



**ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF SOME MEDICINAL
PLANTS FROM HIMACHAL PRADESH AGAINST *ESCHERICHIA COLI***

KUMAR R^{1*}, JANDAİK S² AND PATIAL P³

Department of Biotechnology Shoolini University of Biotechnology and
Management Sciences, Solan, Himachal Pradesh, India

*Corresponding Author: E Mail: rakeshsharmamicrobiology@gmail.com

ABSTRACT

An attempt was made to analyse the antimicrobial activity of 8 medicinal plants viz., garlic, neem, ginger, coriandrum, clove, rosemary, mint, turmeric and their antimicrobial activity was checked against *E.coli*. Antimicrobial study was carried out by well diffusion method. The overall antimicrobial activity recorded by zone of inhibition around the well which was ranging from 9-13mm in case of plant extracts and in case of antibiotics zone of inhibition recorded was from 19-23mm. Three concentrations of methanolic plant extracts i.e 50µg/ml, 100µg/ml and 200µg/ml were used for detection of antimicrobial activity and out of these three concentrations 200µg/ml concentration shows better antimicrobial activity against *E.coli*. The phytochemical analysis of these plant extracts were carried out to detect the presence of different phytochemicals present in the extract. Different phytochemicals detected in the extracts of all the plants were alkaloids, flavonoids, tannins, reducing sugars, phenols, saponins and terpenoids which contribute for the antimicrobial action of above medicinal plants.

Keywords: Antimicrobial activity, phytochemical analysis, *E.coli*

INTRODUCTION

Medicinal plants are classified as greatest bio-resource of drugs. Plants are used from many years in traditional system of medicine, in modern medicines, nutraceuticals, pharmaceutical and chemical entities in synthetic drugs.¹ The

bioactive compounds presents in the medicinal plants led them to be used in industries such as pharmaceutical, food and cosmetic industries as botanical drugs, dietary supplements and functional foods. Plants are also used in ethno pharmacy for

treatment of various diseases such as hypertension, cholesterol and diarrhea and scientific validation of these medicinal plants was provided by identifying and isolating of the bioactive phytochemical present in these plants.² Phytochemical compounds presents in plants are known as secondary metabolites and have many subgroups having various bioactivities such as antioxidant, antimicrobials and anticancer activities.³ Recent research has shown that natural products isolated from plants are used as an alternative to the existing drugs for the treatment of diseases in developing countries.⁴ Medicines which are extracted from plants has been used in traditional health treatments for thousands of years in many parts of the world and they have agents to fight with microbial diseases.^{5,6,7} In the present study methanolic extract of 8 medicinal plants were studied for their antimicrobial activity against *Escherichia coli* strains. Phytochemical screening of these extracts was also done for detection of phytochemicals present in them. The plants discussed in the present study includes garlic, ginger, turmeric, neem, mint, clove, coriandrum and rosemary. The therapeutic use of these plants has been reported.

Garlic has been used as a medicinal agent from thousands of years. Garlic shows antimicrobials, antithrombotic, antiarthritic and antitumor activity.⁸ Garlic is also used in hardening of the arteries and high blood pressure (Hypertension).⁸ Ginger is also used in the treatment of migraine headache without causing any side effects.⁹ Turmeric helps in preventing coronary and heart diseases. Turmeric is used in treatment of liver infections and it is also used in the treatment of jaundice and hepatitis.^{10,11} From many years medicinal properties of neem has been recognized in Indian tradition. Every part of neem tree possesses some medicinal properties.¹

MATERIAL AND METHODS

Collection of plant material:

Plants from different families were selected for the study. Plants were collected from physical garden of Shoolini University, Solan and from the local markets of Himachal Pradesh. A total of 8 plants were collected belonging to different families and possess medicinal properties and shows synergistic activity with different antibiotics against different bacteria. Only the required plant parts are collected either in dry or in fresh form and stored in sterile containers at 4⁰C (Table 1).

Table 1: List of plants selected for antimicrobial activity and phytochemical analysis.

