# Anti-Urease Activity of Essential oil and Phenolic compounds of *Thymus vulgaris*, *Melaleuca alternifolia* and *Betula pendula* against *Proteus mirabilis* isolates

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## Abstract

The present study was carried out to evaluate the activity essential oil and phenolic compounds of *Thymus vulgaris*, *Melaleuca alternifolia* and *Betula pendula* extracts against urinary tract infections caused by urease-producing from seven strains of bacteria *Proteus mirabilis* isolated from urinary tract infection. Microdilution technique has been used to evaluate antibacterial operations in sterile 96-well microtiter plates. Activity of urease and inhibition of urease assessed by indophenol test method. The results showed that *M. alternifolia* extract was the best antibacterial activities against all strains at 1.25 - 25 mg/ml. Also, 0.31 mg/ml of phenolic compounds and essential oil of *M. alternifolia* extract serves as effective dual acting 100% urease inhibitor to prevent and eliminate urease. Besides, the results of phytochemical detection show that Coumarins, Terpenes, Alkaloids, Tannins, Flavonoids and Sesquiterpenes were present in *M. alternifolia* extract, which may be have anti-urease possessing activity. Furthermore, these plants could be act as a source of antimicrobial compounds against *P. mirabilis* which are commonly impaired by the development of bladder and kidney stones.

***Keywords:*** *Urease inhibitor, MIC, Total phenolic compounds, Phytochemical screening*

## Introduction

Urinary tract infections are the severe health problem that affects millions of individuals every year. These are very prevalent infections that occur when bacteria enter the urinary bladder and multiply anywhere along the normal sterile urinary tract [1]. *Proteus mirabilis*, is well-known for its urease production and distinctive ability to differentiate into elongated swarm cells and characteristic bull's-eye pattern of motility on agar plates. *P. mirabilis* could be observed in a huge range of environments, along with soil, water and sewage., but it is predominantly a commensal of the gastrointestinal tracts of humans and animals [2]. *Proteus* genus are widely distributed in the natural environment, including polluted water, soil, and manure. Due to their proteolytic activity, the ability to hydrolyze urea to ammonia and carbon dioxide, as well as the oxidative deamination of amino acids, these bacteria are involved in the decomposing of the organic matter of the animal origin. They are also present in the human gastrointestinal tract of humans and animals [3].

Plants are rich source of antibacterial agents, most of which probably evolved as chemical defense against predation or infection. The present antibacterial review of the plant extracts demonstrates that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. Due to the ongoing restructuring of natural products and processes to maintain human and environmental health, the economic environment and the significance of medicinal plant materials have risen tremendously in latest years [4]. In general, the medicinal plant containing essential oil, alkaline, flavonoids, and phenol contents may posses' strong anti-microbial properties [5]. Therefore, the current study was designed to evaluate the anti-bacterial activity of the essential oil and phenolic compounds of *T. vulgaris* and *M. alternifolia* against seven isolate of bacteria *P. mirabilis* and urease inhibitor agent with dual actions, which are commonly impaired by the development of bladder and kidney stones.

## Material and Methods

### Plant materials

The Plant samples were obtained from Baghdad local market, Baghdad ☐ Iraq. Plants were identified in herbarium, Department of Biology /College of Science /University of Baghdad. The plant's parts dried and grinded into powder by mechanical grinder, it kept at 4°C until further investigations.

### Bacterial Strains and Culture Conditions

Seven of *P. Mirabilis* strains were used in this study. These strains were isolated from urinary tract infections (UTIs). All strain was kindly provided by Department of Biology /College of Science /University of Baghdad. All strains were confirmed by using UTI chromogenic agar and Vitek2 compact. Strains were maintained in Mueller-Hinton agar.

### Extraction of crude phenols

Crude phenols were extracted according to Ribereau-Gayon [6].

### Extraction of the essential oils

The 100 g of the air-dried and ground parts of the tested plants were submitted for 4 hours to water-distillation using a Cleavinger apparatus. The obtained essential oils were dried over sodium sulphate anhydrous, then stored at 4°C until tested and analyzed [7].

### Detection of Triterpenes/Steroids (Liebermann-Burchard Reagent).

One mL of acetic anhydride and 5 drops of concentrated sulfuric acid ($H_2SO_4$) were added to the extract. A color change from violet to blue confirms the presence of steroids and formation of blue-green ring indicated the presence of terpenoids [8].

### Detection of Coumarins.

Three mL of 2N NaOH was added to 2mL of aqueous extract. Formation of yellow color indicated the presence of coumarins. Confirmation test was performed by adding 1mL of 5N HCl; in this case a colorless solution formed at the upper layer is considered positive [9].

### Screening for Sesquiterpene Lactones.

The Baljet reaction (1% Picric acid in 10% sodium hydroxide) was used to detect sesquiterpene lactones in the extracts. Reagents were mixed at a 1 : 1 ratio and added to 1mL of extracts (2-3mg). The transformation of the sodium picrate solution's yellow color to orange-red color confirmed the positive reaction [10].

