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# Estimation and Evaluation of Antibacterial Efficacy of Phenolics Isolated from Iraqi *Myrtus communis* Leaves

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**ABSTRACT**

*Myrtus communis* is considered as one of the major medicinal plants Iraqi abundant widely in plant Kingdom. The current study was established to isolate the phenols from the leaves of this plant and extraction percentages were calculated. Qualitative tests were applied for cold aqueous, cold ethanolic and phenolic extracts for *Myrtus communis*. The concentrations of phenols which were represented by 50,100,125 and 150 gm/ml recorded inhibition zone diameters equal to 20,20,21 and 22 mm respectively against growth *Escherichia coli* while the same concentrations values showed inhibition zone diameters were 20,22,23 and 25 mm respectively against *Staphylococcus aureus*. The concentrations prepared from the phenols showed a very good biochemical and medicinal ability to inhibit the biological and chemical system of these pathogenic bacteria, so the phenolic compounds of *Myrtus communis* can be applied for treatment of infections and inflammatory caused by these pathogens instead of the synthetic drugs having multi-side effects.



## 1. INTRODUCTION

The importance of traditional medicinal plants comes from their various biochemical and clinical functions for treatment of a wide spectrum of diseases causing infections and inflammatory in human being. The increased demand of use of the chemical extracts belonging to different medicinal plants led to treat many pathogenic cases [1], [2]. The metabolitic active chemical compounds are biochemically anabolized in the medicinal plants by secondary metabolism through a lot of metabolic chemical pathways catalized by multi-enzymes. The active compounds present in the various parts of these plants include many chemical families such as phenols, flavonoids, alkaloids, glycosides, tannins, saponins, steroids, essential oils, coumarins and terpenes [3], [4].

Multi-researchers indicated, estimated and proved the biochemical and medicinal potential of the active secondary metabolites against various pathogenic micro-organisms like bacteria, fungi and parasites causing to many diseases infect humans and animals. In addition to presence of active chemical compounds, the medicinal plants have no side effects as in synthetic antibiotics and this feature resulting from synergistic interaction among all active metabolites existing in all parts of these plants [5- 7].

Myrtle (*Myrtus communis*) is one the flowering genus belongs to medicinal plants belonging to Myrtaceae family. This plant was classified and described by the botanist Linnaeus in 1753. Nearly 600 names have been decided to this genus of plant. Also other botanic genera are concerned as synonyms and the subfamily of the myrtle is Myrtoideae. *Myrtus communis* is evergreen shrub and it is found in Europe, Asia and Africa. Myrtle tree is 3-4.5 metres length, dense foliage and it has smooth park. Also this medicinal plant has an aromatic smell specially in glossy leaves [8- 10].

Different studies were established about the active secondary metabolitic compounds abundant in the botanic parts of *Myrtus communis* and these studies proved presence of essential oils, phenolic acids, tannins flavonoids, linalool, trans-caryphyllene oxide and β-caryphyllene [11], [12]. Phenolic compounds are active chemical metabolites present in different medical plants and they are considered as one of natural chemical families which have a biochemical and medicinal roles to treat various diseases caused by multi-pathogenic microorganisms such as bacteria, parasites and fungi [13], [14]. Chemically, phenols are aromatic compounds have benzene ring contains one or more hydroxyl group. The existence of this functional chemical group in the phenolics will give the biochemical activity of these potent compounds. Also hydroxyl group can inter in different chemical reactions with various chemical compounds in pathogenic micro-orgasm then it inhibits the biological and physiologycal roles of these componts [15], [16]. The current research was established to estimate and evaluate the biochemical and medicinal efficacy of phenolics from Iraqi *Myrtus communis* leaves against some pathogenic bacteria.

## 2. Materials and Methods

### *2.1 Plant preparation*

Myrtle (*Myrtus communis* L.) leaves were collected from Abu Al-Khaseeb district-Basrah Governorate in south of Iraq. The plant was taxonomies by a specialist botanist in biology department –college of education for pure sciences at university of Basrah. Then the leaves were washed carefully by distilled water and then they were dried in dark place at room temperature, ground and put in dark glass containers until the day of use.

### *2.2 Pathogenic microorganisms*

Two types of pathogenic bacteria stains represented by *Escherichia coli* (-) and *Staphylococcus aureus* (+) were used in this research.

### *2.3 Culture Medium*

The two kinds of bacterial strains were treated with nutrient agar as a culture medium depending on the characterization information determined by company of manufacturing.

