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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI-MICROBIAL PROPERTIES OF MEDICINAL PLANTS OF DHUNKHARKA COMMUNITY

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ABSTRACT

The primary source of medicine for underprivileged populations without access to contemporary medical care is medicinal plants. The phytochemical and biological screening of several plants obtained from Dhunkharka village in the Kavrepalanchowk district of Nepal is the subject of this study. Stephanie Flavanoids and glycosides were abundant in glandulifera, Cuscuta reflexa, Bergenia ciliata, Melia azadirachta, Drymaria diandra, Jasminum humile, Astelbe rivularis, Oxalis corniculata, and Viola serpens.

Methanolic extract was employed for antibacterial and phytochemical screening purposes. Even though the Dhunkharka population uses a variety of plants, only an extract from Oxalis corniculata and Jasminum humile had a minor antibacterial action on the human pathogenic bacterial strain E. coli. Although the sensitivity of each bacterial strain varies, the methanolic extract of Astelia rivularis had the strongest antibacterial efficacy against Escherichia coli.

Keywords: Nepal, phytochemicals, ethnobotany, medicinal plants, harmful bacteria, and antibacteria

1. INTRODUCTION

Primary health care of about 80 % of the world's population is dependent on the use of medicinal plants derived from traditional medicine , Rajan Shrestha, Tirtha Maiya Shrestha and Rajendra Gyawali* Department of Pharmacy, Kathmandu University, Dhulikhel, Kavrepalanchowk, Nepal ABSTRACT Medicinal plants serve as the main source of medicine to poor communities that do not have access to modern medical services. This research deals with the phytochemical and biological screening of different plants collected form Dhunkharka village of Kavrepalanchowk district, Nepal. Stephania glandulifera, Cuscuta reflexa, Bergenia ciliata, Melia

azadirachta, Drymaria diandra, Jasminum humile, Astilbe rivularis, Oxalis corniculata, and Viola serpens were rich in flavanoids and glycosides. Methanolic extract was used for phytochemical screening and antimicrobial activities. Though there are several plants used by Dhunkharka community, only Jasminum humile and Oxalis corniculata extract showed slight antibacterial effect on human pathogenic bacterial strain E. coli. Methanolic extract of Astilbe rivularis exhibited highest antibacterial effect

against E. coli while each bacterial strain varies in its sensitivity. [1]. Still, due to the inaccessibility to modern facilities, large number of peoples of Nepal depends upon a wide range of natural medicines for their primary healthcare using ethnobotanical knowledges. Such traditional knowledge survives because it is transferred from one generation to another. Because of the innumerable biologically active compounds that are found in plants possess antibacterial properties, now days numerous investigations are going on in isolation of potent compounds for antimicrobial therapy [2] Traditional healers in Dhunkharka community (Latitude 27 . Extracts of plants and phytochemicals are getting more importance as potential sources for inhibiting different diseases during the recent decade. Ethnobotanical plants have a greater number of positive results than randomly selected plants.

Traditional healers in Dhunkharka community (Latitude 27 O 31′52.22″N to 270 31′42.15″ and Longitude 850 29′44.57″ to 850 29′35.79″) of Kavrepalanchwok district, Nepal depend upon many of the medicinal plants available in the area. So it provides immense scope for the characteristic detailed study of these local and medicinally significant plants. Despite many studies on medicinal plant resources of Nepal, a large number of medicinal plants and associated indigenous uses still wait proper documentation and evaluation of their therapeutic properties. This is perhaps because of the fact that these studies do not fully represent the wide range of environments in Nepal and also due to lack of scientific resources for proper validation of bioactive potentiality. Indeed, few studies were carried out on different aspects of ethnobotany of Kavre district [3-5]. Recently, some studies have been carried out on antimicrobial properties of Nepalese medicinal plants to assess to their properties [6,7] . Due to species climatic and geographical conditions, temperate and alpine plants of the Himalaya offer greater possibilities of having novel molecules and even largest quantities of the active compounds. Therefore to evaluate the phytochemical profiles and efficacy of traditional medicine we have recently documented several medicinal plants from various geographical locations of Nepal based on the ethnopharmacological information [8-12] . Thus the present investigation represents a preliminary screening of medicinal plants in Dhunkharka community of Kavrepalanchwok district for their phytochemical profile and antibacterial properties against human pathogenic bacterial strains.

