



(RESEARCH ARTICLE)



## Comparison the effect of plant extracts and antibiotics on gram negative bacteria isolated from patients with urinary tract infection in Tikrit city

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### Abstract

**Background:** With increasing bacterial resistance for antibiotic, there is an increasing interest in medicinal herbal as a new source of an antimicrobial agents and with less side effects than conventional drugs.

**Objective:** Was to evaluate the potential of *Syzygium aromaticum* and rosemarinus officinalis extract on *Proteus mirabilis* isolates in vitro.

**Methods:** Six pathogenic gram negative bacilli belonging to three different genus (*E.coli*, *Proteus mirabilis*, and *Enterobacter aerogenes*) and two isolates from each species were used in this study and four concentration (100%, 75%, 50%, 25% mg/ml ) from each plant extracts (aqueous and ethanolic of *Syzygium aromaticum* and rosemarinus officinalis extracts ) were prepared to be antibacterial agent. antibacterial activity was measured by using wells method and five antibiotics using to compare with the activity of antibacterial agent.

**Results:** The result of the study shown that the ethanolic extracts of *Syzygium aromaticum* is a strong antibacterial agent (with diameter of inhibition zone 25 mm) against *Proteus mirabilis*. *Enterobacter aerogenes* with (15mm) of inhibition zon, *E.coli* did not show any inhibition zone also the aqueous and ethanolic of rosemarinus officinalis extracts and aqueous of *Syzygium aromaticum* extracts did not show any activity towards any gram negative bacilli also the results of the study show resistance isolates of *E.coli* and *Proteus mirabilis* toward several antibiotic while *Enterobacter aerogenes* is sensitive for more antibiotics that used in this study.

**Conclusions:** Our study concluded that the extract of *Syzygium aromaticum* has high potential as antibacterial agent against UTI that caused by *Proteus mirabilis*, clove (*syzygium aromaticum*) can be used in pharmaceutical for the production of new synthetic agents. There is an increasing interest in phytochemicals as new sources of natural antioxidant and antimicrobial agents. The natural compounds which have biological activities are interest to pharmaceutical industries especially for treatment of human disease of microbial origin and several pathological disorders such as cancergenesis, coronary atherosclerosis, as well as in ageing processes.

**Keywords:** *Syzygium aromaticum*; *Rosemarinus officinalis*; Antibiotic sensitivity test; Urinary tract infection

### 1. Introduction

Urinary tract infections are the majority common infections in children and women worldwide. It may lead to severe illness and complications, if not diagnosed and treated [1]. UTIs are caused by both Gram-positive and Gram-negative bacteria, as well as by some fungi. The most common causative agent for UTIs are uropathogenic *Escherichia*

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*coli* (UPEC), *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp [2].

Antibiotics such as sulfamethoxazole, ciprofloxacin, ampicillin and trimethoprim are the most generally therapeutics used for UTIs [3].

Medicinal plants are the essential bioresource of drugs for conventional systems of medicine, food supplements, pharmaceutical intermediates, nutraceuticals, folk medicines, and chemical entities for synthetic drugs. In plants and vegetable, natural phenolic compounds may rising the risk of some disease because of their free –radical inhibition and antioxidant properties imparted by the benzene ring and the hydroxyl group in their structure [4]. Clove is from Myrtaceae family are used in Ayurveda, Chines medicine and Western herbalism [5]. Many spices around the world have been used for a lot of medicinal objectives and as food conservative, and out of those *Syzygium aromaticum* (clove) is extensively usage it has pick up anti-inflammatory antioxidant, antimicrobial, antimutagenic, anti-ulcerogenic and antithrombotic properties [6]. scientifically, Rosemary named *Rosmarinus officinalis* associated to the family of Lamiacea and is well-known as apice and medical plant in most countries [7]. Rosemary obtain rising attention due to its anti –inflammatory, antimicrobial and antioxidant constituent, it has been cultivated throughout world and accepted as one of the spices with best antioxidant activeness [8]. Rosemary contains tannins, oleoresin, camphor, pinene, and bornyl acetate also involve great amount of salicylates. In traditional medicine, this herb is utilised for digestive, anti-asthmatic, headache, sedative, relieving, anti-rheumatic, visual acuity, circulatory disorders and irritant effects [7].

Many pathogenic organisms had become resistant nowadays for many manufactured antibiotics so various researches have been done to improve and focus on the pharmltical properties of plants and their parts. the aim of this study was:

- To evaluate the potential of *Syzygium aromaticum* and *rosmarinus officinalis* extract on gram negative bacilli isolated from patient with urinary tract infection.
- Compare the effects of *Syzygium aromaticum* and *rosmarinus officinalis* with some current antibiotics.



