RESEARCH

Anti Escherichia Coli Activity of Some Medicinal Plants of High Altitude of Nepal

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ABSTRACT

Background: Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. The aim of the present study was to find out the bioactive chemical constituents such as flavonoids, alkaloids, tannins, saponins, carbohydrate and to find out the anti *E. coli* activity of the ethanolic extracts of traditionally used ten medicinal plants of Nepal at an altitude of 1500 ft from above the sea level.

Methods: Ethanolic extracts of ten commonly used medicinal plants were analyzed phytochemically and evaluated for their significant antimicrobial activity against the clinical isolates of *Escherichia coli*. Mean zones of inhibition were calculated for each of the extracts.

Results: The results revealed that though all the plants of the high altitude showed some degree of antimicrobial activity, the leaf extract of Syzygium cumini (5.7±0.3 cm), Chromolaena odorata (5.2±0.4 cm), Ocimum sanctum (4.7±0.6 cm) and Justicia adhatoda (3.2±0.3 cm) were most effective against the clinical isolates of E. coli, whereas the other six plant extracts were least effective against the clinical isolates of E. coli. Qualitative phytochemical analysis of the extracts revealed the presence of bioactive components. Seven of the plant extracts contain alkaloids, six of them contain glycosides, four of them contain flavonoids, three of them contain carbohydrate, oil and fats, two of them contain tannins, whereas only one of them contains saponins.

Conclusion: The result of this study justified the folkloric usage of the studied plants and concluded that these plants extract have great potential in finding new clinically effective antimicrobial compounds.

Keywords: Analysis, Antimicrobial, Effect, Phytochemical, Plant extracts

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INTRODUCTION

Herbal medicine contributes immensely to the quality of life for thousands of years. Plants are an essential part of traditional medicine in almost any culture. Currently the world population is about 5-6 billion and the increase in this population is expected to rise up to 7.5 billion by the year

2020.[1, 2] According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal properties of plants are due to the active chemical constituents present in different parts of the plant.[3]

These chemical constituents produce a definite physiologic action on the human body. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants.[4]

Medicinal plants are a source of great economic value all over the world. The World Health Organization also supports the use of medicinal plants provided it is proven to be efficacious and safe.5 Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. In high altitude country like Nepal thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vague since ancient times. Among the 7000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are said to be found in Nepal.6 Unfortunately, the medicinal values of all the plants are not yet explored.

Escherichia coli are Gram-negative, facultatively anaerobic bacilli, commonly found in the lower intestine of warm-blooded organisms.[7] They constitute 0.1% of gut flora, and the pathogenic strains of the bacterium can cause disease by transmitting through the faeco-oral route.[8] Virulent strains of *E. coli* can cause Urinary tract infection (uropathogenic *E. coli*), gastroenteritis, and neonatal meningitis. In rare instances they are also responsible for septicemia, mastitis, haemolytic-uremic syndrome and Gram-negative pneumonia.[9]

According to WHO and Centre for Disease Control (CDC) *E. coli* is one of the most commonly isolated organisms from different samples in different diseases.[10] The problem is further compounded by the emergence of resistance to antimicrobial agents that are commonly used against *E. coli*, making the treatment expensive and prolonged.

Therefore, in the present study, an effort has been made to analyse the phytochemical constituents and anti *E. coli* activity of the ethanolic extracts of the ten medicinal plants found in the high altitudes of Nepal.

MATERIALS AND METHODS

The fresh plants were collected from different sites of Parsa (Birgunj), Bara (Simra) and Makwanpur (Hetauda) districts (altitude about 1500ft from above the sea level), during May 2012 to August 2012, and processed at Clinical Microbiology laboratory of National Medical College and Teaching Hospital, Birgunj, Nepal. These plants were identified on the basis of available literatures[11, 12] and in the Department of Botany, Vidyasagar University, India. Leaves were washed with distilled water, shade dried, powdered and stored in an airtight container until further use.

Preparation of extracts

Solvent extracts were prepared by transferring 1g of the powder to sterile wide-mouthed screw-capped bottles containing the solvent (50%, v/v). It was allowed to soak for 24 hours at room temperature then heated for an hour at 100°C. The mixture was then centrifuged at 2000 rpm for 10 minutes at 4°C.[13] The supernatants were filtered through a sterile funnel containing sterile whatmann filter paper no.1 and then filtrate was sterilized using syringe filter containing 0.2µ cellulose acetate membrane (Sartorius, India).[13] The percentage yield of the extract was determined using the expression:

Percentage yield = Weight of extract X 100/ Weight of ground plant Material

Phytochemical Evaluation of the Extract

The phytochemical evaluation of the plant extracts for the major constituents was undertaken as per the

standard methods.[14, 15] The methods for detecting the bioactive constituents were described below.

