

## RESEARCH / INVESTIGACIÓN

# Antimicrobial Activity of Herbal Mixture Extract Combination on Microorganisms Isolated from Urinary Tract Infection

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**Abstract:** Urinary tract infection (UTI) is the second most common infection after respiratory tract infection. Its prevalence is more in women as compared to men. Approximately 50% of women have an infection of the Urinary tract in their life-time. The bacterial infection is one of the most important bioactivity; using their ability to imitate and then distribute international fitness problems into the 21<sup>st</sup> century. Thus a recent study was undertaken to investigate the antibacterial activity of a mixture of three medicinal plants against UTI infectious isolates. The three considered plants were (*Aloe vera*, *Artemisia herba alba* and *Teucrium polium*), which were used in Iraqi medicine for many centuries. The effectiveness of this combination was investigated using *in vitro* well diffusion method. The extract was tested against four isolated pathogenic bacteria (*Staphylococcus aureus*, *Klebsiella spp.*, and *Proteus spp.*). The aqueous extract exhibited antibacterial activity against gram-positive and gram-negative bacteria. The mixture extract had the highest effect against *S. aureus* and *Proteus spp.*, followed by a lower effect on *Klebsiella spp.* In conclusion, the antibacterial effect of the tested plant extracts confirmed a higher impact on Gram-positive bacteria as compared to Gram-negative bacteria. Therefore, it can be concluded that the usage of these plants as a traditional medicine form can be considered as a strong assistant to regular medicine drugs and treatments.

**Key words:** Urinary tract infection (UTI), Antimicrobial Activity, Herbal Mixture Extract.

## Introduction

The herbal remedy is a developing sector of health care and demands attention<sup>1</sup>. Plants served as a valuable source of traditional treatment over the years<sup>2</sup>. World Health Organization (WHO) estimated that around 80% of the worldwide population remember at least one traditional remedy by extracting active components<sup>3</sup>. This, due to medicinal flora, bears various advantages (a low price or much fewer facet effects) compared to modern conventional drugs, as are expensive yet acknowledged in hazardous side effects<sup>4</sup>.

The prevalence of Urinary tract infections (UTI) by bacteria is problematic worldwide and among all age range and gender. Several pathogens belong to gram-positive and gram-negative bacteria regard as a fundamental everyday health hazards<sup>5</sup> to cause UTI; the most pathogens for UTI infection include strains of uropathogenic *Escherichia coli*, *Klebsiella pneumonia*, *Enterococcus spp.*, *Staphylococcus saprophyticus*, *St. aureus*, group B *Streptococcus*, *proteus mirabilis*<sup>6-10</sup>. Among all bacterial species *E. coli* is known to be the most common in complicated and uncomplicated UTI especially in diabetic patients<sup>6</sup>.

Treatment of UTI subordinate the severity of infection; it can be ranging from a single-dose antibiotic treatment to rescue nephrectomy for pyonephrosis in diabetic patients with septic shock<sup>11</sup>. Third-generation beta-lactam antibiotics such as ciprofloxacin, cefixime, and ceftriaxone are commonly used in UTI. Indiscriminate antibiotic use resulted in the development of resistance to one or multiple antibiotics that give a severe challenge upon disease treatment or even treatment failure<sup>12</sup> beside other adverse effects on the liver and bone marrow<sup>13,14</sup>. The tremendous use of antimicrobial has induced resistances among various bacterial kinds and, as much be counted concerning fact<sup>1</sup>, the efficacy of these compounds is remarkably decreased. A long time put in appearances concerning antibiotic stopping pathogens has been a global problem in the latest. According to inquire, the undesirable facet

consequences about half of antibiotics instituted us because of latter sources in conformity will combat these problems<sup>15</sup>. Literature cited that many strains of *E. coli* and *K. pneumoniae* isolated from UTI have extended-spectrum to Beta-lactam antibiotics, carbapenem-resistant, and polymyxin; moreover, the resist could be transferred to other infectious bacteria through horizontal gene transfer systems as transformation, transduction, and conjugation<sup>16</sup>.