**Figure 1** *Rosmarinus officinalis*. L



**Figure 2** *Syzygium aromaticum*

## 2. Materials and methods

### 2.1. Bacterial strains and Identification

Six pathogenic gram negative bacilli belonging to three different genus (*E.coli*, *Proteus mirabilis*, and *Enterobacter aerogenes*) and two isolates from each species were used in this study. Isolates were obtained from patient with UTI (from clinical laboratory of Tikrit Military Hospital, Tikrit, Iraq ). All isolates were grown on blood agar and macConkey agar plate. Isolates were identified by colonial morphology on culture, gram stain and classical biochemical tests [9].

### 2.2. Preparation of ethanolic and aqueous extract of syzygium aromaticum and rosmarinus officinalis

The plants were got from a market and were ground to powder. Fourty gram of each powder solved in (160) ml of ( ethanol 95 % for prepare ethanolic extract and water for prepare aqueous extract ) were allocated in room temperature for 24 hours, the solvents were filtered by Whatman filter paper and then the extract was settled in petri dish at room temperature for drying. The sediment obtained were reserved in a freezer until future tests [10].

### 2.3. Evaluation of antibacterial activity

Four concentration (100%, 75%, 50%,25% mg/ml) from each plant extracts (aqueous and ethanolic of *Syzygium aromaticum* and *rosmarinus officinalis*) were prepared.

Well diffusion method was followed [11]. First prepared Muller Hinton agar plates, the broth cultures of selected bacteria were incubated at 37 °C for 18 hours to obtain a uniform culture. Four wells of 8 mm in diameter in each plate were cut out using a sterile well cutter. In to each of the wells by using a micropipette, 75 µl of the plant extract was added and for 30 mins allowed to diffuse at room temperature. These plates were then incubated at 37 °C for 18 hours. The diameter of the inhibition zones is measured in millimeters, then antibacterial activity was determined.

#### 2.4. Antibiotic susceptibility test

Available five current antibiotic discs were selected and tested in this study (see table 1) against isolated bacteria by using disc diffusion method (Kirby–Bauer method) [12]. The broth culture of 18 hours has to match a defined standard (McFarland turbidity standard) and by a sterile cotton swabs was swabbed onto the Muller Hinton agar. The antibiotic discs of appropriate concentration were placed on the agar surfaces by using a sterile forceps. At 37 °C for 18 hours, the plates were incubated in an inverted manner. The diameter of the inhibition zones was measured in millimeters, after the incubation.

**Table 1** Antibiotics discs used in current study

No.	Name of antibiotic	Code	Concentration of antibiotic
1	Amoxicillin – Clavulanic acid	Aug	30 µg
2	Ceftazidim	Caz	10 µg
3	Cefepime	Fep	10 µg
4	Meropenem	Mem	10 µg
5	Levofloxacin	Lev	5 µg

### 3. Result

Antibacterial activity of plant extracts against gram negative bacteria and compare its with the antibiotic :

Medicinal plants are the important source of antimicrobial agents in various countries [13]. Ethanolic extract of *Syzygium aromaticum* was evaluated for the antibacterial activity against isolates of gram negative bacteria isolated from patient with UTI by using four concentration (100 %,75%,50% and 25% mg / ml) for each plant extracts and the results are given in the Table (2). The antibacterial activity has observed in the ethanolic *Syzygium aromaticum* extract against *Proteus mirabilis* and *Enterobacter aerogenes* with varied activity according to the concentration. The maximum inhibition zone for *Proteus mirabilis* (two isolates) is 25mm in con. %100 mg/ml see fig (3), 15mm for *Enterobacter aerogenes* 2 (one isolates) in con. 100% see fig (4). Aqueous extract of *Syzygium aromaticum* did not show any antibacterial activity against any gram negative bacteria also ethanolic and aqueous extract of *rosmarinus officinalis* failed to show any antibacterial activity against any gram negative bacteria (without any inhibition zone) see table (3) and fig (5). Five different standard antibiotics were used in this study to compare with the antibacterial agent of the ethanolic and aqueous extracts. The results of the comparison showed that in the some tested isolates the antibacterial activity of *Syzygium aromaticum* ethanolic extracts were best than the antibiotics in inhibitions of bacteria such as its activity against *Proteus mirabilis* see table (4) and fig (3) also the results of antibiotic susceptibility showed that *E.coli* isolates has resistance to most antibiotic that used in this study such as Ceftazidim, Cefepime, and Amoxicillin – Clavulanic acid but is show susceptibility towards meropenem see table (4) and fig (6) also the extract plant did not show any activity towards *E.coli* isolates these results are agreement with [14] who stated that *E.coli* strains is resistant to several antibiotics but he found in the same study that (640 mg/ml) from *Syzygium aromaticum* is enough to exhibit *E.coli*. While *Enterobacter aerogenes* showed great inhibition zone toward most antibiotic in this study in compare with the plant extracts.. [15] It is noted that the gram negative tested bacteria in their response toward the extracts were proportionality and demonstrate gradual increasing in inhibition zone with the ascending of extract concentrations, these reality were in accord with some studies.