S.NO.	Plant	Vernacular name	Botanical name	Family	Part used
1	Garlic		<i>Allium sativum</i>	Liliaceae	Bulb
2	Rosemary		<i>Rosamarinus officinalis</i>	Lamiaceae	Leaf
3	Turmeric		<i>Curcuma longa</i>	Zingiberaceae	rhizome
4	Ginger	Adrak	<i>Zingiber officinale</i>	zingiberaceae	Rhizome
5	Mint		<i>Mentha longifolia</i>	Lamiaceae	Leaves

6	Neem		<i>Azadirachta indica</i>	Meliaceae	Leaves
7	Coriandrum		<i>Coriandrum satium</i>	Umbelliferae	Leaf
8	Clove	laung	<i>Syzygium aromaticum</i>	Myrtaceae	Dried buds

Preparation of plant extract:

The parts of plants used for extract preparation were first washed with tap water and then washed with 0.1% HgCl_2 to remove the contamination and after that washed with distilled water. The plant parts washed were then dried for 4 to 5 days in shade. Then the dried plant parts were grinded in to fine powder with the help of mortar and pestle. Plants powder was stored at 4°C until use.

Soxhlet extraction:

Powdered plants were subjected to soxhlet extraction with methanol as a solvent. Methanol is widely used as a solvent, because many of the compounds dissolve in it easily, which is important for the plant material, moreover methanol easily evaporates. So, it can be separated from the extract and it is also easily available at low cost. The initial concentration of 0.1g/ml (200 ml methanol + 25 g powder) was made. Apparatus was run for 18-24 hours to get final concentrated slurry. Then extract was poured in china dish. Methanol was evaporated from the extract by incubation at $35-38^\circ\text{C}$. Powders obtained were weighed and stored in a sterile tube at 4°C till use¹⁷.

Microbial strains:

The strains of *Escheritia coli* used in the present study were procured from Sabine

Schuster and Winfried V. Kern, Center for Infectious Diseases and Travel Medicine, University Hospital, and Department of Medicine, *Albert-Ludwigs-University, Freiburg*, Germany and from MTCC and NCTC. The *E.coli* strains used were one knock out strain 1- DC14, wild type strain K-12 AG100 and two standard strains with NCTC number 12923 and MTCC number 1302.

Antimicrobial activity:

Antibacterial activity was measured using well diffusion method according to National Committee for Clinical Laboratory Standard. Tryptose soya broth was inoculated and incubated at 37°C for overnight. Presence of turbidity in broth was adjusted according to 0.5 McFarland standards (McFarland solution was prepared by dissolving 99.5 mL of 1% H_2SO_4 and 0.5 mL of 1.175% BaCl_2 and stored in dark at room temperature). Muller Hinton agar plates were prepared. Sterilized swabs were dipped in standardized bacterial suspension with inoculum size of 1.5×10^8 cfu/mL prepared above and excess of culture was removed by turning the swab against the side of the tube. Inoculum was spread evenly over the entire surface of Muller Hinton Agar plates. These plates were allowed to dry for at least 15 min. Wells 6 mm in diameter were

punched in agar and were filled with 30 μ L of plant extract (3 concentrations 50 μ g/ml, 100 μ g/mL and 200 μ g/mL of plant extract were used) and antibiotic alone was used as positive control and DMSO was used as negative control. The three replicates of each plate was performed. The plates were incubated at 37°C for 24hrs.

Phytochemical analysis:

The methanolic plant extracts which are selected by screening were then analysed for the presence of all the major groups of phytochemicals by using standard phytochemical assays. The phytochemical screening of extracts from different plants was carried out to determine the presence of active secondary plant metabolites. The plant extract were analysed for the presence of reducing sugars, alkaloids, saponin, flavonoids, tannins, anthraquinones, phlobatannin, steroids, terpenoids and cardiac glycosides according to previously described procedures.^{15,16}

(a) Fehling's test for reducing sugars:

The methanolic herbal extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction. Change in colour from blue to red indicates the presence of reducing sugars.