### *2.4 Preparation of aqueous and ethanolic extracts:*

The cold aqueous extract belonging to *Myrtus communis* leaves was prepared where 50 gm of leaves powder was treated with 500mlof distilled water in 1L conical flask. The mixture was put on magnetic stirrer for 16 hours then it was filtered and the precipitate was removed then the filtrate was let to dry. Finally the crude extract was collected with yield equal to 12.02 gm. By the same procedure, cold ethanolic extract was prepared but by using 95% ethanol as a solvent and the extract weight was 4.23 gm [17].

### *2.5 Isolation of phenolics from Myrtus communis*

Fifty grams of *Myrtus communis* were treated with 150 ml of 95% ethanol and then the mixture was heated on hot plate at 75°C for 30 minutes. After cooling, the contents were placed in the separation funnel and 50ml

of ethyl acetate was added and mixed well. Then the mixture was shaken well, and finally brown-white precipitate as fine powder. Filtration process was carried out and the phenols crude was formed with weight equal to 6.26gm [18].

### *2.6 Qualitative analysis of chemical extracts*

The cold aqueous, cold ethanolic and phenolic extracts were underwent several tests represented by ferric chloride (1%w/v) for phenols, lead acetate(1%w/v) for tannins, Molisch for carbohydrates, Benedict for glycosides, Potassium hydroxide (5N) for flavonoids ethanolic sulphuric acid with formaldehyde for alkaloids ferric chloride (10%w/v) for saponin and ninhydrin (1%) for amino acids [15], [17].

### *2.7 Determination of antibacterial efficacy of phenolics*

Several concentrations of phenols isolated from *Myrtus communis* leaves are represented by 50,100,125 and 150 mg/ml were prepared. The method of nutrient diffusion was applied for by addition of 0.2 ml of the culture medium to each pathogenic bacteria suspension has optical density equal to 0.1. The all concentrations were put in all petri dishes containing bacteria present in digs and they were placed in the incubator at 37$^{o}$C for 24 hours. Finally inhibition zone diameters were measured [19].

## 3. Results and Discussion

The medicinal importance of *Myrtus communis* is because of existence of various active natural metabolites as chemical compounds in the different parts of this plants. So the all extracts prepared belonging to the *Myrtus communis* had various extraction percentages according to the chemical nature of the extracts and they were calculated with values equal to 24.04, 8.46 and 12.52% for cold aqueous, cold ethanolic and phenolic extracts respectively. The extraction percentages were calculated from the following law:

$$\text{Extraction percentages} = \frac{Weight\ of\ crude\ extract(gm)}{Weight\ of\ plant\ powder\ (gm)} \times 100$$

The results of the extraction percentages are indicated in table (1).

**Table (1):** Extraction percentages of extracts belonging to *Myrtus communis* leaves

| No. | Plant powder weight (gm) | Extract kind | Crude extract weight (gm) | Extraction percentages (%) |
|---|---|---|---|---|
| 1 | 50 | Cold aqueous | 12.02 | 24.04 |
| 2 | 50 | Cold ethanolic | 4.23 | 8.46 |
| 3 | 50 | Phenolic | 6.26 | 12.52 |

From the table (1), it was noticed that the extraction percentage of phenols extract is very good and this ensures presence of many active phenolic compounds in the leaves of *Myrtus communis*. Also the extraction percentage of cold aqueous extract is higher than the same in cold ethanolic extract therefore this chemical case proves abundance of more phenolic compounds in cold aqueous extract and in the same time, this ensures attendance of high polar phenols because they were extracted and isolated by using distilled water as solvent has the high polarity [20].

Table (2) shows the preliminary qualitative detections belonging to the cold aqueous, cold ethanolic and phenolic extracts by using different chemical reagents.

**Table (2):** Qualitative detections of the extracts of the *Myrtus communis* leaves.

| Conclusion | Test results | phenolic extract | Ethanolic cold extract | Cold aqueous extract | Reagent |
|---|---|---|---|---|---|
| Phenols are Present | Blulish-green colour | + | + | + | $FeCl_3$(1%) |
| Tannins are Present | White-light brown p.p.t | - | + | + | $Pb(Ac)_2$(1%) |
| Carbohydrates are Present | Violet ring | - | + | + | Molisch |
| Glycosides are Present | Red p.p.t | - | - | + | Bendecit |
| Flavonoids are Present | Yellow p.p.t | + | + | + | Ethanolic KOH (5N) |
| Alkaloids are Present | Turbidity with p.p.t | - | + | + | $H_2SO_4$+formaldehyde |
| Saponins are Present | Yellow-green p.p.t | - | + | + | $FeCl_3$(10%) |
| Amino acids are Absent | No Violet colour | - | - | - | Ninhydrin (1%) |