This necessitates relies on a safe and low-cost medicinal plant having antibacterial activities with a promising future. More than half a million plants worldwide have medical issues essential to treat or prevent many infections<sup>17</sup>. A variety of secondary metabolite produces in plant tissues with therapeutic values with less toxicity and side effects and could be a good substitute for traditional synthetic or semisynthetic chemical antibiotics and overcome multidrug-resistant bacteria<sup>18</sup>. Since several plant antimicrobial contains different functional groups, their antibacterial activity attributed to multiple mechanisms. Therefore, the prospect of developing resistance to plant constitutes is relatively smaller<sup>19</sup>. The antibiotic resistance phenomenon exhibited by the pathogenic microorganisms were not reported in medicinal flora because of their strong antimicrobial activity<sup>20</sup>. Phytochemical compounds and small secondary metabolites have a significant value for medicinal plants. The most important of these bioactive constituents are alkaloids, tannins, flavonoids, and phenol, all of which are accoutered for new antibacterial agents<sup>21,22</sup>. It is believed that crude extracts from some medicinal plants are more biologically active than isolated compounds due to their synergistic effects<sup>23</sup>.

The researchers are showing interest in natural products with bactericidal activity<sup>24-26</sup>. Humans use plant extracts for a wide variety of diseases, of dense developing countries; it depends on traditional medical practitioners or their collections

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regarding medicinal vegetation in conformity with treatment to them<sup>27</sup>. Herbals execute apply for an important position in conserving biodiversity. These plants are genuinely familiar to bucolic human beings anybody according to their scarcity yet their disappearance<sup>28</sup>. Indeed, medicinal plants lead the very necessary health ponderous position and then symbolize a giant source concerning profits for many families within the countryside and cities<sup>29</sup>.

Urinary tract infection is the second most common infection after respiratory tract infection worldwide. Its prevalence is more in women than men<sup>30</sup>; approximately 50% of women have an infection of the Urinary tract in their life. The current study focuses on discovering antibacterial outcomes regarding aqueous extracts of a natural combination of about 3 medicinal plants (*Aloe vera*, *Artemisia herba alba*, and *Teucrium polium*) against UTI causing bacteria.

## Materials and methods

### Isolation and identification of bacteria from UTI.

Twenty out samples of urine were collected from patients with suspicious clinical symptoms like dysuria, loin pain, fever, frequent urination, and need to urinate with an empty bladder<sup>7</sup>; patients were visited Ibn Al-Nafees Hospital/ Baghdad and asked about taken antibiotic prior visiting the hospital during last seven days. The ethical committee approved the study of the University.

All urine samples were cultured over blood agar, Mac-Conkey agar, and mannitol salt agar. Bacterial characters were identified using Gram stain, urease test, oxidase, catalase, hemolysis of RBCs, and Indole Methyl red Vokes Proskauer (IMVC)<sup>31</sup>.

### Aqueous extraction of Plant Material

This research was conducted from January to February 2020. Plants of (*Aloe vera*, an Herbal (Sheh), and *Teucrium polium* (Algeada) toughness were obtained from the local Iraqi market and were identified at the College of Agriculture Engineering of Baghdad University - Iraq. The plants were kept at room temperature (20-25°C) till usage. Equal weights of each force in (*Aloe vera*, *Artemisia herba alba* (sheh), and *Teucrium polium* (Algeada) were ground then mixed. The aqueous extract regarding that combination was prepared along with the useful resource; 2 g regarding mixture, including 200 ml of sterilized distilled water for 15 minutes, left overnight in a refrigerator. Meanwhile, the extract panel was filtered and refrigerated in a glass container as described (32) with modification.

