

Comparative Analysis of Medicinal Plants and herbs antibacterial activity on E.Coli

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ABSTRACT

In recent years, the natural products derived from medicinal plants have proven to be a rich source of biologically active compounds and it is very useful for developing new drugs. Plant-based green medicines are currently in greater demand. This work is focused on to analyze the antibacterial activity of different plants and herbs extract against E. coli causing Urinary Tract Infections (UTI). Initially, the juice is extracted from part of plant and herbs like leaves, seeds, barks and stems. Extracted liquid is mixed with ethanol and chloroform separately. After 24 hours the disk diffusion procedure is performed. Next, Colony forming units are determined by the streak plate method to identify the susceptibility and resistance of E. coli. The antibacterial activity of plants is based on the phytochemicals present in it. Due to this the properties of phytochemicals are found. To analyze the performance of the experiment, ten different UTI samples are collected and tested with sixteen different plants and herbs. From this *Pedalium murex*, Cucumber, Legume, neem and balipoovu show higher inhibition zones and better antibacterial activity against E.coli bacteria.

Keywords: E.coli bacteria, Zone of inhibition, Disc diffusion method, Ethanol extraction, Phytochemicals

1. INTRODUCTION

Urinary tract infection (UTI) is a serious health problem that affects millions of people worldwide. UTI is the second most common disease affecting humans. According to a WHO report, the current rate of UTI infection is increasing year by year, and people who use public, unhygienic toilets are the main source of infection. Women are more susceptible to this infection due to the shorter urethral structure. As reported by the WHO, 40 to 45% of adult women and 25 to 30% of young women are affected. In the United States, 10.5 million people are affected. 60% of women are affected 30 times more often than men. Urinary tract infections lead to major health problems such as bladder disease, kidney failure, and even death. Allopathic medicine is the most popular among people. These drugs treat the infection well in the short term but have side effects in the short and long terms. Some of the allopathic medicines used to treat UTIs are Amoxicillin, Urebhil, Bacterium, Cefentil, and Fosfomycin, but they cause side effects like stomach upset, vomiting, dizziness, blurred vision, diarrhea, fever, and joint pain. To solve this problem, many pharmacists are using herbal ayurvedic drugs called 'green medicine' that are safer and have fewer side effects than expensive synthetic drugs.

According to the World Health Organization (WHO) report, 80% of the world's population currently uses herbal medicines, this kind of ayurvedic treatment has therapeutic value in its own way. The antibacterial activity of four different plant-based methanol extracts was tested against 12 pathogenic microorganisms and two reference bacterial strains

Sarita Manandhar, Shisir Luitel, and Raj Kumar Dahal (2019). Ethyl acetate leaf extract from *Capparis zeylanica*'s excellent antibacterial activity is analyzed [1,2]. Forty-nine antibacterial activities of ethanol are studied in vitro. Extracts from 37 plant species used in traditional Indonesian medicine for the treatment of *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are proposed [3]. The antibacterial activity of medicinal plants against human pathogens and the minimum inhibitory concentration (MIC) of plant extracts are evaluated [4]. The antibacterial effects of tea tree, rosemary, eucalyptus, and lavender essential oils are against four pathogens, *E. coli* and *S. aureus*. Screened for Tyhpi, *C. Koseri* is under investigation [5]. to measure the antibacterial activity of five plant extracts against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* using agar medium diffusion [6]. The antibacterial activity of polyphenols and alkaloids in 20,000 different plant species in the Middle East is being studied [7].

The antibacterial activity of various tinctures is measured by the disc diffusion method in agar. This measures the diameter of the growth inhibitory zone of the culture using a template ruler [8]. Drug resistance of antibacterial activity of *Ricinus communis* Linn leaf is tested [9]. The antibacterial activity of aqueous extracts of *Bidens sulphurea*, *Bidens pilosa*, and *Tanacetum vulgare* is a commonly used species of Asteraceae for the treatment of genitourinary infections, is studied [10]. *Cynodon dactylon* exhibits moderate inhibitory

action against 13 bacterial species when tested for invitro growth inhibitory action. Ethanolic extracts of *Curcuma longa*, *Curculigo orchoides*, *Cinnamomum camphora* show the highest antibacterial activity are discussed [11].

