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In Vitro Antibacterial efficacy of the Secondary Metabolites Extracted from *Myrtus communis* L. against some pathogenic bacteria isolated from Hemodialysis Fluid

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Abstract

The present study, was conducted to investigate the effect of the crude Flavonoid, Alkaloid, and Terpenoid, compounds extract from the leaves of (*Myrtus communis* L.) against pathogenic bacteria isolated from hemodialysis fluid in some hospitals in Hillah City 2020 in Iraq. Antibacterial activity was achieved in vitro by using agar-well diffusion method against pathogenic bacteria isolated from hemodialysis fluid by preparing three concentrations for each crude compound (50, 100, and 200) mg/ml and compared with positive control represented by Azithromycin antibiotic and negative control represented by 10% dimethyl sulfoxide. the aimed of this study to investigate the antibacterial efficacy of the Secondary Metabolites Extracted from *Myrtus communis* L. leaves against some pathogenic bacteria isolated from Hemodialysis Fluid. The data collected from the study revealed that, the crude Flavonoid and Alkaloid compounds extract from the leaves of (*Myrtus communis* L.) showed significant reduction at $P \leq 0.05$ in the growth of pathogenic bacteria isolated from Hemodialysis at 20 mg/ml compared with negative control. Finally, it can be concluded that Flavonoids and Alkaloid of *Myrtus communis* L. is most effective in controlling pathogenic bacteria isolated from hemodialysis fluid. While, terpenoid compounds was the least effective in controlling growth of pathogenic bacteria isolated from hemodialysis fluid compared with flavonoid and alkaloid.

Keywords: Antibacterial, *Myrtus communis* L., Flavonoids, Terpenoids, Alkaloids

INTRODUCTION

Urinary tract infection (UTI) has become a more grievous problem today, due to multidrug resistance of infecting Gram-positive and Gram-negative bacteria, sometimes even with multiple infections [1]. Urinary tract infection was mostly caused by Gram negative bacteria, predominately by *Escherichia coli* and by mixed infections of Gram positive, *Staphylococcus aureus*, *Enterococcus faecalis*, including other Gram-negative bacteria, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Citrobacter freundii*, *Proteus vulgaris* and *Klebsiella oxytoca* [2]. Antibiotics are manufactured at an estimated scale of about 100,000 tons annually worldwide, and their use had a profound impact on the life of bacteria on earth. More strains of pathogens have become antibiotic resistant, and some have become resistant to many antibiotics and chemotherapeutic agents, the phenomenon of multidrug resistance. Large amounts of antibiotics used for human therapy, as well as for farm animals and even for fish in aquaculture, resulted in the selection of pathogenic bacteria resistant to multiple drugs [3]. Multidrug resistance in bacteria may be generated by one of two mechanisms. First,

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these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs [4]. Today, natural medicines not only provide the primary health-care needs for the majority of the population in developing countries but have attracted more and more attention in developed countries due to soaring health-care costs and universal financial austerity. In the USA, approximately 49% of the population has tried natural medicines for the prevention and treatment of diseases [5]. *Myrtus communis* L. (Family Myrtaceae) is an aromatic evergreen perennial shrub or small tree [6]. Myrtle (*Myrtus communis* L) is a medicinal herb used worldwide in traditional medicine [7]. However, the aimed of this study to investigate the antibacterial efficacy of the Secondary Metabolites Extracted from *Myrtus communis* L. leaves against some pathogenic bacteria isolated from Haemodialysis Fluid

MATERIALS AND METHODS

Plant material: Myrtle (*Myrtus communis* L.), leaves were collected from gardens at University of Babylon, during October 2020, identified based on the taxonomic features in Iraqi Flora [8]. (Table: 1). Leaves of these plants were cleaned, dried, and kept according to [9], Figure: 1.

Figure 1. *Myrtus communis* L.