### Test of Carboxyl Group.

The presence of carboxyl groups was evidenced by adding 10 drops of 10% sodium bicarbonate solution; visible bubbles of carbon dioxide were considered a positive reaction [11].

### Test for Tannins.

Extracts were treated with 1mL of 5% ferric chloride which was added. The presence of tannins was indicated by the formation of bluish black or greenish black precipitate [12].

### Shinoda Test. (Test for flavonoids)

Few fragments of magnesium metal ribbon (3-4 pieces) were added to 1mL of extract, followed by dropwise addition of concentrated hydrochloric acid. Formation of pink or red color indicated the presence of flavonoids [13].

### Test for Saponin.

Two mL of distilled water was added to extracts suspended in ethanol and was shaken vigorously. The formation of copious foam layer indicates the presence of saponins [14].

### Determination of Minimum Inhibitory Concentration (MIC)

MIC of plant extracts against studied bacteria was determined by microdilution method in sterile 96-wells microtiter plates according to the protocol described previously McBain *et al.* [15]. Different plant extracts concentrations (100, 50, 25, 12.5, 6.25, 3.125 and 1.625 µg/ml) (W/V) were prepared to containing bacterial cells comparable to McFarland standard no. 0.5 in a final volume of 200 µl. Sterile distilled water, broth and plant extracts were used as a negative control while sterile distilled water, broth and bacteria were used as positive control. After 24 hrs. at 37° C, the MIC of each sample was determined. The MIC considered the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after 24 hours incubation [16].

**Urease inhibition activity assay**

The phenolic solution assay mixture consisted of urea (30 mM) and (200 µl) crud extract. The reactions were initiated by the addition of 50 µl of urease enzyme solution in distilled water. Urease activity was determined by measuring ammonia concentration after 15 minutes of enzymatic reaction. The ammonia was determined using 500 ml of solution A (contained 5.0 g

phenol and 25 mg of sodium nitroprusside in 500 ml of distilled water) and 500 ml of solution B (contained of 2.5 g sodium hydroxide and 4.2 ml of sodium hypochlorite 5% in 500 ml of distillated water) at 37°C for 30 minutes. The absorbance was read at 625 nm. Activity of uninhibited urease was designated as the control activity of 100% [17].

Urease solution (200 µl) was mixed with the (200 µl) essential oil of the two plants. Each mixture was incubated at 37°C for 30 min. Urease activity was determined by measuring ammonia production using the indophenol method as described (Weatherburn MW) [18]. Briefly, 5m1 each of essential oil reagent A and 5ml of alkali reagent B were added to each well. The increasing absorbance was measured after 30 min at wavelength of 625 nm using uv-visible spectrophotometer. All reactions were performed in duplicates in final volume of 10.4 ml [19]. % Inhibition= 100 - (Absorbance of Test Compound/Absorbance of Control)*100

## Statistical Analysis

expressed as mean ± standard deviation (SD) for analysis performed in duplicate at least three times. Statistical analysis of the data was performed by Duncan□s test: similar letters refer to a non-significant difference, while the different letters refer to a significant difference.

## Results and Discussion

**Effect of medicinal plants against bacteria.**

**Effect of TPC (Total Phenolic Compounds)**

Antibacterial activity TPC against seven strains of *P. mirabilis* isolated from UTIs were revealed in Table 1. TPC showed different inhibited concentration ranged from 6.25 to 25 mg/ml. *M. alternifolia* leaves extracts showed a good result of antibacterial activity against all tested microorganism strains using Microdilution assay method (Table 1). The MIC of *M. alternifolia* extract ranged from 6.25 to 12.5 mg/mL, while *T. vulgaris* and *B. pendula* show activity against microorganism strains but in higher concentration.

**Table 1. MIC of TPC against *P. mirabilis*.**

| Concentration of extract (mg/mL) | | | |
|---|---|---|---|
| **Bacterial strains** | **Thymus vulgaris** | **Melaleuca alternifolia** | **Betula pendula** |
| P. mirabilis1 | 25 | 12.5 | 25 |
| P. mirabilis2 | 25 | 12.5 | 25 |
| P. mirabilis3 | 25 | 6.25 | 25 |
| P. mirabilis4 | 25 | 12.5 | 12.5 |
| P. mirabilis5 | 25 | 12.5 | 25 |
| P. mirabilis6 | 25 | 6.25 | 25 |
| P. mirabilis7 | 25 | 12.5 | 12.5 |
| mean ± SE | 25.0 ± 0.0a | 10.71 ± 1.15b | 21.43 ± 2.31a |

### Effect of EOC (Essential Oil Concentration)

The growth of *P. mirabilis* strains were effectively inhibited for all isolates at MIC between 0.39 and1.25mg/ml of essential oils Table 2. The lowest MIC was 0.39 mg/ml for *T. vulgaris* essential oil, while the highest MIC was 25 mg/ml for *B. pendula* essential oil as shown in Table 2 .