(b) Test for Alkaloids:

30 ml of sample extract was evaporated to dryness in an evaporating dish on water

bath. 5 ml of 2N HCl was added and stirred while heating on the water bath for 10 min., cooled, filtered and the filtrate was treated with few drops of Mayer reagent. The samples were then observed for the presence of turbidity or precipitation.

(c) Test for Saponin:

About 2g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

(d) Test for Tannins:

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colour.

(e) Test for Flavonoids:

5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H₂SO₄. A yellow colour observed in extract indicates the presence of flavonoids.

(f) Test for Anthraquinone:

10 ml of N/2 potassium hydroxide containing 1 ml of 3% hydrogen peroxide solution was added to 1 g of the powdered plant material. The suspension was boiled

for 3-5 min. then cooled, filtered and 5 ml of filtrate was acidified with 10 drops of benzene. A 5 ml aliquot of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. A pink to red colour of the alkaline layer indicates the presence of anthraquinone.

(g) Test for Phlobatannins:

Deposition of a red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

(h) Test for Steroids:

2 ml of acetic anhydride was added to 0.5 g methanolic extract of plant sample with 2 ml H₂SO₄. The colour changed from violet to blue or green indicates the presence of steroids.

(i) Test for Terpenoids (Salkowski test):

5 ml of extract was mixed with 2 ml chloroform, and concentrated H₂SO₄ (3 ML) was carefully added to form a layer. A reddish brown colour of the interface was formed to show the positive result for the presence of terpenoids.

(j) Test for Cardiac glycosides (Keller-Killani test):

5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

RESULT

Antimicrobial activity of plants and antibiotics:

Results obtained in the present study relieved that the tested eight medicinal plants extracts exhibits potential antibacterial activity against *Escherichia coli*. When tested by the well diffusion method, the methanolic extracts of the plants viz., garlic, neem, ginger, coriandrum, clove, rosemary, mint, turmeric showed significant activity against the tested micro organism (Table.2). The highest antibacterial activity recorded in garlic (13mm) and lowest in mint and coriandrum (9mm) (table 2).

Table 2: Effect of plant extract on *Escherichia coli*

S.n	Plants	Zone of inhibition in mm
1	Garlic	13 ±1
2	Neem	11 ± 1
3	Ginger	10 ±2
4	Rosemary	11 ±1
5	Mint	9 ±1
6	Clove	10 ±0
7	Coriandrum	9±0
8	Turmeric	11±1

Table 3: Effect of antibiotics on *Escherichia coli*

S.NO	Antibiotics	Zone of inhibition in mm
1	Tetracycline	23±2
2	Ciprofloxacin	20±1
3	Erythromycin	19±1