The antibacterial activity of hydromethanolic extracts of 9 medicinal plant extracts tested against different Gram-positive and Gram-negative reference bacterial strains. Moreover the zone of inhibition is carried out using the disc diffusion method and minimum inhibitory concentration (MIC) [12]. There is a variation in the phenolic content, flavonoids, and alkaloids of plants. Antibacterial activity is founded against four PDR bacterial and fungal strains. The fatty acid composition of leaf extracts is also detected and explained [13]. The minimal inhibitory concentrations of various plants are exposed by evaluating the antibacterial activities of 13 flavonoids and six organic acids. [14]. Antibioresistance reversal properties are analyzed using by ethanol extract of the bark of *E. chlorantha* [15].

The proposed work is focused on to analyze the antibacterial activity of different plants and herbs extract against *E. coli* causing Urinary Tract Infections (UTI). Samples are collected from “Doctor’s diagnostic pathology and Hematology center” at Dindigul. This work is carried out with the support and guidance of Shri Annai Siddha and Ayurveda Clinic, Dindigul. Culture preparation for checking the antibacterial activity and Disc diffusion method procedures are explained in section 2. The phytochemical analysis test methods are in section 3. Results of the experiment are discussed in section 4. The work is concluded in section 5.

2. METHODOLOGY

The objective of this work is to determine the sensitivity and resistance of *E. coli* bacteria which causes urinary tract infections (UTI) when exposed to plants and herbs. To achieve the output the procedures are given below.

2.1 Sample Culture Preparation

Infected urine samples are collected from ten individuals at Dindigul “Doctor’s Diagnostic Center's microbiology laboratory”. From the collected urine samples, the culture is prepared and the *E. coli* is grown in the Biochemistry & Microbiology Laboratory at PSNA College of Engineering and Technology. The culture preparation is done in a petri dish using the streak plate method.

2.1.1 Procedure involved

- To take ten empty Petridish with agar gel medium.
- Collect the infected urine samples
- Take *E. coli* bacterium with the help of sterilized small loop and swabbed in the petridish
- The petridish is placed 24 hrs in incubator at 34 degree Celsius.
- After 24 hrs the culture is taken from incubator
- Now the *E. coli* bacteria are grown and spreaded over the petridish.

2.1.2 Medicinal plant and herbs extract

Different plants are collected to study the sensitivity and resistance of its extract against E.coli. The English, Tamil and botanical names, Plant's parts and family used for this analysis are given in the table 1.

Table 1 Selected sample plants and herbs names

S.NO	English name	Tamil name	Botanical name	Plant's part used	Family
1	Ginger	Inji	Zingiber officinale	Rhizome	Zingiberacea
2	Garlic	Vellaipoondu	Allium sativum	Garlic bulb	Amarilidaceae
3	Tulasi	Tulasi	Ocimum tenuiflorum	Leaf	Mint
4	Legume	Avaram poo	Senna auriculata	Flower	Leguminosae
5	pedalium murex	Yanai nerunjil	Tribulus terrestris	Leaf	Zygophyllaceae
6	Turemeric	Manjal	Curcuma longa	Rhizome	Family of zinger
7	Lemon	Elumichai	Citrus lemon	Fruit juice	Rutaceae
8	Pomegranate	Maadhulai	Punica granatum	Fruit peels	Punicaceae
9	Drumstick	Muraungai keerai	Moringa Oleifera	Leaf	Moringaceae
10	Mint	Puthina	Mentha	Leaf	Mint
11	Curry leaves	Karuvepillai	Murraya koenigii	Leaf	Rutaceae
12	Cumin	Seeragam	Cuminum cyminum	Seeds	Umllelifers
13	Balipoovu	Sirupeellai	Aerva lanata	Leaf	Amaranthaceae
14	Cucumber	Vellari	Cucumis sativus	Juice	Cucurbits
15	Goosberry	Nelli	Phyllanthus emblica	Fruit juice	Phllanthaceae
16	Neem	Vepillai	Azadirachta indica	Leaf	Milliaceae

2.2 Disc diffusion method

Plants and herbal grinded powders or juice are mixed with 2 ml of ethanol. After centrifugation at 1000 rpm for 10 minutes and then the extract is filtered through Whatman filter paper. Similarly, the same procedure is performed with chloroform. Paper discs are immersed half an hour with individual plant extracts. After that, these paper discs are placed in the petridish with the grown E. coli culture. Next two drops of plant extract are added to developed E. coli culture. The cultured Petridishes are placed into the incubator for 48 hours to analyze the sensitivity, resistance, and zone of inhibition.

