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ORIGINAL ARTICLE

Phytochemical analysis and Antimicrobial activity of some medicinal Plants against *E.coli* Bacteria from Clinical Isolates from infected broiler chicken

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ABSTRACT
Medicinal plants produce wide variety of phytochemical properties which are used in the pharmaceutical industry for drug manufacturing processes. The components or extracts of the plants were used to treat many diseases including bacterial infections. In the current study, methanol extracts of eight medicinal plants (Terminalia chebula, Alpinia offinarum, Myristica fragrans, Piper longum, Piper cubeba, Anacyclus pyrethrum, Glycyrrhiza glabra and Piper nigrum) were examined for antibacterial activity by agar well diffusion method using bacterial strain such as Escherica coli. The results revealed that the methanol extracts of Terminalia chebula leaves showed better antibacterial activity than the other plants tested. Phytochemical screening revealed the presence of Alkaloid, Phenol, Flavonoid, Steroid, Glycoside, Saponin, Tannin, Cardiac glycoside, Terpenoid, Carboxylic acid, Protein and Carbohydrate. From the results, it could be encouraging the researcher for the identification of active substances which are used in the design and development of new antibacterial drug.
Keywords: Antibacterial Drug, Medicinal plants, Phytochemical and Antioxidant analysis

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INTRODUCTION

Since ancient times, plant-derived medicines were used for the treatment of various diseases. Medicinal treatment by using plant extracts gained popularity in the late 1990s. Still plants are vital sources of medicines especially in developing countries for discovering new drugs [4]. Many efforts have been put to discover new antimicrobial components from various kinds of natural sources. Several Indian medicinal plants are having fair number of antimicrobial activity [2].

In recent years due to failure of chemotherapy an increasing emergence of antibiotic resistant organisms is in record. Therefore, it is essential to prevent the spread of these organisms and to improve the treatment methods [5]. Recently, several studies have reported that ethno medicinal plants are having diverse pharmacological and biological properties [13].

In this study, eight plant species (*Terminalia chebula, Alpinia offinarum, Myristica fragrans, Piper longum, Piper cubeba, Anacyclus pyrethrum, Glycyrrhiza glabra*and *Piper nigrum*) were selected. These plants have been used for many pharmaceutical purposes for various diseases. Most of the parts like root, fruit, leaves and seeds are used for the treatment of various ailment categories. Its leaf extract has been tested for toxicity, mutagenicity, antimutagenicity and antimicrobial activity [4]. The present study explores the antibacterial activity and the phytochemical analysis of the eight different medicinal plants against pathogenic bacteria.

MATERIAL AND METHODS

Plant materials

Eight medicinal plants such as *Terminalia chebula, Alpiniaoffinarum, Myristicafragrans, Piper longum, Piper cubeba, Anacyclus pyrethrum, Glycyrrhiza glabra, Piper nigrum* were collected from the local areas of Nagercoil, Kanyakumari, Tamilnadu.

Extract preparation

Collected plant materials were washed thoroughly and air dried in shade until dried completely. After drying, the plant materials were macerated with mixed grinder. The plant extracts were prepared by percolation method [16].

Antibacterial assays

Bacterial Strains

The bacterial strain *E. coli* isolated from nasal passage of infected broiler chicken.

Identification of Bacteria

The isolated organisms were subjected to the following biochemical tests and were identified by comparing with the standard data provided in Bergey's manual of Systematic Bacteriology [11].

Antibacterial assay

The antibacterial assay was carried out by agar well diffusion method [2].

Phytochemical analysis

Freshly prepared extracts were subjected to standard phytochemical analysis to find the Presence of the following phyto-constituents like protein [9], steroids [8], alkaloids [15], resins and saponins [5].

RESULTS

Isolation of bacteria from broiler chicken

Bacterial strain was isolated from the fluid oozed out from the nasal passage *Escherichia coli* of infected broiler chicken

Identification of bacteria by biochemical analysis

Based on the morphological and biochemical characteristics, the bacterial strain was Gram negative, rod-shaped and motile bacterium, urease and oxidase positive, voges Proskauer and citrate negative, indole and methyl red positive, nitrate and catalase positive, fermented sugars and agar positive, so the organism was identified as *E, coli* sp. (Table. 3.1).

Table: 3.1: Biochemical analysis for identification of bacteria

S. No	Biochemical test	Positive/Negative
1	Morphology	Rods
2	Motility	+
3	Gram stain	-
4	Capsule stain	-
5	Glucose fermentation	+
6	Lactose fermentation	+
7	Indole	+
8	Methyl Red	+
9	Voges Proskauer	-
10	Citrate	-
11	Nitrate	+
12	Catalase	+
13	Oxidase	-
14	Eosin Methylene Blue Agar	+
15	MacConkey Agar	+

Antibacterial assay

Results obtained for the antimicrobial tests performed on ethyl acetate, acetone, hexane, diethyl ether and methanol extracts of *Terminalia chebula, Alpiniaoffinarum, Myristica fragrans, Piper longum, Piper cubeba, Anacyclus pyrethrum*, *Piper nigrum* and *Glycyrrhiza glabra* are presented in Table 3.2. The results showed that the Acetone extract of *Alpiniaoffinarum, Myristica fragrans, Piper longum,* plants showed a broad spectrum of activity, being active to *E. coli*in the antimicrobial assays. There was no inhibition activity in the methanol and ethyl acetate extract of *Myristica fragrans* against *E.coli*.