### Screening of the active compound

Many tests were applied to screen the different active compounds in the aqueous extract of plant mixture; these tests include the detection of each flavonoid, tannins, glycosides, saponin, and terpin and steroid. The procedures were briefly described below:

Flavonoids were detected in cold aqueous extract; the detecting solution composed of an equal volume of 50% ethanol and 1M KOH. About 5 ml of extract was mixed with 5 ml of detecting solution at 25°C, and the color was changed to the yellow indicating presence of flavonoid<sup>33</sup>.

The system below<sup>34</sup> was used to detect tannins; in its procedure, 50 ml of each ban was back in conformity withstand equally broken on couple conical flasks. For the first one, administration lead acetate (CH<sub>3</sub>COOPb) (1%; w/v) was once

introduced to plant extract afterward; the jelly pellet was used following keep viewed a direct reaction. The second flask, ferric chloride (FeCl<sub>2</sub>) (1%; w/v) was used for screening tannins. Navy-blue shade was involved in the emergence concerning tannins.

### Glycosides

These techniques were once taken according to the method described by (35); non hydrolyzed extract: Equal aggregation concerning the bury recover was once as soon as blended with the Fehling test in the test tube; development of purple precipitate shows a quality result for glycosides.

### Hydrolyzed extract

Few drops of HCl were added to 5ml of the aqueous extract of the plant since they were left at a water bath of 20 minutes, the acidity was once neutralized using NaOH solution, amount of aggregation respecting the Fehling test was added. The improvement concerning crimson precipitate suggests a positive result.

### Saponins

This method was made under conformity with (35). Couple methods detected saponins:

The first method was applied by shaking the tube containing extract; the formation of foams standing indicates a first-rate result.

While the second method was done by adding five ml of plant extract over 1-3 ml of 3% ferric chloride solution, positive results were pointed out upon bright precipitate formation<sup>33</sup>.

### Terpenes and steroids

few drops of chloroform were drop wised to 1 ml of the, then a decline over acetic anhydride and a decline on sulfuric acid; brown precipitate appeared that represents the availability of terpenes. While dark blue color appeared after about five minutes, it suggests the availability of steroids<sup>33</sup>.

### Antibacterial activity of aqueous herbal extracts

Agar well diffusion approach on Mueller -Hinton agar was once used in imitation of the search for antibacterial activity<sup>36</sup>. Bacterial cultures were crashed out from the nutrient agar plate and were suspended in sterilized peptone water. Turbidity was evaluated and compared with McFarland standard tube number 1, which equivalents approximately to 1X10<sup>8</sup> CFU/mL. The cotton swab was immersed in bacterial suspension and spread over Muller Hinton agar, which let for 10 minutes to ensure bacterial adherence. Meanwhile, the borer applicator was sterilized by flame, cooled, and pressed on the top of seeded Muller Hinton agar to make well with a 6 mm radius, let distance about 15 mm between wells the aspect of the plate. Each well was filled with 25, 50, 75, and 100 µl, plates were stand for 10 minutes and were incubated for 24 h at 37 °C. Three replicas of each plate were prepared, and the diameter of the inhibition zone was recorded from the edge of the well.

## Results and Discussion

### Isolation and identification of bacteria

The recent result of isolation and identification regarding bacterial isolates from patients with UTI symptoms showed the prevalence of *Klebsiella spp*, *Proteus*, and *S. aureus*; the characters of each isolate listed in the table (1). Although this

was a preliminary study with a small number of UTI patients, the study showed that G<sup>-ve</sup> bacterial species were more prevalent than G<sup>ve</sup> bacteria. Many other researches referred to that different bacterial species might encounter with the UTI inpatients, and some of these bacteria develop antibiotic-resistant to one or more of the traditional and extended-spectrum of beta-lactam antibiotic<sup>37</sup> Mohamed H. Mourad and one of the most important reason is the recurrent uses of antibiotic even without a physician prescription. This following (38) found that 54.8% of isolates were gram-negative, while 45.2 % were gram-positive.

higher concentration of plant mixture extract for all bacterial isolates under study. Also, study found that *Staph. aureus* was more susceptible than other bacterial species followed by *Klebsiella spp.* (figure2).