It was noticed that the cold aqueous extract has phenolic compounds, tannins, carbohydrates, flavonoids, glycosides, alkaloids and saponnins but the cold athanolic extract belonging to *Myrtus communis* leaves contains phenols, tannins, carbohydrates, flavonoids, alkaloids and saponins whereas the phenolic extract has only phenols and flavonoids. This ensures isolation of phenolic compounds in high purity because non-phenolic compounds were not detected qualitatively in the extract. Various qualitative and quantitive studies in regard to *Myrtus communis* plant indicated and proved of existence of phenols with different chemical classes (phenolic acids, tennins and flavonoids) in the several parts of this medicinal plant [21], [22]. Since various researches showed and insured abundance of different active chemical metabolites including phenols therefore the myrtle is considered as antibacterial, antifungal and antiparasitic plant. Thus phenols which were isolated separated and characterized from *Myrtus comminis* were carried out successfully to treat many diseases resulting from infections by pathogenic microorganisms [23], [24]. Phenolics as active chemical compounds present in plants, are biochemically anabolized by shikimic acid as starting materials by using several various metabolic pathways catalyzed by multi-enzymes are responsible for production of these natural metabolites. So *Myrtus communis* has biochemical activity and great biological efficiency because it has these secondary metabolites [25].

Because of *Myrtus communis* has antibacterial potential therefore it was used in the current research through presence of phenols which were extracted and isolated from the leaves of this plant. Table (3) represents results of antibacterial activity of these phenolics against growth of two pathogenic bacteria represented by Escherichia coli and Staphylococcus aureus.

**Table (3):** Antibacterial Efficacy of phenolics isolated from *Myrtus communis* L. leaves against the two pathogenic bacteria.

| Inhibition zone diameter(mm) | | Phenolics concentration (mg/ml) |
|---|---|---|
| *Staphylococcus aureus* | *Escherichia coli* | |

| | | |
|---|---|---|
| 20 | 20 | 50 |
| 22 | 20 | 100 |
| 23 | 21 | 125 |
| 25 | 22 | 150 |

The concentrations of 50,100,125 and 150 mg/ml of *Myrtus communis* leaves phenolics recorded inhibition zone values equal to 20,20,21 and 22 mm respectively against growth of *Escherichia coli* while the same concentrations value of phenols showed different values of inhibition zone diameters represented by 20,22,23 and 25 mm towards *Staphylococcus aureus* bacteria. From table (3), it was found that the increase of concentration value has led to increasing in values of inhibition zone diameters so this case are explained by inhibition or killing most pathogenic bacteria when the phenolics concentration has increased.

The biochemical metabolic mechanism of phenolic compounds for inhibition of growth of various pathogens including Escherichia coli and Staphylococcus aureus is explained by many mechanisms one of them is chemical capability of natural metabolites phenolics to bind chemically with nucleic acids represented by DNA and RNA leading to decrease or inhibit the biochemical and physiological functions of these acids in the pathogenic bacteria cell [26], [27]. Also the hydroxyl group(s) abundant in natural phenols have chemical ability for destruction of the wall and membrane belonging to pathogens cell leading to change in the tertiary structure of proteins existing in the cell of pathogenic bacteria. Some studies proved the high ability of different phenols in medicinal plants including Myrtus communis to distract the primary metabolism of lipids, carbohydrates and proteins present in the living cell of pathogenic bacteria leading to consumption of biochemical energy of these pathogens. Also the phenolic metabolites are capable for decrease and/or finish the biochemical roles of various enzymes of microorganisms including pathogenic bacteria causing to inflammatory and infections in human body [28], [29].

## 4. Conclusions

*Myrtus communnis* phenolics were extracted, isolated and purified successfully and they were established and carried out for estimation of antibacterial Efficacy against two pathogens represented by *Escherichia coli* and *Staphylococcus aureus* coli. The concentrations prepared from the phenols showed a very good biochemical and medicinal ability to inhibit the biological and chemical system of these pathogenic bacteria, so the phenolic compounds of *Myrtus communis* can be applied for treatment of infections and inflammatory caused by these pathogens instead of the synthetic drugs having multi-side effects.

## 5. References

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