**Table2. MIC of EOC against *P. mirabilis*.**

| Concentration of extract (mg/mL) | | | |
|---|---|---|---|
| **Bacterial strains** | **Thymus vulgaris** | **Melaleuca alternifolia** | **Betula pendula** |
| P. mirabilis1 | 0.78 | 1.25 | 25 |
| P. mirabilis2 | 0.39 | 1.25 | 25 |
| P. mirabilis3 | 0.78 | 1.25 | 25 |
| P. mirabilis4 | 0.78 | 0.78 | 25 |
| P. mirabilis5 | 0.39 | 1.25 | 12.5 |
| P. mirabilis6 | 0.78 | 1.25 | 12.5 |
| P. mirabilis7 | 0.78 | 0.78 | 25 |
| mean ± SE | 0.67 ± 0.07c | 1.12 ± 0.23b | 21.43 ± 6.10a |

### Urease enzyme inhibition assay

Herein, urease enzyme inhibitory activity of TPC and EOC were elucidated as the most potent ones including *T. vulgaris*, *M. alternifolia* and *B. pendula*. As it is presented in Table 3, TPC and EOC value 0.32 mg/mL of *M. alternifolia* were the most effective compound (100%) urease enzyme inhibition, followed by 0.30 mg/ml of *B. pendula* TPC (93.7%) and by 0.32mg/ml of by *B. pendula* EOC value 100%, respectively. While, the lowest activity was 90.6% for *T. vulgaris* TPC.

**Table 3. Urease enzyme inhibition**

| Plant extracts | Activity | % enzyme inhibition |
|---|---|---|
| *T. vulgaris* TPC | 0.29 | 90.6% |
| *M. alternifolia* TPC | 0.32 | 100% |
| *B. pendula* TPC | 0.30 | 93.7% |
| *T. vulgaris* EOC | 0.31 | 96.5% |
| *M. alternifolia* EOC | 0.32 | 100% |
| *B. pendula* EOC | 0.32 | 100% |

### Phytochemical screening

Phytochemical screening results of different functional groups of selected plant extracts were summarized in Table 4. Coumarins, Terpenes, Alkaloids, Tannins, Flavonoids and Sesquiterpenes were found in *M. alternifolia* and *T. vulgaris* extract. While, Tannins, Flavonoids, Alkaloids and Sesquiterpenes compounds have been described in *B. pendula* extract.

**Table 4. Phytochemical screening results of selected extracts.**

| Compounds | M. alternifolia | T. vulgaris | B. pendula |
|---|---|---|---|
| Terpenes | +++ | +++ | +++ |
| Flavonoids | +++ | ++ | + |
| Coumarins | +++ | ++ | - |
| Alkaloids | ++ | +++ | + |
| Tannins | ++ | ++ | - |
| Saponins | - | - | - |
| Carboxyl group | - | - | - |
| sesquiterpenes | +++ | +++ | +++ |

**+: low intensity reaction, ++: medium intensity reaction, and +++: strong intensity reaction**

## Discussion

In general, the results presented here contribute to the knowledge of antimicrobial activities and chemical composition of the tested TPC and EOC obtained from aromatic plants. Some of these compounds were recognized to exhibit therapeutic activity and metabolic activity [20]. Actually, the flavonoids, have been reported to be synthesized by plants in response to microbial infection and have been shown to have antibacterial activities. Also, Tannins were reported have demonstrated activity against bacteria [21]. Also, the antimicrobial activity was found in the *M. alternifolia*, *T. vulgaris* and *B. pendula* extracts, and this may be due to the presence of Terpenes. This result is supported by the study carried out by Alberto *et al*., who reported that the terpinen-4-ol have antioxidant and anti-bacterial activity [22]. In this work, the urease enzyme inhibitor, have been shown that the EOC extracts were the most effective inhibition growth of *P. mirabilis* than TPC, this may be due to the high concentration of phytochemical compounds. Besides, each phytochemical has different functional and mechanism in the antimicrobial action. For example, the phenolic compounds were much active than aldehydes, while the aldehydes were much active than ketones, and the ketones were more active than alcohols, ethers and hydrocarbons [22]. Also, this result agrees with the study of Hanene *et. al.* [23], whom demonstrated the finding of MIC antibacterial activity of EOC extract of *T. vulgaris* against wide range of pathogenic gram-negative bacteria [24]. On the other hand, the *M. alternifolia* was more effective inhibiter of urease enzyme, which may be due to the high concentration of flavonoids and coumarins compared with the other plants. However, the Coumarins possess a variety of biological properties, including antimicrobial, antiviral, anti-inflammatory, antidiabetic, antioxidant, and enzyme inhibitory activity. On the other hand, possible the synergistic effects of phytochemical compounds in the oil may also increase their antimicrobial activity.

## Conclusion

Some of the plant extracts assessed in the current study had potential activity for antimicrobial and urease inhibitors against isolated UTIs bacteria, which may be an alternative to controlling the development of microbial urease or may be used as a model for searching for fresh drugs.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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