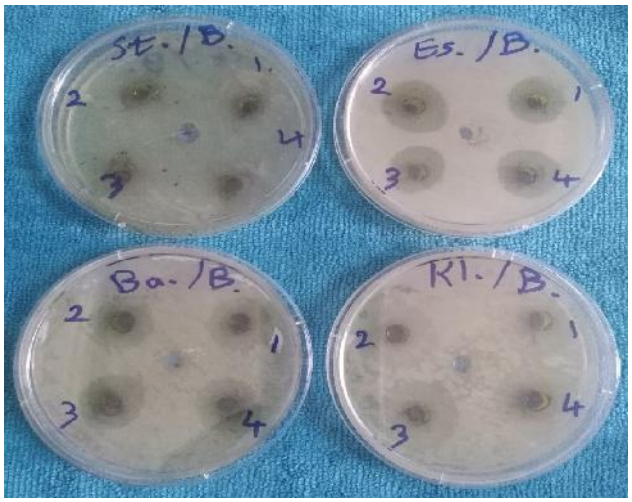

FIGURE 1- The antimicrobial activity of *B. spectabilis* leaves extract at the concentrations: 0 in center, 1(15), 2(30), 3(45), 4(60mg/ml) against gram positive and negative bacteria.

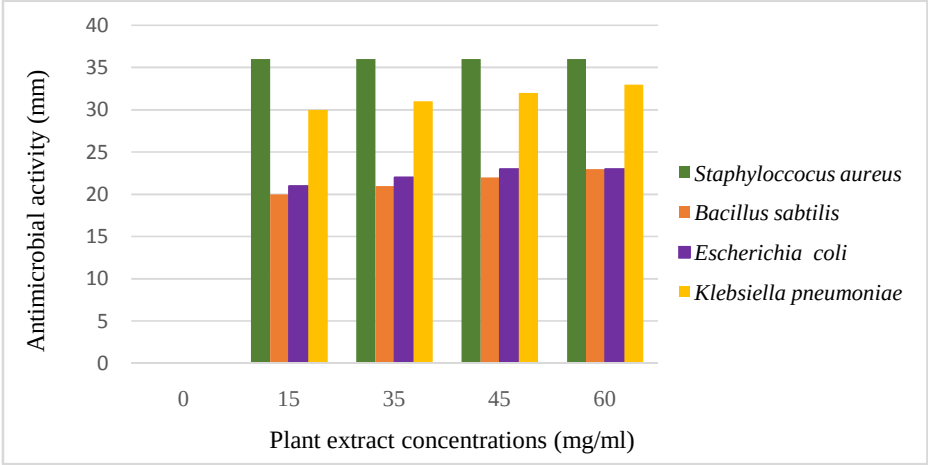

FIGURE 2- The antimicrobial activity of *B. spectabilis* leaves extract against four strains of bacteria.

Myrtus communis extract inhibited the growth of *Escherichia coli* more than *Bacillus sabtilis* that was more than *Staphylococcus aureus* which was more than *Klebsiella pneumoniae* (Table 3, figure 3 and 4). The main secondary metabolites in this plant are essential oils, volatile oils, terpens and phenols that have antimicrobial and antioxidant properties [20].

TABLE 3- Inhibition zones (mm) caused by *Myrtus communis* leaves extract

Bacterial strains	*Myrtus communis* L. ethanolic extract concentrations (mg/ml)				
	0	15	30	45	60
Staphyloccocus aureus	0	22	26	28	30
Bacillus sabtilis	0	20	25	30	32
Escherichia Coli	0	34	36	36	36
Klebsiella pneumoniae	0	20	22	25	30

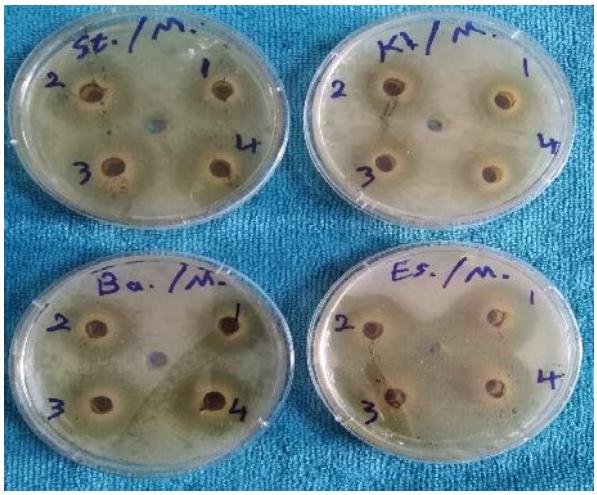

FIGURE 3- The antimicrobial activity of *M. communis* leaves extract at the concentrations: 0 in center, 1(15), 2(30), 3(45), 4(60mg/ml) against gram positive and negative bacteria.