**Table 2** Antibacterial activity of *Syzygium aromaticum* ethanolic extract against gram negative bacteria

Isolates	100%	75%	50%	25%
<i>E.coli 1</i>	0	0	0	0
<i>E.coli 2</i>	0	0	0	0
<i>Proteus mirabilis 1</i>	25 mm	23 mm	20 mm	15 mm
<i>Proteus mirabilis 2</i>	25 mm	23 mm	19 mm	15 mm
<i>Enterobacter aerogenes 1</i>	0	0	0	0
<i>Enterobacter aerogenes 2</i>	15mm	13mm	12mm	0

**Table 3** Antibacterial activity of rosmarinus officinalis ethanolic, aqueous and *Syzygium aromaticum* aqueous extract against gram negative bacteria

Isolates	100%	75%	50%	25%
<i>E.coli 1</i>	0	0	0	0
<i>E.coli 2</i>	0	0	0	0
<i>Proteus mirabilis 1</i>	0	0	0	0
<i>Proteus mirabilis 2</i>	0	0	0	0
<i>Enterobacter aerogenes 1</i>	0	0	0	0
<i>Enterobacter aerogenes 2</i>	0	0	0	0

**Table 4** Comparisons of antibacterial activity of *Syzygium aromaticum* ethanolic extract with standard antibiotics (inhibition zone mm)

Isolates	S.a	Lev	Caz	Aug	Fep	Mem
<i>E.coli 1</i>	0	13	R	R	R	32
<i>E.coli 2</i>	0	12	R	R	R	30
<i>Proteus mirabilis 1</i>	25	18	R	R	R	18
<i>Proteus mirabilis 2</i>	25	13	R	R	R	19
<i>Enterobacter aerogenes1</i>	0	38	R	25	R	40
<i>Enterobacter aerogenes2</i>	15	38	R	28	R	36

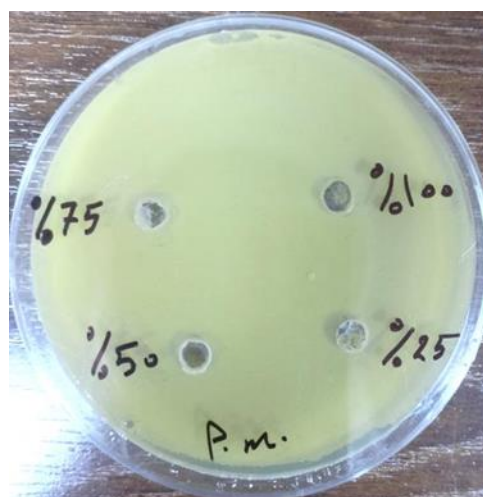
Abbreviation : S.a, Ethanolic extract of *Syzygium aromaticum*, Lev Levofloxacin, Caz Ceftazidim, Mem, Meropenem, Fep, Cefepime, Aug, Amoxicillin – Clavulanic acid.



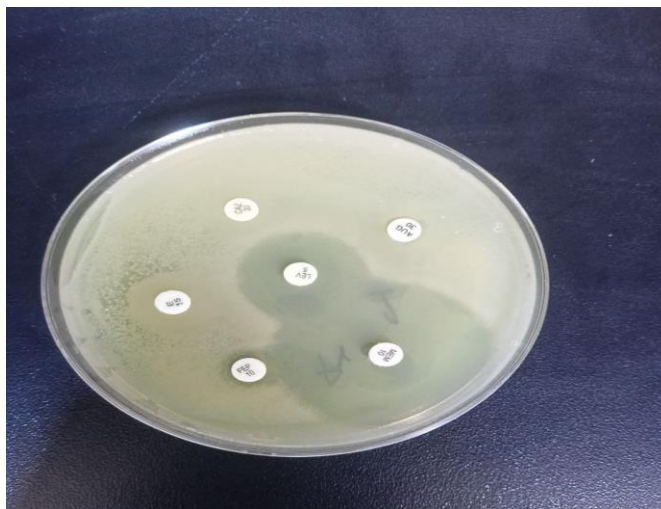
**Figure 3** Inhibition zones (mm) of *Syzygium aromaticum* ethanolic extract against *Proteus mirabilis*