Detection of Alkaloids

5ml plant extracts were dissolved individually in dilute (2%) Hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

a) **Mayer's Test**: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.

b) **Dragendroff's test**: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

Detection of Carbohydrates

The plant extracts (5ml) were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) **Molisch's Test**: 2 ml of the filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of Carbohydrates.

b) **Benedict's test**: The filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugars.

Detection of Glycosides

The plant extracts were hydrolysed with diluted HCl (2%) and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

Detection of Phenols

Ferric Chloride Test: 5 ml of the extracts were treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of Oils and Fats

Stain Test: 2ml of the plant extracts were pressed between two filter papers.

An oily stain on filter paper indicates the presence of fixed oil.

Detection of Saponins

Froth Test: 5ml of the extracts were diluted with 20ml. distilled water and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam test: 5ml of the extracts were shaken with 10 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of Tannin

Gelatin Test: 1% gelatin solution containing 2% sodium chloride was added to the extract. Formation of white precipitate indicates the presence of tannins.

Detection of Flavonoids

a) **Alkaline Reagent Test**: 5ml of the extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) **Lead acetate Test**: Extracts were treated with few drops of lead acetate solution.

Observation of yellow color indicated the presence of flavonoids.

Isolation of *E. coli*

The clinical isolates of *E. coli* were isolated from the urine, pus and fecal samples in the department of microbiology of National Medical College & Teaching Hospital of Birgunj, Nepal. For isolation of *E. coli*; clinical samples such as urine, pus and feces were inoculated onto MacConkey's agar media (Hi Media, India). The inoculated culture plates were incubated at 37°C in an incubator

for 24 hours. These consecutive clinical isolates of *E. coli* were identified on the basis of colony morphology, gram staining, catalase, indole production and methyl red positive tests.[16]

Preparation of the Inoculum

Five colonies grown on MacConkey's agar (Hi Media, India) (*E. coli*) media were obtained. These colonies were inoculated in 2.5 ml Muller-Hinton broth in a test tube by rotating the inoculating loop at least ten times with the tip touching the bottom of the test tube. The test tube was incubated at 37° C for 3 hours. The turbidity is also matched with 0.5 McFarland Standard.

Statistical analyses

To ensure consistency of all findings, antimicrobial activity of plant extracts was performed in triplicate under aseptic condition. Data were statistically analyzed and expressed as mean ± Standard deviation (SD) by SPSS 10, Chicago, Inc.

RESULTS

Ten medicinal plants (as shown in Table 1) used in this study are widely used in folkloric medicine of Nepal in treating different diseases. The percentage yields of extracts and the phytochemical constituents of the plants are shown in Table 2 and 3 respectively. The highest yield of ethanolic extract was found in case of *C. odorata* (L.) (32.4% ± 0.65) and the lowest in *Curcuma longa* (L.) (9.3% ± 0.65). Phytochemical analysis conducted for the ten plant extracts reveals the presence of flavonoids, alkaloids and saponins as the major constituents (Table 3). The other secondary metabolites like tannins, glycosides and carbohydrates were present in trace amount in some of the plant extracts (Table 3). In this study, ethanolic extracts of these ten plants were studied. The clinically isolated gram negative bacteria *E. coli* was found maximally inhibited by the ethanolic extract of *Syzugium cuminii* (L) *Chromolaena odorata* (L.) *Ocimum sanctum* (L.), and *Justicia adhatoda* (L.).

The zones of inhibition of ethanolic extract of different medicinal plants, which are able to produce zone of inhibition are shown in Table 4. Though all the plants of the high altitude showed some degree of antimicrobial activity against *E. coli*, it was seen from the Table 4 that *C. odorata* (L.), *S. cumini* (L.), *O. sanctum* (L.) and *J. adhatoda* (L.) possess significant antimicrobial activity against *E. coli.*

Table 1: List of medicinal plants used in antimicrobial assay

Botanical Name	Vernacular Name	Family	Month of collection
Curcuma longa (L.)	Haledo	Zingiberaceae	May,2012
Emblica officinalis (L.)	Amlaki	Euphorbiaceae	June,2012
Ocimum sanctum (L.)	Tulsi	Lamiaceae	June,2012
Sapindus mukorossi (Gaertn.)	Reetha	Sapindaceae	July,2012
Chromolaena odorata (L.)	Banmara	Asteraceae	July,2012
Justicia adhatoda (L.)	Asuro	Apocynaceae	July,2012
Origanum majorana (L.)	Ramtulsi	Lamiaceae	August,2012
Syzygium cumini (L.)	Jamun	Myrtaceae	August,2012
Rauvolfia serpentina (L.)	Sarpagandha	Apocynaceae	August,2012
Allium sativum (L.)	Lasun	Liliaceae	August,2012