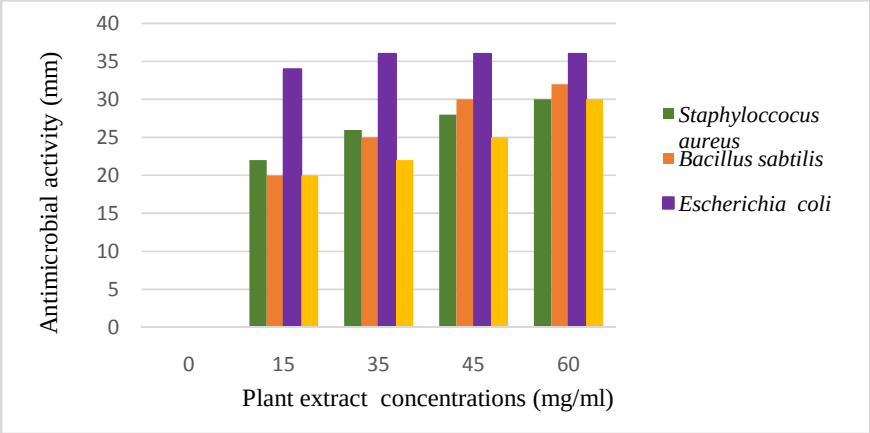

FIGURE 4- The antimicrobial activity of *M. communis* leaves extract against four strains of bacteria.

The effect of the two plant extracts on biofilm formation were illustrated in (table 4, 5 and figure 5), *Myrtus communis* extract was more effective than *Bougainvillea spectabilis* extract in inhibiting biofilm formation. There was compatibility between these results and the results of the plant extracts on microbial activity. *Bougainvillea spectabilis* extract had the highest effect on *Staphylococcus aureus* then *Klebsiella pneumoniae* then *Escherichia coli* then *Bacillus sabtilis. Myrtus communis* had the highest effect on *Escherichia coli* then *Bacillus sabtilis* then *Staphylococcus aureus* then *Klebsiella pneumoniae.* Many studies have improved the role of plants extracts on retarding biofilm formation [21,22]. The effect of *Bougainvillea spectabilis* and *Myrtus communis* leaves extracts may be due to their contain of essential oils, alkaloids, phenols and terpens that was reported in many studies as antibacterials [23,24,25].

TABLE 4- effect of plant extracts on biofilm formation (O.D. at 630 nm)

Plant extract treatment	Bacterial strains			
	Staphyloccocus aureus	*Bacillus sabtilis*	*Escherichia coli*	*Klebsiella pneumoniae*
Before treatment	0.262	0.242	0.269	0.230
Bougainvillea spectabilis treatment	0.141	0.175	0.182	0.143
Myrtus communis treatment	0.167	0.138	0.113	0.162

TABLE 5- effect of plants extracts on biofilm formation inhibition (%)

Plants extracts	Bacterial strains			
	Staphyloccocus aureus	*Bacillus sabtilis*	*Escherichia coli*	*Klebsiella pneumoniae*
Bougainvillea spectabilis	46.183 %	27.685 %	32.342 %	37.826 %
Myrtus communis	36.629 %	42.975 %	57.992 %	29.565 %