**Figure 4** Inhibition zones (mm) of *Syzygium aromaticum* ethanolic extract against *Enterobacter aerogenes* 2



**Figure 5** No Inhibition zones of *rosmarinus officinalis* aqueous extract against *Proteus mirabilis*



**Figure 6** Antibiotic sensitivity test of *E.coli* isolates

#### 4. Discussion

In developing countries about 60% to 90% of the population use herbal remedies [16]. Natural plant was utilized as food items, a flavouring odor agent in beverages and cosmetics [17]. In this study it was clearly that there is a positive relation between the concentration of the extract and the inhibition zones, the results showed that the inhibition zones were increased with the increasing of concentrations see table (2) while some tested isolates did not response to any concentrations of plant extracts may be these isolates were required higher concentration from plant extracts.

The current study demonstrate resistance of tested isolates to many antibiotic especially *E.coli* and *Proteus mirabilis*. It appears that the development of resistance is occurring at a faster rate than in years past [18] these due to several pathway of resistance which is can be by decrease uptake of drug or porins channels are absent, and plasmid –associated synthesis of enzymes [19,20]

Plasmid mediated resistance it occurs in many different species, especially gram negative rods. Plasmids frequently mediate resistance to multiple drug and Plasmids have a high rate of transfer from one cell to another, usually by conjugation. Transposon-Mediated Resistance, transposons are genes that are transferred either within or between larger pieces of DNA such as the bacterial chromosome and plasmid [21].

Synthetic chemicals are extensively used against microorganisms, therefore they developed resistance to several antibiotics due to the indiscriminate use of commercial antibiotics. additionally, these antibiotics occasionally has side effect, cause allergic reactions, and causing immune suppressing so the use of plant extracts is protected to the human health. Plant material from rosemary is of commercial importance for its vital oil constituent and its antioxidant compounds [10]. This plant's most known pharmacological benefits are identified by its phenolic ingredients : carnosic acid, caffeic acid, carnosol and rosmarinic acid. As they naturally have essential antioxidant action scavenging effects, carnosic acid and carnosol cover almost 90% of the antioxidant action of rosemary among the phenolic components. For various disease rosmary extracts has been suggested as a possible therapeutic agent. More than 8000 phenolic structures have been described, they have a serious function as protection from pathogens, parasite, and provide intensify to the color of plants [7]. Many pharmacological characteristics involving antimicrobial and antiviral activity, growth factor stimulation, and inhibition of hepatotoxicity have been recorded for this plant [9]. Previous researches have been demonstrated that eugenol is the major component of cloves (*syzygium aromaticum*) that has antibacterial activity [22]. the cloves are antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic and antiparasitic. Research has reported that clove oil is an effective mosquito repellent, also was used as a topical application to promote healing and relieve pain. Several constituents of cloves has been identified, mainly eugenol, eugenyl acetate, alpha-humulene, acetyleugenol, 2-heptanone, isoeugenol, methyleugenol, biflorin, kaempferol, phenyl1 propanoides, myricetin, rhamnocitin and methyl salicylate, gallic acid, ellagic acid and oleanolic acid [23]. In many parts of the world clove used for malaria, cholera, tuberculosis and parasite that cause illnesses for human. cloves also use to alleviate muscle spasms, skin ulcer and sties in the eyes. Mechanism of action of Cloves extract on pathogens is by damaging the bacterial cell, damaging membrane which lead to cell death and inhibit growth of both gram positive and negative bacteria [16]. In various industries Clove has a strategic role including the pharmaceutical, food and beverage, vegetable pesticides, cigarettes, fisheries active packaging, cosmetic and other chemical industries [22]. [24] stated the

materials of clove compounds can induce non-specific and specific immunity and activate cellular components of the immune system, such as phagocytic functions without affecting both cellular and humoral immunity

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## 5. Conclusion

Our study concluded that the extract of *Syzygium aromaticum* has high potential as antibacterial agent against UTI that caused by *Proteus mirabilis*, clove (*syzygium aromaticum*) can be used in pharmaceutical for the production of new synthetic agents to treat the infectious disease caused by pathogens. There is developing interest in phytochemicals as modern sources of natural antimicrobial agents and antioxidant. The natural compounds which have biological activities are interest to pharmaceutical industries especially for treatment of human disease of microbial origin and several pathological disorders such as cancergenesis and coronary atherosclerosis. cancergenesis,coronary atherosclerosis processes.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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