Table 2: Percentage yield of extracts

Plant name	Weight of raw material (gm)	Weight of the extract (gm)	% yield * Extractive value
Curcuma longa (L.)	90	8.4	9.3 ± 0.65
Emblica officinalis (L.)	78	18.3	23.4 ± 0.54
Ocimum sanctum (L.)	77.4	17.2	22.2 ± 0.79
Sapindus mukorossi (Gaertn.)	105	22.4	21.3 ± 0.65
Origanum majorana (L.)	64	19.4	30.3 ± 0.61
Syzygium cumini (L.)	115	27.8	24.2 ± 0.78
Chromolaena odorata (L.)	120	38.9	32.4 ± 0.65
Justicia adhatoda (L.)	100	21.1	21.1 ± 0.48
Rauvolfia serpentina (L.)	96	15.6	16.2 ± 0.61
Allium sativum (L.)	80	16.4	20.5 ± 0.57

*Values are in terms of Mean ± SEM of results done in triplicate

DISCUSSION

The present study was conducted to obtain preliminary information on the antimicrobial activity of ten traditional medicinal plants of Nepal. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. Through this *in vitro* study, we could see that ten plant extracts inhibited the growth of clinical isolates of *E. coli* but their effectiveness varied.

Based on the mean zone of inhibition, the test organism *E. coli* was found to be most susceptible to *Syzygium cumini* (L.) (5.7 ± 0.3 cm) extract and least susceptible to *Emblica officinalis* (L.) (1.1 ± 0.4 cm) (Table 4). Similarly a study conducted by Chhetri *et al.,* in 2008 found that *E. coli* was most susceptible to *Rhododendron setosum* (L.) and least towards *Curcuma longa* (L.).[17] The findings indicate the existence of antimicrobial compounds in the crude ethanolic extracts of ten plants under study and showed a good correlation between the reported medicinal uses of these plants against different diseases in local communities of Nepal. The inhibitory effect of these ten plants against the clinical isolates of *E. coli* might be attributed to the presence of some active constituents in the plant as in other herbal plants.

The phytochemical analysis of the study reveals that the extract of the ten plants (ethanolic) contained bioactive compounds (flavonoids, alkaloids, saponins, tannins, carbohydrates, glycosides & oils) (Table 3). These bioactive compounds are believed to be responsible for the observed antimicrobial effects. Some workers have observed the antimicrobial effects of plant extracts to the presence of these bioactive compounds.[18,19] The presence of these bioactive compounds is an indicator that the ten plants can be a potential source of precursors in the development of synthetic drugs.

The result of the present study provides a scientific evidence for the local use of the ten medicinal plants studied and ushers for the selection of plants with antimicrobial activities for further phytochemical work in the isolation and identification of the active compounds.

Table 3: Phytochemical constituents of the ten plant extracts

Components / Plant extracts	Alkaloids	Flavonoids	Saponins	Tanins	Carbohydrates	Glycosides	Oils& Fats
Chromolaena odorata (L.)	++	+++	-	-	-	-	-
Ocimum sanctum (L.)	++	-	-	+++	-	+	-
Sapindus mukorossi (Gaertn.)	+	-	+++	-	+	-	-
Syzygium cuminii (L.)	++	-	-	+++	+	-	-
Allium sativum (L.)	-	-	-	-	-	+	+++
Curcuma longa (L.)	-	+++	-	-	-	++	-
Rauvolfia serpentina (L.)	+++	++	-	-	-	-	+
Justicia adhatoda (L.)	++	+	-	-	+	+	-
Emblica officinalis (L.)	-	-	-	-	-	+	+++
Origanum majorana (L.)	+	-	-	-	-	+	+++

'+' = Positive; '++' = Moderate Positive; '+++' = Strongly Positive; '-'= Not Detected

CONCLUSION

The plant extracts under study demonstrated great potential against the clinical isolates of *E. coli* and supported their folkloric use against the diseases caused by this organism. Furthermore, it has opened the avenues for the discovery of new clinically effective antimicrobial compounds. Therefore, the future investigations should be directed towards the determination of chemical structure of the active constituents and toxicological evaluation with the aim of formulating novel chemotherapeutic agents to cope up with increasing prevalence of drug resistant diseases caused by virulent strains of *E. coli*.

Table 4: Zone of inhibition of the plant extracts against *Escherichia coli*

Plants	Extract solution (ZOI) *
Curcuma longa (L.)	2.1 ± 0.3 cm
Emblica officinalis (L.)	1.1 ± 0.4 cm
Ocimum sanctum (L.)	4.7 ± 0.6 cm
Sapindus mukorossi (Gaertn.)	1.6 ± 0.3 cm
Origanum majorana (L.)	2.3 ± 0.5 cm
Syzygium cumini (L.)	5.7 ± 0.3 cm
Chromolaena odorata (L.)	5.2 ± 0.4 cm
Justicia adhatoda (L.)	3.2 ± 0.3 cm
Rauvolfia serpentina (L.)	2.1 ± 0.3 cm
Allium sativum (L.)	1.1 ± 0.4 cm

**Values are in terms of Mean ± SEM of results done in triplicate.*

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