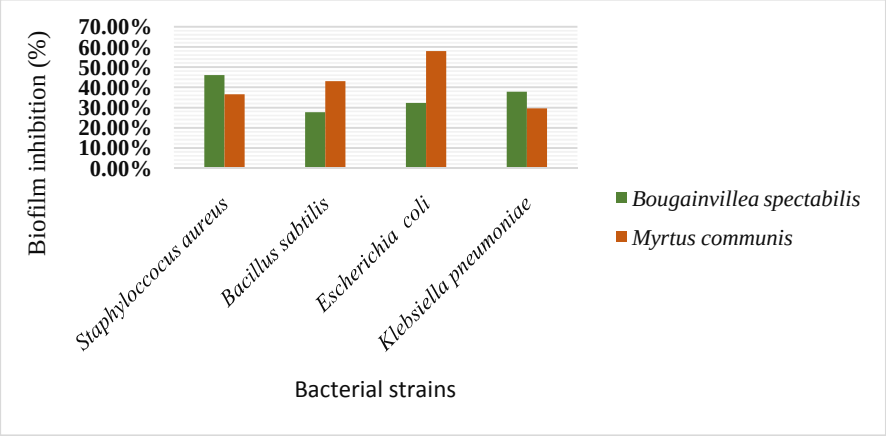

FIGURE 5- Effect of plant extracts on biofilm formation (%).

The effect of *Bougainvillea spectabilis* and *Myrtus communis* leaves extracts on antioxidant activity was illustrated in (table 6, 7 and figure 6); the results revealed that both plants had the ability to reduce the effect of free radicals. *Myrtus communis* extract was more effective than *Bougainvillea spectabilis* extract, this may be due to its contain of saponins and volatile oils that was found in the phytochemical analysis.

The antioxidant capacity of plant extracts is strongly related to phenolic compounds, such as anthocyans, flavonoids and phenolic acids[26]. Results of Phytochemical analysis of *Bougainvillia spectabilis* leaves in this study revealed the presence of many compounds that was considered as antioxidants [27, 28], these results are compatible with previous studies[29,30]. Aromatic medicinal plants such as myrtle, are good source of natural antioxidants, the antioxidant activity of myrtle berries were studied[31], the main compounds that considered to have this property were anthocyanins and flavonoids[32], these results are compatible with those obtained by [33,34].

TABLE 6- effect of plants extracts on antioxidant activity, O.D. at 517nm

	Leaves extracts concentrations (mg/ml)			
Plant extracts	15	30	45	60
Bougainvillea spectabilis	0.36	0.26	0.18	0.10
Myrtus communis	0.33	0.24	0.15	0.07
	(O.D. for blank 0.4)			

TABLE 7- effect of plants extracts on antioxidant activity (%)

plants extracts	Leaves extracts concentrations (mg/ml)			
	15	30	45	60
Bougainvillea spectabilis	10%	35%	55%	75%
Myrtus communis	17.5%	40%	62.5%	82.5%

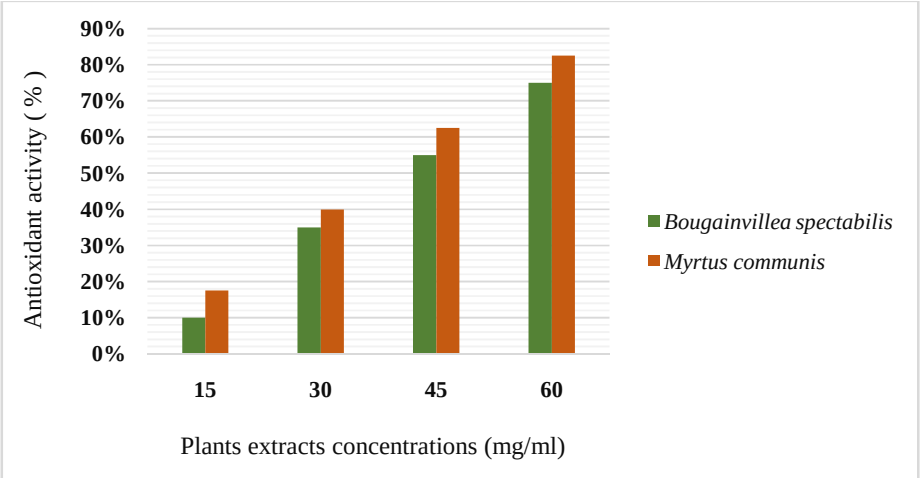

FIGURE 6- Antioxidant activity (%) of *B. spectabilis* and *M.communis* leaves extracts.

CONCLUSION

The current research showed that *Bougainvillea spectabilis* and *Myrtus communis* leaves extracts posses significant amount of phtochemicals and *in vitro* antibacterial and antioxidant activity that is encouraging to be used as alternative medicines.

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