2. MATERIALS AND METHODS

Plant Materials

The whole aerial plant parts (Table 1) were collected from Dhunkharka Community of Kavrepalanchok District in Bagmati zone, Nepal during March, 2011. The altitude of research area is about 1820-1921 m above Sea level. Voucher specimens identified by Tirtha Maiya Shrestha and Dr Rajendra Gyawali (Department of Pharmacy, Kathmandu University, Dhulikhel, Nepal) have been deposited in Department of Pharmacy, Kathmandu University. The collected plant materials were dried in shade and stored at room temperature before the experiments.

Preparation of the plant extract

Extraction was carried out using methanol. The whole dried plant sample was blended in home blender and powdered sample was

initially soaked in methanol in a conical flask and allowed to stand for 15 days with occasional shaking. After 15 days, the solvent along with components were collected and was filtered using Whatman N° 1 filter paper. Traces of the methanol from the extract were removed by keeping the extract on a water bath at low temperature. The extracts obtained were then weighed and percentage of yield evaluated and was kept aseptically until use.

Phytochemical screening

The methanolic extracts of different plant samples was screened for the presence of Alkaloids, Flavanoids, Tannin, Coumarins and Glycoside according to standard procedures of analysis [13,14].

Microbial cultures and growth conditions

Human pathogenic bacteria were provided by Dhulikhel Hospital, Kathmandu University Teaching Hospital, Nepal. Klebsiella sp. ECI10A, Serratia sp. (AF-5A), Escherichia coli (0157: H) were used as test microorganisms. Cultures of bacteria were grown for 24 h in 50 ml of nutrient broth (Himedia, India) at 37 °C and were maintained at 4 °C. The microorganisms were kept under refrigeration (4 °C) until use. Subcultures of the organisms were grown in nutrient broth (Himedia, India) at 37 °C, 24 h before each experiment.

Antimicrobial assay

Methanolic extracts of the plant parts were dissolved in 1 % (v/v) DMSO and tested for antimicrobial activity using the agar disk diffusion method. Sterile, 6 mm diameter Grade 1 Whatman filter paper discs were impregnated with extracts. And placed in duplicates onto MacCkonkey agar (Himedia, India) plates, surface spread with 1.5 × 10 were used as test microorganisms. Cultures of bacteria were grown for 24 h in 50 ml of nutrient broth (Himedia, India) at 37 °C and were maintained at 4 °C. The microorganisms were kept under refrigeration (4 °C) until use. Subcultures of the organisms were grown in nutrient broth (Himedia, India) at 37 °C, 24 h before each experiment. Antimicrobial assay 6 cells / ml (adjusted to the 0.5 McFarland turbidity standards) bacteria cultures. The plates were then incubated for 24 h at 37 °C. The experiments were carried out in duplicate of each for the three times. The results (mean value) were recorded by measuring the zones of growth inhibition surrounding the discs. Inhibition zone values were corrected i.e. disk diameter was subtracted from the value of the inhibition zone. DMSO single considered as a control. For comparative purposes standard ciprofloxacin (30 mcg: disc), was included in the assay. Experiments were conducted as per the procedure given in literature.

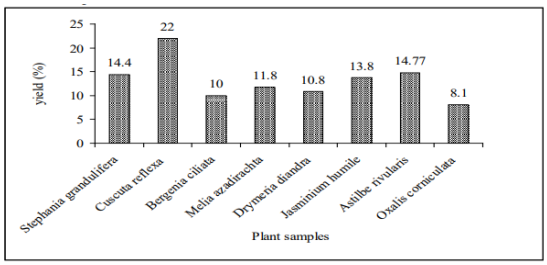
3. RESULTS AND DISCUSSION

The major objective of this research was to enumerate the medicinal plants from local community of Kavre and investigate antibacterial property of those plants against human pathogenic bacteria. The preliminary results obtained from research showed that total nine plants are consumed as an aspect of ethnomedicinal use of local communities of research area. Out of them most of the plants were found used for the treatment of stomach related illness. Three plants were used for antipyretic and two were used in laxative purposes (Table 1). Multiple-remedies made by above plants were used for several conditions including one used for jaundice, cooling, and astringent etc purposes.