An increase rate of re-emerging infections, has carried an inquire for instant and more safe natural antimicrobial compounds; however, these compound need more investigation for their effecting mechanism. Plants are valuable supply of pharmaceutical substances, due to the fact they have an almost limitless potential according to synthesize compounds including one of a kind antimicrobial endeavor against more than

Bacterium spp	Blood agar	MacConky agar	Mannitol-salt agar	Nutrient agar or milk agar	Gram stain	Catalase test	Urease test
<i>Klebsiella spp.</i>	White colony, no hemolysis	Pink mucoid colony	*	White colony	G-ve rod	#	-
<i>Proteus spp.</i>	Swarming , no hemolysis	Pale colony NLF	*	White colony	G-ve rod	#	+ pink
<i>S. aureus</i>	B-hemolysis	*	Mannitol fermenter	White colony	G + grape like clusters	+ Bubbles	#

G-ve (Gram negative), G + (Gram positive), # (negative reaction), \*(negative reaction), - (negative Results), + (positive results), NLF (Non Lactose Ferment).

**Table 1.** The morphological and characteristics of bacteria on different cultural media with some biochemical test.

Chemical analysis of the aqueous extract of plant mixture under study showed that a weight of 1.58g representing 5.95%, could achieve from 25g regarding the extracted plant material. Screening of bioactive components revealed that the extract contains some flavonoids, phenols, tannins, saponins, glycosides, and terpenes have been detected. Aqueous extracts were low in steroids (Table 2). *A. herba-alba* is a prosperous source of flavonoids certain as like hispidulin as well as cirsilineol. Flavonoids isolated out of some medicinal vegetation have been established in conformity with possessing anti-inflammatory effect<sup>39</sup>.

Active compound	Quality result
<b>Phenols</b>	+
<b>Tannins</b>	+
<b>Flavonoids</b>	+
<b>Terpenes</b>	+
<b>Saponins</b>	+
<b>glycosides</b>	+
<b>steroids</b>	-

+ (Positive reaction), - (Negative reaction)

**Table 2.** Chemical analysis of active compounds in aqueous extract of a plant mixture.

**Antibacterial activity**

Each extract was tested against bacterial isolates (*S. aureus*, *Klebsilla.spp.*, and *Proteus.spp.*). The extracts' different concentrations illustrated increased effectiveness against the studied microorganisms represented by the increased inhibition zones in figure (1).

The measurement of the average diameter of inhibition zone indicated that highest bacterial inhibition reached with

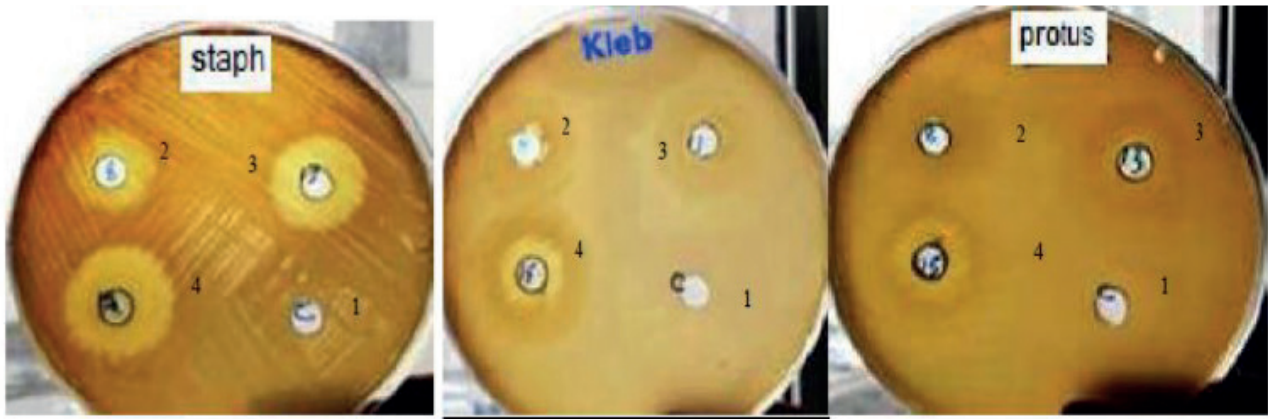
a few pathogenic as well as opportunistic microorganisms<sup>40</sup>.