3. Phytochemical Analysis

To identify the eradication nature of plant extracts against E. coli the present analysis carried out the phytochemical test. The different phytochemical test such as Phenol test, Tannin test, Flavonoids test, Betacyanin test, quinone test, Terpenoids test are carried out on plants to

analyze the E.coli bacteria eradication rate. Different test procedures for phytochemical is given below:

Phenol test: 1ml of plant extract and 2ml of distilled water 10% of ferric chloride are added together the appearance of green color indicates the presence of phenol.

Tannin test: 1ml of extract and 2ml of 5% ferric chloride are added together the appearance of greenish black indicates the presence of tannin.

Flavonoids test: 5ml of dilute ammonia and 1ml of extract and concentrated sulphuric acid is added together the appearance of yellow color indicates the presence of flavonoids.

Betacyanin test: 2ml of plant extract and 1ml of 2N sodium hydroxide are added together and heated for 5 minutes the appearance of yellow color indicates the presence of betacyanin.

Quinone test: 1ml of extract and 1ml of concentrated sulphuric acid are added together the appearance of red color indicates the presence of quinone.

Terpenoids test: 0.5 ml of extract and 2 ml of chloroform and concentrated sulphuric acids are added together the appearance of red brown indicates the presence of terpenoids.

Analysis of the phytochemical nature of the plants and herbs details are listed in table 2

Table 2: Analysis of the phytochemical nature of the plants and herbs

S.no	Plants	Phenol	Tannin	Flavonoids	Betacyanin	Quinone	Terpenoids
1	Ginger	-ve	-ve	+ve	+ve	+ve	+ve
2	Garlic	+ve	-ve	+ve	+ve	+ve	+ve
3	Pedalim murex	-ve	+ve	+ve	+ve	+ve	+ve
4	Curry leaves	+ve	+ve	+ve	+ve	+ve	+ve
5	Tulasi	-ve	-ve	+ve	+ve	+ve	+ve
6	Turmeric	-ve	+ve	+ve	+ve	+ve	+ve
7	Lemon	-ve	+ve	+ve	+ve	+ve	+ve
8	Pomegranate	-ve	+ve	+ve	+ve	+ve	+ve
9	Drumstick	-ve	-ve	+ve	+ve	+ve	+ve
10	Mint	+ve	+ve	+ve	+ve	+ve	+ve
11	Legume	-ve	+ve	+ve	+ve	+ve	+ve
12	Cumin	-ve	-ve	+ve	+ve	+ve	+ve
13	Balippovu	-ve	-ve	+ve	+ve	+ve	+ve
14	Cucumber	-ve	-ve	+ve	+ve	+ve	+ve
15	Gooseberry	-ve	+ve	+ve	+ve	+ve	+ve
16	Neem	-ve	+ve	+ve	+ve	+ve	+ve

5. RESULTS AND DISCUSSION

Analysis of sensitivity and resistance of the plants and herbs against E.coli bacteria is carried out by measuring their zone of inhibition is shown in figure 1.