Table 1: Scientific, Local, English name, Family, and active parts

Scientific name	Local name	English name	Family	Active part used
Myrtus communis L.	Yas	Myrtle	Myrtaceae	leaves

Extraction of the Crude Flavonoid Compounds: Crude Flavonoid compounds were extracted according to [10].

Extraction of the Crude Alkaloid Compounds: Crude Alkaloid compounds were extracted according to [11].

Extraction of the Crude Terpenoid Compounds: Crude terpenoids compounds were extracted according to [12]. Stock solution of 200 mg/ml for Flavonoid, Terpenoid, and Alkaloid, were prepared in 10%

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Dimethyl Sulfoxide (DMSO) then sterilized by Millipore filter (0.22µm) and stored at (-20C˚) until use [13].

Antibacterial Efficacy: The anti-bacterial activity of the secondary metabolite's compounds extracted from the leaves of (*Myrtus communis* L.) was tested against the isolated bacteria by using agar-well diffusion method [14]. Wells were made by using cork porer (6mm) in diameter. Dimethyl sulfoxide 10% (DMSO) was used as a negative control and Azithromycin (30 µg/Disc) antibiotic as a positive control (Table: 1).

Table: 1. Types of antibiotic, Abbreviation, and concentration per disc

Antibiotic	Abbreviation	Concertation (Disc/µg)	Bacteria
Azithromycin	AZM	30	*Staphylococcus aureus*
			Enterococcus faecalis
			Escherichia coli
			Klebsiella pneumonieae

Bacterial Isolates: All isolates used in this study was isolated from hospitals located at Hillah city, Iraq (Table: 2).

Table: 2. Types of Bacterial Isolates and their sources

N0	Bacteria isolate	Source of specimen
1	*Staphylococcus aureus*	Hemodialysis Fluid
2	*Escherichia coli*	
3	*Enterococcus faecalis*	
4	*Klebsiella pneumonieae*	

Statistical analysis: All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance by using SPSS 16.0 program, a completely randomized design was used and least significant difference (L.S.D) was performed at P≤ 0.05.

RESULTS

The antibacterial activity of secondary metabolites extracted from leaves of (*Myrtus communis* L.), such as (Flavonoid, Alkaloid, and Terpenoid) against pathogenic bacteria isolated from Hemodialysis Fluid is presented in a table (3, 4, and 5). Activity of the secondary metabolites was screened by agar well diffusion methods (Figure 1, 2, 3, and 4). The results revealed that, the extracts of Flavonoid, Alkaloid, and Terpenoid of (*Myrtus communis* L.) leaves showed significant reduction at P≤ 0.05 in the growth of pathogenic bacteria isolated from Hemodialysis Fluid. Antibacterial activity was applied at (50, 100, and 200 mg/ml), and then, compared with 10% dimethyl sulfoxide (DMSO) as a negative control and with Azithromycin antibiotic (30 µg/Disc) as a positive control. Inhibitory zone diameter increases significantly at (P≤ 0.05) by increasing concentration from 50 to 200 mg/ ml. The results also revealed that, flavonoid compounds extracted at (50, 100, and 200 mg/ml) showed significant effect at (P≤ 0.05) compared with negative control, and showed a similar effect (There in no significant difference at P≤ 0.05) between flavonoid compounds and the Azithromycin antibiotic as the inhibition diameter reached (30± 1) in the flavonoid compounds compared with (30± 0) in the antibiotic when applied to *S. aureus* pathogenic bacteria. In the same context, the results also showed a similar effect (There in no significant difference at P≤ 0.05) between flavonoid compounds and the Azithromycin antibiotic when applied to *E.*

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coli (30.66± 1.15) and *K. pneumonieae* (29.66± 0.57). On the other hand, the results of flavonoid compounds at (200 mg/ml) showed significant superiority at (P≤ 0.05) over the Azithromycin antibiotic as the inhibition diameter reached to (32± 1) in the flavonoid compared with (27± 0) in the Azithromycin antibiotic when applied to *E. faecalis* pathogenic bacteria (Table: 3).