The use of methanolic extract is common to .Yield percentage of methanolic extracts of different plants were also evaluated and presented (Fig 1). Among the yield value of nine different extracts expressed in percentage, Cuscuta reflexa showed highest yield of 22 % and Oxalis corniculata showed lowest yield of 8.1 %. Yield value quantifies the amount of active constituents relative to amount of the crude drug material which was found co-related in present study. This is a first report we are documenting since there are no previous documentation on standard yield values of above mentioned plants. Thus, we assumed that such variability in yield value may be due to either the plant contains variable amount of active constituents or relative number of soluble compounds in methanol is variable.

In this report first the methanolic extracts of plants Stephania glandulifera, Cuscuta reflexa, Bergenia ciliata, Melia azadirachta, Drymaria diandra, Jasminum humile, Astilbe rivularis, Oxalis corniculata, and Viola serpens were subjected to phytochemical screening (Table 2). Methanol crude extracts of all above species were found rich in alkaloids, tannin and coumarins. But Melia azadirachta was found positive only for flavanoids and glycoside. Oxalis corniculata was tested positive for flavanoids, tannin, coumarin, and glycoside.

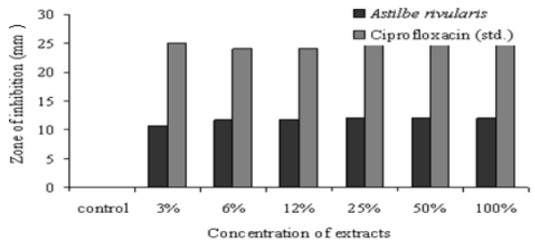
Fig 1: Yield percentage of methanolic extract of plants collected plants from Dhunkharka community of Kavrepalanchwok district, Nepal.



Methanol crude extracts of different plants were tested for antimicrobial activity. Methanol crude extracts (100 % w/v in DMSO) of nine plants did not show antibacterial activity against Klebsiella sp. and Serratia sp. (Table 3). However, following extracts showed inhibitory effect against E. coli; Cuscuta reflexa (ZOI = 7mm), Jasminum humile (ZOI = 8mm), Astilbe rivularis (ZOI = 13mm) and Oxalis corniculata (ZOI = 7mm). Astilbe rivularis showed potential action against the test organism E. coli (ZOI =13mm), anti-microbial test was focused on exposure of test organism to different concentrations (0, 3, 6, 12, 25, 50 and 100) % of methanol crude extracts of Astilbe rivularis (Fig 2). Astilbe rivularis showed maximum potential against E. coli at concentration of 100 % methanol crude extract. Graph showing comparison between varying strengths of methanol crude extracts of Astilbe rivularis with their corresponding ZOI taking Ciprofloxacin (30 mcg) as standard is illustrated. In local community Astilbe rivularis is being used in the treatment of diarrhea and dysentery. So, the use of Astilbe rivularis as an antimicrobial agent is justified. Since, only Astilbe rivularis showed good inhibition zones, antimicrobial assay was carried out at varying concentration for Astilbe rivularis (0, 3, 6, 12, 25, 50 and 100 gm/100ml) using the above procedure. Its remarkable effect has been found against E. coli and shown in Figure 2. In agreement with previous findings these plants had been used in local

folk medicinal remedies in different forms for various afflictions. Fig 1: Yield percentage of methanolic extract of plants collected plants from Dhunkharka community of Kavrepalanchwok district, Nepal. [11,12] , in present study, it was demonstrated that the methanolic extracts of selected plant species exhibited highest antibacterial effect towards the E. coli than some others and each bacterial strains varies in its sensitivity to the plant extracts.

Fig 2: Antimicrobial activity of methanolic extract of Astilbe rivularis varying concentration against E. coli using Ciprofloxacin (30mcg) as standard. Concentration of extract: 100 % (w/v in 1 % DMSO) and Ciprofloxacin (30mcg) as standard.