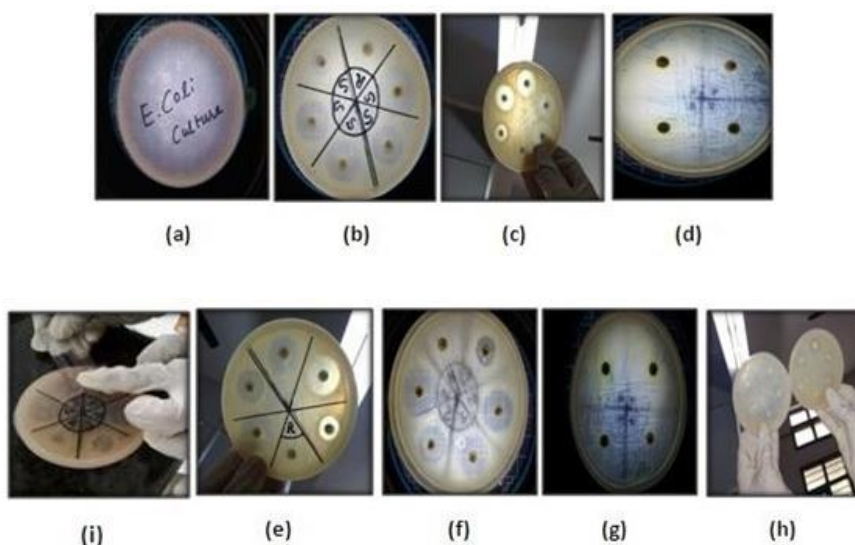


Figure 1 (a) E.coli culture (b) Sensitivity and resistance analysis from ethanol extract of Ginger, Garlic, Pedallium murex, Curry leaves, Turmeric, Tulasi. (c) Sensitivity and resistance analysis from ethanol extract of Lemon, Pomegranate, Drumstick, Mint, Legume, Balippovu (d) Sensitivity and resistance analysis from ethanol extract of Cucumber, Gooseberry, Neem, cumin (e) Sensitivity and resistance analysis from chloroform extract of Ginger, Garlic, Pedallium murex, Curry leaves, Turmeric, Tulasi (f) Sensitivity and resistance analysis from chloroform extract of Lemon, Pomegranate, Drumstick, Mint, Legume, Balippovu (g) Sensitivity and resistance analysis from chloroform extract of Cucumber, Gooseberry, Neem, cumin (h) Observation of eradication rate of chloroform and ethanol extracts. (i) Zone of inhibition

In the proposed work, sixteen medicinal plants & herbs and ten different infected urine samples are collected. The final result shows that the two plant Curry Leaves and Henna extracts are resistant to E.coli and the remaining fourteen are sensitive with a different zone of inhibition. The comparison of the zones of inhibition of sixteen different plant ethanol and chloroform extracts with ten infected urine samples is shown in Tables 3 and 4. The overall average inhibition zone rate is shown in figure 2.

Table 3: Zone of inhibition of ethanol extract of sixteen plant and herb on ten infected urine samples (S1-S10)

S.no	Plants	Zone of inhibition of Ethanol extract in mm									
		S1	S2	S3	S 4	S5	S 6	S7	S8	S 9	S 10
1	Ginger	23	20	23	22	22	20	19	22	23	21
2	Garlic	18	19	15	18	19	17	18	19	17	16

3	Pedaliium Murex	20	24	23	24	25	22	24	22	24	24
4	Cury leaves	R	10	9	R	R	2	10	5	R	3
5	Tulasi	R	2	R	1	4	R	6	R	R	R
6	Tumeric	22	21	20	22	23	24	25	22	22	23
7	Lemon	5	7	10	5	5	5	5	5	5	5
8	Pomegranate	20	21	22	20	20	22	24	22	20	21
9	Drumstick	12	10	12	12	12	11	12	10	9	12
10	Mint	13	18	17	17	13	12	13	11	13	12
11	Legume	17	19	20	17	17	12	13	13	16	17
12	Cumin	10	19	17	10	16	12	10	10	11	10
13	Balippovu	18	19	19	19	18	18	20	18	21	18
14	Cucumber	13	10	12	13	24	11	21	21	13	10
15	Gooseberry	13	17	10	13	13	12	12	13	10	12
16	Neem	24	23	23	22	24	25	22	22	20	23

Table 4: Zone of inhibition of chloroform extract of sixteen plant and herb extracts on ten infected urine samples (S1-S10)

S.no	Plants	Zone of inhibition of chloroform extract (S1-S10)
1	Ginger	No Zone of inhibition
2	Garlic	No Zone of inhibition
3	Pedaliium murex	No Zone of inhibition
4	Cury leaves	No Zone of inhibition
5	Tulasi	No Zone of inhibition
6	Tumeric	No Zone of inhibition
7	Lemon	No Zone of inhibition
8	Pomegranate	No Zone of inhibition
9	Drumstick	No Zone of inhibition
10	Mint	No Zone of inhibition
11	Legume	No Zone of inhibition
12	Cumin	No Zone of inhibition
13	Balippovu	No Zone of inhibition
14	Cucumber	No Zone of inhibition
15	Gooseberry	No Zone of inhibition
16	Neem	No Zone of inhibition

6. CONCLUSION

The experimental results shows plant-ethanol extract involved high eradication rate but chloroform shows no eradication on E.coli. Based on the analysis report and suggestion given by the ayurvedic clinic the following findings are given in the form of statements. (i) Zinger, garlic, lemon, and mint, have a stronger effect; it is not commonly used medicines because it may cause discomfort in patients with esophageal and ulcer problems, but it can be used by healthy people. (ii) Tulasi is also not as effective for UTIs because of its heat content. (iii) Gooseberry is not recommended for patients with colds by birth. (iv) According to Ayurvedic medicine, the most recommended herbs and plants for UTI for all common patients are cucumbers, leguminous plants, neem, balipoovu, drumstick, and Pedaliyum murex, of which cucumber can be taken with curd and the other four plants are taken as boiled water extracts. (v) Pedaliyum murex and Balipoovu are proven high inhibition zone and useful plants for UTI infection. The UTI can be cured within one week if these two medicinal plants are taken continuously.

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