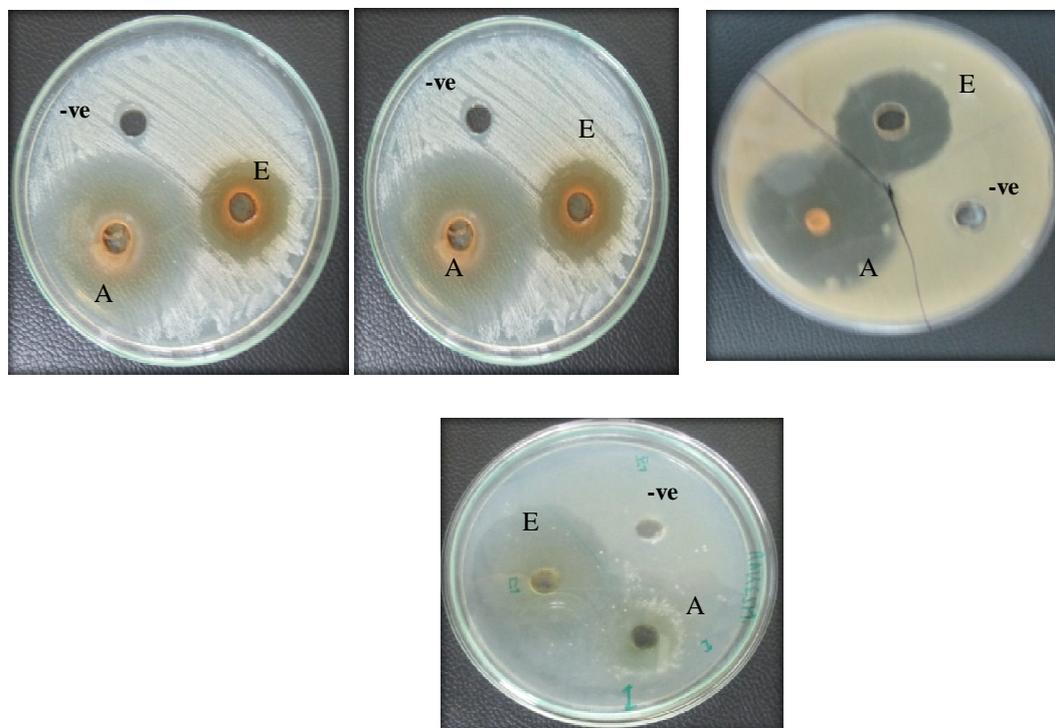


Fig 1: A- Antibiotic alone, E= Extract only and -ve = negative control (DMSO). Antimicrobial activity expressed in terms of clear zone (mm) produced around the well (6 mm) by different plant extract and antibiotics after incubation at 37°C for 24 hours of incubation.

Phytochemical analysis:

The methanolic extracts of medicinal plants revealed the following phytochemicals (Table.4).

Table 4: Different group of phytochemicals present in methanolic extract of plants

S.No	Phytochemical tests	Phenols	Tannins	Reducing sugars (fehling’s test)	Saponins	Flavonoids	Terpenoids
1	Garlic	+	-	+	-	+	-
2	Neem	+	+	-	+	+	+
3	Ginger	+	-	-	-	-	+
4	Coriandrum	-	-	+	-	-	+
5	Mint	+	+	-	+	+	-
6	Clove	-	+	-	+	+	+
7	Rosemary	+	+	-	-	+	+
8	Turmeric	+	+	+	+	+	-

+ = positive, - = negative

DISCUSSION

Traditional medicines involve a large number of plant species. In the present study we selected a small subset of 8 plants belonging to different families and just

attempted to check whether they possess antimicrobial activity or not. Antibacterial activity and phytochemical analysis of 8 medicinal plants were studied against *Escherichia coli*. Extract of all 8 medicinal

plants possesses good inhibitory activity against *Escherichia coli*. Garlic shows higher antibacterial activity against *E.coli* as shown in earlier studies as well.^{13,14} Screening of these 8 medicinal plants suggest that these plants are potential source of antimicrobial agents. This in vitro study corroborated the antimicrobial activity of these 8 medicinal plants i.e. Garlic, ginger, neem, mint, coriandrum, turmeric, rosemary and clove in Ayurveda. Results from our study suggest that leaves, bark and other parts of these plants possess a good antibacterial activity against *E.coli*. Hence these plants can be used in combination with antibiotics for the treatment of infection caused by bacteria. Based upon the phytochemical analyses of the eight plants phenols were present in garlic, neem, ginger, mint and rosemary. Tannins were present in neem, mint, clove, rosemary and turmeric. Reducing sugars were present in garlic, coriandrum and turmeric. Saponins were present in neem, mint, clove and turmeric. Flavonoids were present in garlic, neem, mint, clove, rosemary and turmeric. Terpenoids were present in neem, ginger, coriandrum, clove and rosemary.

CONCLUSION:

The results from our study suggest that methanolic extract of these 8 medicinal plants shows antibacterial activity against

Escherichia coli. They can be used in combination with different antibiotics for the treatment of infections. The present study leads to the establishments of some valuable compounds which can be used in the formation of new and more potent antimicrobial drugs of natural origins. Further experimental studies are needed on plants to identify new compounds which show activity against pathogenic bacteria responsible for diseases in humans.

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