Table: 3.2 Antibacterial activity of herbal plants in different solvents against *E. coli*

S. No	Herbals	Zone of inhibition				
		Methanol	Ethylacetate	Acetone	Hexane	Diethyl ether
1	*Terminalia chebula*	**10±0.02**	8±0.23	8±0.05	9±0.13	6±0.06
2	*Alpiniaoffinarum*	8±0.09	6±0.19	**11±0.02**	8±0.09	9±0.02
3	*Myristicafragrans*	-	-	**12±0.16**	5±0.05	7±0.09
4	*Piper longum*	8±0.05	4±0.08	**10±0.19**	7±0.16	8±0.05
5	*Piper cubeba*	-	8±0.02	6±0.11	6±0.01	6±0.00
6	*Anacyclus pyrethrum*	4±0.12	-	3±0.01	2±0.08	2±0.04
7	*Glycyrrhiza glabra*	8±0.06	7±0.11	5±0.26	4±.011	6±0.15
8	*Piper nigrum*	3±0.14	-	3±0.21	6±0.17	8±0.26

Phytochemical analysis

The preliminary phytochemical analysis of leaves of *T. chebula* was examines in order to identify various chemical compounds. In these analysis, Alkaloid, Phenol, Flavonoid, Steroid, Glycoside, Saponin, Tannin, Carboxylic acid and Protein showed positive results in methanol solvent. Leaves extracts in hexane solvents showed negative results in all phytochemicals tested especially tannin (Table. 3.3).

Table: 3.3 Phytochemical analysis of *T.chebula* extract in different solvents

S. No	Phytochemicals	*T. Chebula* extract				
		Methanol	Ethylacetate	Acetone	Hexane	Diethyl ether
1	Alkaloid	+	+	+	-	+
2	Phenol	+	-	+	-	-
3	Flavonoid	+	+	+	-	-
4	Steroid	+	-	-	-	+
5	Glycoside	+	-	-	-	+
6	Saponin	+	-	-	-	-
7	Tannin	+	+	+	+	+
8	Cardiac glycoside	-	-	-	-	-
9	Terpenoid	-	-	-	-	-
10	Carboxylic acid	+	-	+	-	-
11	Protein	+	-	-	-	-
12	Carbohydrate	-	-	-	-	-

'+' – positive; '-'negative

The leaves extract of *A. offinarium* showed positive results for alkaloids, phenol, flavonoid, steroid, tannin, cardiac glycoside, terpenoid, protein, carbohydrates and glycosides in the methanol solvents. While saponin and carboxylic acid showed negative results in the methanol extract. Among methanol, ethylacetate, acetone, hexane and diethyl ether, methanol extract showed more phytochemical constituents than the other solvents (Table. 3.4).

Table: 3.4: Phytochemical analysis of *A.offinarium* extract in different solvents

S. No	Phytochemicals	*A.offinarium* extract				
		Methanol	Ethylacetate	Acetone	Hexane	Diethyl ether
1	Alkaloid	+	+	-	-	+
2	Phenol	+	-	-	-	-
3	Flavonoid	+	-	+	-	-
4	Steroid	+	+	-	-	+
5	Glycoside	+	+	-	-	+
6	Saponin	-	-	-	-	-
7	Tannin	+	-	-	+	-
8	Cardiac glycoside	+	-	-	-	-
9	Terpenoid	+	+	+	-	+
10	Carboxylic acid	-	-	-	-	-
11	Protein	+	-	-	-	-
12	Carbohydrate	+	-	+	-	-

'+' – positive; '-' negative

The *M. fragrans* extract showed positive results for Alkaloid, Phenol, Flavonoid, Steroid, Glycoside, Saponin, Tannin, Carboxylic acid, Protein and Carbohydrate in the Methanol, Ethyl acetate, Acetone, Hexane and Diethyl ether solvents (Table 3.5).