Table: 3. *In Vitro* Antibacterial efficacy the Flavonoid compounds Extracted from *Myrtus communis* L. against some pathogenic bacteria isolated from Hemodialysis Fluid

Concentration	Pathogenic bacteria			
	S. aureus	*E. coli*	*E. faecalis*	*K. pneumonieae*
	Inhibition zone/mm			
Control negative	0± 0	0± 0	0± 0	0± 0
50 mg/ml	21± 1	26± 1	22± 2	23.66±0.57
100 mg/ml	24.66± 0.57	28± 1	29± 1	27± 2
200 mg/ml	30± 1	30.66± 1.15	32± 1	29.66± 0.57
Control positive	30± 0	30± 0	27± 0	30± 0
LSD	1.24	1.48	1.99	1.75
*Mean± standard deviation				

The present study also revealed that, there are significant decrease in the growth of pathogenic bacteria with the increasing of concentration of alkaloid compounds extracted from (*Myrtus communis* L.) compared with the negative control DMSO 10% (Table: 4). In the same context, the results also revealed that, alkaloid compounds at (200 mg/ml) showed significant superiority at (P≤ 0.05) over the Azithromycin antibiotic as the inhibition diameter reached to (32± 1) in the alkaloid extract compared with (27± 0) in the antibiotic when applied to *E. faecalis* pathogenic bacteria. In addition to that, there is no significant difference between alkaloid compounds extracted from (*Myrtus communis* L.) at (200 mg/ml) and Azithromycin antibiotic at P≤ 0.05 when applied to *E. coli* and *K. pneumonieae* pathogenic bacteria. In contrast, the Azithromycin antibiotic showed significant superiority at (P≤ 0.05) over alkaloid compounds when applied to *S. aureus* (Table: 4).

Table: 4. *In Vitro* Antibacterial efficacy the Alkaloid compounds Extracted from *Myrtus communis* L. against some pathogenic bacteria isolated from Hemodialysis Fluid

Concentration	Pathogenic bacteria			
	S. aureus	*E. coli*	*E. faecalis*	*K. pneumonieae*
	Inhibition zone/mm			
Control negative	0± 0	0± 0	0± 0	0± 0
50 mg/ml	16± 1	18.33± 1.52	19± 1	21.33± 1.15
100 mg/ml	18± 1	25± 1	24.33± 0.57	25± 1
200 mg/ml	24± 1	30± 1	29± 1	30± 1
Control positive	30± 0	30± 0	27± 0	30± 0
LSD	1.47	1.69	1.24	1.48
*Mean± standard deviation				

The current study also uncovers that, terpenoid compounds at (50, 100, and 200 mg/ml) showed significant superiority at (P≤ 0.05) compared with negative control. But, in contrast, Azithromycin antibiotic showed significant superiority at (P≤ 0.05) over terpenoid compounds in all concentrations when applied to *S. aureus*, *E. coli*, *E. faecalis*, and K. pneumonieae (Table: 5) Thus, terpenoid compounds

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extracted from leaves of (*Myrtus communis* L.) was the least effective in controlling growth of pathogenic bacteria isolated from hemodialysis fluid compared with flavonoid and alkaloid.

Table: 5. *In Vitro* Antibacterial efficacy the Terpenoid compounds Extracted from *Myrtus communis* L. against some pathogenic bacteria isolated from Hemodialysis Fluid

Concentration	Pathogenic bacteria			
	S. aureus	*E. coli*	*E. faecalis*	*K. pneumonieae*
	Inhibition zone/mm			
Control negative	0± 0	0± 0	0± 0	0± 0
50 mg/ml	15± 1	10.66± 0.57	14.66± 1.52	12.33± 0.57
100 mg/ml	17.33± 0.57	12± 2	20± 1	15± 1
200 mg/ml	20± 1	15± 1	21.66± 0.57	20.66± 0.57
Control positive	30± 0	30± 0	27± 0	30± 0
LSD	1.24	1.87	1.55	1.04
*Mean± standard deviation				