The essential factors up to expectation to antibacterial reactivity are the type of diffusion strategies of active substances used and the tendency to inter bacterial cell, pH, and temperature of the surroundings<sup>41</sup>. The pH may also result in the antibacterial inhibitory effect of *R. sativus* had an excellent antibacterial effect at acidic pH, which has declined by increasing pH toward alkaline. This might be because antibacterial compounds in cationic forms can also engage the negatively charged bacterial cells<sup>42</sup>.

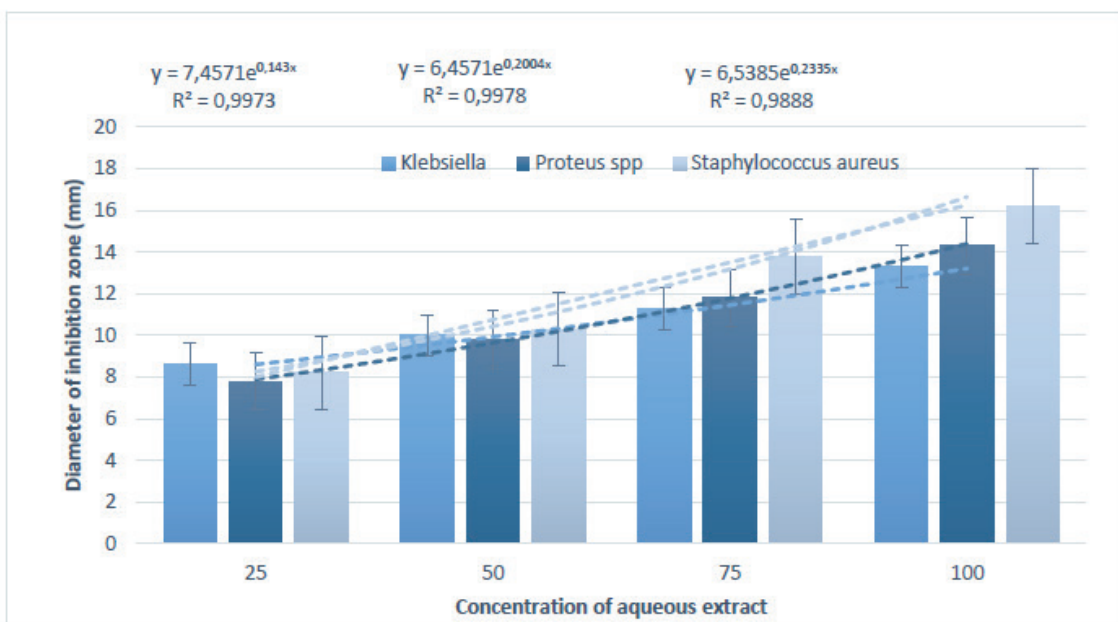
Successful extraction concerning bioactive compounds beside row material depends on the solvent back into the extraction procedure. The recent study's main success is using aqueous extract for three plant material that contained different active compounds with a synergistic effect in one preparation, a phenolic compound known to alter microbial cell permeability, leading to change damage the macromolecules inside the bacterial cell. Many enzymes responsible for either the bacterial reproduction and growth or virulence factor might lose their activity in response to phenolic compounds<sup>43,44,45</sup>. The saponin activity against monocrystalline sugar; reduces sugar within the bacteria reducing power intake to the bacterial cell, leading to growth decline and death<sup>46</sup>. The presence of tannins and flavonoids caused a toxic effect and an inhibition of different types of enzymes and transporter proteins found in the cell membrane and inside cell<sup>47,48</sup>.