Conclusively, In Nepal, generally there are several medicinal plans waiting for documentation and evaluation of their therapeutic properties. These researches assist the finding of publications and

also conclude that though there are several plants used by local community of Kavre, only Astilbe rivularis extract showed effective antibacterial activity towards human pathogenic bacterial strain E. coli. So this plant could be useful and effective towards the control of stomach illness caused by E. coli. However, more data is necessary to evaluate the safety of the following plants used to treat stomach related disorders

Table 1:Plants collected based on ethnobotanical uses in Dhunkharka community of Kavrepalanchwok district, Nepal.

Scientific Name (Code)	Local Name	Parts Used	Traditional Use
Stephania glandulifera	Guzzargaano	Rhizome	Postnatal abdominal pain , stomach disorders
Cuscuta reflexa	Akash bel	Whole plant	Anti-itching, expectorant, antipyretic
Bergenia ciliata	Pakhanbhed	Rhizome	Diarrhea, Carminative, roundworm infestation
Melia azadirachta	Bakaino	Fruit	Anti helminthtic, Aphrodisiac, Pelvic Pain
Drymaria diandra	Abijaalo	Whole plant	Laxative and cooling effect, antipyretic
Jasminium humile	Jaai	Leaves	Ringworm infestation, stomach disorders
Astilbe rivularis	Thulol okhati	Rhizome	Diarrhea, dysentery, powder is astringent.
Oxalis corniculata	Chari-Amle	Whole Plant	Scurvy
Viola serpens	Ghatteghaans	Whole plant	Antipyretic, boils, laxative, leaves as emollient.

Table 2: Phytochemical screening results of methanolic extracts of different plants from Dhunkharka community of Kavrepalanchwok district, Nepal.

Methanol crude extract of	Parts Used	Alkaloids	Tannins	Flavonoids	Coumarins	Glycosides
Stephania glandulifera	Rhizome	++	++	++	++	++
Cuscuta reflexa	Whole plant	++	++	++	++	++
Bergenia ciliata	Rhizome	++	++	++	++	++
Melia azadirachta	Fruit	--	--	++	--	++
Drymaria diandra	Whole plant	++	++	++	++	++
Jasminium humile	Leaves	++	++	++	++	++
Astilbe rivularis	Rhizome	++	++	++	++	++
Oxalis corniculata	Whole Plant	--	++	++	++	++
Viola serpens	Whole plant	++	++	++	++	++

Table 3:Antimicrobial activity of methanolic extracts from different plants againt human pathogenic bacteria, E. coli, Klebsiella sp. and Serratia sp. Concentration of extract: 100 % (w/v in 1 % DMSO) and Ciprofloxacin (30mcg) as standard.

Plant	Zone Of Inhibition(ZOI) (mm)					
	Ciprofloxacin			Methanolic extract in DMSO		
	E. coli	*Klebsiella sp.*	*Serratia sp.*	*E. coli*	*Klebsiella sp.*	*Serratia sp.*
Stephania glandulifera	25	26	23	6	-	-
Cuscuta reflexa	24	23	22	-	-	-
Bergenia ciliata	25	25	22	-	-	-
Melia azadirachta	26	23	22	-	-	-
Drymaria diandra	25	23	21	-	-	-
Jasminium humile	23	25	22	8	-	-
Astilbe rivularis	25	25	24	13	-	-
Oxalis corniculata	22	23	22	7	-	-

4. CONCLUSION

The phytochemical screening and evaluation of antimicrobial properties of medicinal plants from the Dhunkharka community has provided valuable insights into the potential therapeutic benefits of traditional remedies. Through rigorous screening processes, we identified a diverse range of phytochemical compounds present in these plants, indicating their rich medicinal value. Furthermore, our antimicrobial assays revealed promising antibacterial and antifungal activities in

several plant extracts, suggesting their efficacy in combating microbial infections. These findings underscore the importance of preserving and utilizing indigenous knowledge of medicinal plants for healthcare purposes. Moving forward, further research and clinical trials are warranted to explore the therapeutic potential of these natural remedies and their possible integration into modern healthcare practices.

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