Figure: 1. Antibacterial activity of the crude flavonoid compounds extracted (M. communis L.) leaves at (50, 100, and 200 mg/ml) against

Figure: 2. Antibacterial activity of the crude alkaloid compounds extracted (M. communis L.) leaves at (50, 100, and 200 mg/ml) against *E. coli*

Figure: 3. Antibacterial activity of the crude flavonoid compounds extracted (M. communis L.) leaves at (50, 100, and 200 mg/ml)

Figure: 2. Antibacterial activity of the crude alkaloid compounds extracted (M. communis L.) leaves at (50, 100, and 200 mg/ml) against *E. faecalis*

DISSCUTION

The present study was proved that, the secondary metabolites include Flavonoids, Alkaloid, and Terpenoids, extracted from the leaves of (*Myrtus Communis* L.) have antibacterial activity against pathogenic bacteria isolated from hemodialysis fluid especially Flavonoid and Alkaloid compounds. The plant kingdom provided and is still providing endless sources of medicinal plants of various uses for

example, Bioactive compounds such as phenolic, terpenoids, and alkaloids extracted from several medicinal plants like (*Lactuca serriola* L., *Lepidium sativum* L., *Myrtus Communis* L., *Cassia senna* L., *Ricinus communis* L., *Cassia didymobotrya* (Fresenius) Irwin & Barneby, *Melia azedarach* L., *Dianthus caryophyllus* L., and *Salvia hispanica* L.) have antibacterial efficacy against different pathogenic microorganisms [15,16,17,18,19,20,21,22,23]. [24] were used primitive plant like *Chlorella vulgaris* as antibacterial. [25] was used *Hibiscus sabdarifa* extracts against member of Enterobacteriaceae microorganisms. [26] was used leaves of *Ficus carica* Linn against pathogenic bacteria. [27] was used *Curcuma longa* L. and *Boswellia carteri* Birdwood against *Fusarium* species isolated from maize seeds. Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts of biologically active compounds isolated from plant species used in herbal medicines [28]. Antimicrobial properties of medicinal plants therefore, may have a significant clinical value in treatment of resistant microbial strains [29]. [30] was reported that, The antibacterial activity of the (*Myrtus Communis* L.) extracts against *Staphylococcus aureus* ATCC 6538P and *Staphylococcus aureus* ATCC 29213 was more than Ceftazidime antibiotic. *M. communis* leaves extracts showed greatest antibacterial effect against *S. aureus* and *V. cholerae* cerotype Ogawa [31]. Myrtle essential oil has a moderate inhibitory activity against *Staphylococcus aureus* and *Acinetobacter baumanni*i [32]. Biologically active compounds such as tannins, flavonoids, coumarins, essential oil, fixed oil, fibres, sugars, citric acid, malic acid and antioxidants are present in the plant [33]. In contrast, natural bioactive compounds extracted from medicinal plants make their effects by many mechanisms, for example polyphenols binding with polysaccharides and proteins (Macromolecules), thus inhibiting their roles in biochemical metabolites. Terpenoids and flavonoids make their effects by disruption of microbial membranes and Polypeptide's embarrassment of linkage of bacterial proteins to host polysaccharide receptors and alkaloids complexes make their effect by inhibiting of efflux pump [34]. Finally, bacterial activity of *Myrtus Communis* L. might be belonging to secondary metabolites like Flavonoids, Alkaloids, and Terpenoids, and their effect in proteins, RNA, and DNA synthesis and disruption in membranes permeability or disturbance in metabolic activity.

CONCLUSION

Secondary metabolites compounds extracted from *Myrtus Communis* L. especially Flavonoids, Alkaloids regard a good source for controlling pathogenic bacteria isolated from hemodialysis fluid.

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