Many researchers observed that the extraction of the plant active ingredients and the organic solvents methanol, ethyl acetate, and chloroform resulted in a more substantial antibacterial effect, but aqueous extraction, cheaper and need less requirements to prepare 26=32. Gram-negative microorganisms are among the resistant organisms against chemotherapeutic antibiotics than gram-positive bacteria; a survey concerning currently observed antibacterial activity of herbal takes place, showing that >90% of herbal extracts lacked activity against *E. coli*, in compassion to gram-positive bacteria<sup>49</sup>.

The mechanisms of bacterial resistance against antibiotic



**Figure 1.** Bacterial inhibition zone with different concentration of aqueous plants extract (1= 25µl, 2= 50 µl, 3=75 µl, 4=100 µl): Muller Hinton agar.



**Figure 2.** The differences in bacterial susceptibility in response to aqueous plant mixture extract

might be equipped through changing membrane permeability; drug molecules in accordance with a mobile phase can keep transferred through membrane via porins, diffusion through the bilayer then through self-uptake. The porin channels are located among OM (outer membrane) about Gram-negative bacteria. The little hydrophilic molecules ( $\beta$ -lactams then quinolones) can pass the OM only through porins. The minimize among quantity over porin channels leads to decreased access regarding  $\beta$ -lactam antibiotics between the cells, subsequently resistance<sup>50</sup>. G+ve microorganisms like Staphylococcus might harbor transmissible genetic element encoding for antibiotic resistance such as plasmids encoding the penicillinase genes, namely in collecting negative bacteria; conjugation is an essential mechanism regarding drug transfer and effect occur into supreme genera<sup>50</sup>. The main barrier for Gram-positive bacteria towards antibiotics is thick peptidoglycan that protects against osmotic rupture. The simple subunit over the peptidoglycan thing is a disaccharide monomer regarding N-acetylglucosamine (NAG) and N-acetylmuramic (NAM) pentapeptide. The pentapeptide consists of amino water brush residues alternating into L- and D-stereoisomers, then terminating within D-alanyl-D-alanine. A stem peptide regarding moving measure then contract is given to the third amino water brush over

it pentapeptide. Pentapeptides are then same, including stem peptides, according to form a cross-link between polysaccharide chains. This reaction is catalyzed by a transpeptidase. This transpeptidation response is sensitive to prohibition via  $\beta$ -lactams. The penicillin-sensitive reactions are catalyzed by using a family of closely associated proteins, penicillin-binding proteins (PBPs).  $\beta$ -Lactam antibiotics out turn their lethal impact regarding bacteria through inactivation concerning more than one PBPs simultaneously, then for that reason inhibiting cell wall synthesis. The inhibition of PBPs additionally leads to imitation of breakdown on an ideal match, probably at the time over cell division. This agitated morphogenesis is hypothesized after provoking cell dying<sup>51,52</sup>.

## Conclusions

Different active compounds were detected in the aqueous mixed extract of the 3 medicinal vegetation (*Aloe vera*, *Artemisia herba alba* and *Teucrium polium*) permanency life, including phenols and flavonoids tannins, saponins, glycosides afterward terpenes. Aqueous extrusion concerning an herbal aggregate upon 3 medicinal vegetation (*Aloe vera*, *Artemisia herba alba* and *Teucrium polium*) undergo antibacterial effect

against positive but village Gram negative microorganism. Our consequences aid that the aqueous extract regarding this three plant combination well-known shows the synergistic effect as antibacterial activity compared to previous studies that examined each plant alone.

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