Table: 3.5 Phytochemical analysis of *M. fragrans* extract in different solvents

S. No	Phytochemicals	*M. fragrans* extract				
		Methanol	Ethylacetate	Acetone	Hexane	Diethyl ether
1	Alkaloid	+	-	+	-	-
2	Phenol	+	-	+	-	-
3	Flavonoid	+	+	+	+	-
4	Steroid	+	-	+	+	-
5	Glycoside	+	-	+	-	+
6	Saponin	+	+	+	-	+
7	Tannin	+	-	+	+	-
8	Cardiac glycoside	-	-	-	-	-
9	Terpenoid	-	-	-	-	-
10	Carboxylic acid	+	+	+	+	+
11	Protein	+	+	+	-	-
12	Carbohydrate	+	+	+	-	+

'+' – positive; '-' negative

Phytochemical analysis of Diethyl ether extract of *Piperlongum* showed the maximum number of phytochemical constituents than the other plant extracts (Table 3.6). Acetone extracts showed the presence of alkaloid and steroid only.

Table: 3.6 Phytochemical analysis of *P. longum* extract in different solvents

S. No	Phytochemicals	*P. longum* extract				
		Methanol	Ethyl acetate	Acetone	Hexane	Diethyl ether
1	Alkaloid	-	+	+	+	+
2	Phenol	+	+	+	-	+
3	Flavonoid	+	-	-	-	+
4	Steroid	-	+	-	+	+
5	Glycoside	+	-	-	-	+
6	Saponin	+	+	-	-	+
7	Tannin	+	-	+	-	+
8	Cardiacglycoside	-	-	-	-	-
9	Terpenoid	-	-	-	-	-
10	Carboxylic acid	-	-	-	-	-
11	Protein	+	+	-	-	-
12	Carbohydrate	+	+	-	-	+

'+' – positive; '-' negative

P. cubeba contained Alkaloid, Phenol, Flavonoid, Steroid, Glycoside, Saponin, Tannin and Carbohydrate of Methanol extracts (Table 3.7). There were no phytochemical constituents in ethyl acetate, acetone, hexane and diethyl ether extracts.

Table: 3.7 Phytochemical analysis of *P. cubeba* extract in different solvents

Sl. No	Phytochemicals	*P. cubeba* extract				
		Methanol	Ethylacetate	Acetone	Hexane	Diethyl ether
1	Alkaloid	+	-	-	-	-
2	Phenol	+	-	-	-	-
3	Flavonoid	+	-	-	-	-
4	Steroid	+	-	-	-	-
5	Glycoside	+	-	-	-	-
6	Saponin	+	-	-	-	-
7	Tannin	+	-	-	-	-
8	Cardiac glycoside	-	-	-	-	-
9	Terpenoid	-	-	-	-	-
10	Carboxylic acid	-	-	-	-	-
11	Protein	-	-	-	-	-
12	Carbohydrate	+	-	-	-	-

'+' – positive; '-' negative

DISCUSSION

Traditional medicinal plants play a vital source of drug compounds for human health and wellbeing. Plant extracts have significant antimicrobial properties for therapeutic treatment [16, 17]. The medicinal plants extracts exhibiting antimicrobial activity and it was reported to play a significant role in the treatment of disease globally [1, 2, 3,9]. The plants have valuable reservoir of bioactive compounds of substantial medical importance [13]. From this understanding, this study has evaluated the antibacterial activity of eight medicinal plants and their phytochemical constituents. *Terminalia chebula* showed maximum zone of inhibition than the other two plants tested. Samy and Ignachimuthu, [19] reported that, *Cassia auriculata* exhibited significant activity against *E. coli* was contrast with the present study.

Plants have been important source of pharmaceuticals because of their unique characteristics such as less toxic, widely available and less expensive [18]. The researchers interest has to shifted to the use of safer and natural plants to kill the pathogenic bacteria. The antibacterial activity of plants was normally depending on the chemical structure of active compounds (phytochemicals) and their concentration. Such phytochemicals including Alkaloid, Phenol, Flavonoid, Steroid, Glycoside, Saponin, Tannin, Cardiac glycoside, Terpenoid, Carboxylic acid Protein and Carbohydrate are used to treat the bacterial pathogens and act as an active agent. The use of plant extracts and phytochemicals with known antibacterial properties can be of great significance in therapeutic value [6,14]. In the present study, the effect of methanol, ethyl acetate, hexane, acetone and diethyl ether of *Terminalia chebula, Alpinia offinarum, Myristica fragrans, Piper longum, Piper cubeba, Anacyclus pyrethrum, Glycyrrhiza glabra, Piper nigrum* leaves were used to control Gram-positive bacteria *E.coli sp*. Among the different solvents used, methanol extracts of all the eight plants showed more inhibition of *E,coli* sp. and presence of more phytochemical constitutes. Methanol has two chemical and biological characteristics including medium polarity and minimum toxicity on test strains. They can help to extract many biological compounds (polar and non-polar). The results of the present study also confirm the presence of antibacterial substances in the plant extracts and exhibited against the bacterial pathogens.

CONCLUSION

Past decades, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. The traditional plants against pathogens as a result, plants are one of the bases for modern medicine to attain new principles. The plants screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs. Further steps are focused on the isolation of the active components in plant extracts which could be used as in the development of new antimicrobial drugs.

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