

ANTIBACTERIAL ACTIVITY OF SELECTED ETHNOMEDICINAL PLANTS OF SAGARMATHA REGION OF NEPAL

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ABSTRACT

The present studies cover antibacterial activity of 15 crude extracts (5 extracts each from hexane, dichloromethane and methanol) from five ethnomedicinal plants viz. *Anaphalis nepalensis*, *Piptanthus nepalensis*, *Senecio raphanifolius*, *Thermopsis barbata* and *Thermopsis inflata* collected from Sagarmatha region of Nepal against one Gram-positive (*Staphylococcus aureus*) and three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*) bacterial strains. Plants used to treat at least one ailment considered to be caused by bacterial infection were identified and further investigated for their antibacterial activity. Crude extracts were obtained by the successive extraction method and examined *in-vitro* using the agar well diffusion process. Among 15 extracts examined, 13 (86 %) extracts showed antibacterial property against *Staphylococcus aureus* followed by 8 (53 %) extracts against *Escherichia coli* and 6 (40 %) extracts each against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Plant extracts were more likely to inhibit Gram-positive bacteria, *Staphylococcus aureus*. With respect to Gram-negative bacteria, it was more common for plant extracts to inhibit *Escherichia coli* than *Pseudomonas aeruginosa* or *Klebsiella pneumonia*. Overall, 86 % of plant extracts showed activity against Gram-positive test bacteria, and 45 % showed activity against Gram-negative test bacteria. This research results will promote to search for alternative, inexpensive and simply available antibacterial of plant origin.

Keywords: Antibacterial property, medicinal plants, traditional use, Nepal

INTRODUCTION

Medicinal plants are of significant remedial aid for various ailments. About 80% of the world population is wholly or partially dependent on drugs derived from plant origin^[1]. Today, drugs derived from natural sources play a significant role in the treatment of human diseases. Many of the drugs currently used to treat bacterial and other infections were first isolated from natural sources including ethnomedicinal plants^[2].

Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century^[3]. The emergence of artificial antimicrobials leads to lack of interest in plants as a natural source for antimicrobial drugs^[4]. However, in the recent years the condition has modified and the field of bio-prospecting research has augmented.

Several ethnomedicinal plants of Nepal have been identified and their usage documented^[5-7, etc]. These documented plants have been used as antibacterial, antifungal, antiviral and for other general treatments. But, a scientific and systematic investigation on antibacterial properties of

Nepalese medicinal plants is still lacking.

Only a few ethnomedicinal plants from Nepal have been explored scientifically^[8-13], to validate their traditional knowledge in laboratory. In this research, medicinal plants that were used to treat at least one ailment that may be of bacterial origin were collected from the Sagarmatha region of Nepal and tested with suitable bacterial strains in Nepal Academy of Science and Technology, Kathmandu Laboratory to identify their antibacterial properties.

In this research, a total of 15 extracts from five ethnomedicinal plants were examined for their antibacterial activity *in-vitro* using the agar well diffusion method. The plan of screening was to correlate the antibacterial activity to the indigenous uses/knowledge. This is the first step in the exploration for primary health care products that are socially suitable and scientifically accepted^[8-9, 14-18].

MATERIALS AND METHODS

Collection of plant materials and ethnomedicinal uses

Plants species with their useful parts were collected from natural populations from the Sagarmatha region of Nepal in 2013. The herbarium specimens were identified by the first author and a set of voucher herbarium specimens was made for each collection. Plant samples for

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laboratory investigation were air-dried in the shade at room temperature for at least four weeks. All these plants are used traditionally to treat at least one ailment that is likely of bacterial origin (table 1). Ethnomedicinal uses were collected following (Bhattarai *et al.* 2006, 2010) ^[5-6].

Plant extracts

Successive extraction method was used for obtaining antimicrobial extracts ^[16]. The air dried samples were crushed in the electric mixture grinder. Extracts were prepared from the plant organs specified by the traditional healers and the knowledgeable villagers. For each extraction, three different solvents [hexane, dichloromethane (CH₂Cl₂) and methanol (MeOH)] were used in succession. Twenty-five grams of dry milled plant powder was placed in a beaker (500 mL), and 100 mL of hexane was added and the mixture left to soak overnight. The mixture was then vigorously stirred for 10 minutes and allowed to settle for 5 minutes. This process was repeated thrice.

The supernatant liquid was then passed through filter paper to remove solid plant material and the solvent evaporated from the filtrate *in-vacuo* at 34 °C in a rotary evaporator. The residual plant material was further extracted, first with dichlo then with methanol using the same procedure as used for hexane. The crude evaporated extracts were dried at room temperature for 2 weeks. Then 50 mg of each crude extract was dissolved in 1 mL (1000 µL) of the respective solvents (methanol/dichloromethane/hexane) to give a final concentration of crude extract in solvents of 50 mg/mL. From 50 mg/mL crude extract stock solution, each well is impregnated with 20 µL extract (20 µL/well from a stock solution of 50

mg/mL) which is equivalent to 1 mg/well.

Microorganisms used

Four different strains of bacteria were used in the screening process including Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Inoculums of each bacterial strain were suspended in 5 mL of nutrient broth and incubated overnight at 37 °C. The overnight cultures were diluted 1/5 with nutrient broth before use. Bacteria were supplied by the Nepal Public Health Laboratory, Tripureshwor, Kathmandu, Nepal.

Antibacterial assay

The antibacterial assay was studied by agar well diffusion method and the activities of the extracts were compared with controls. Standard paper discs containing erythromycin/chloramphenicol/ampicilline were used for positive controls. Standard filter paper discs were prepared by saturating with methanol/dichloromethane/hexane (known to have a saturation point of 20 µL) for negative control. Tests were based on procedures followed by (Andrews 2005) ^[16], with slight modification.

The overnight diluted culture was used to inoculate the nutrient agar test plate. The test plates were inoculated with the appropriate bacterial overnight culture on a sterile cotton swab. Once inoculated, wells of 6 mm diameter were punched in the agar medium and plant extracts dissolved in the respective solvents (20 µL) were added to each well and controls were added. Plates were incubated for 24 hours at 37 °C and the diameter of the inhibition zone around the test wells or around the control discs were measured to assess antibacterial activity. All the tests were

Table1: Traditional uses of ethnomedicinal plants in Nepal

Plant Scientific name	Plant Part	Traditional uses
<i>Anaphalis nepalensis</i> (Spreng.) Hand.-Mazz.	Whole plant	Asthma, liver diseases, joint pain, prevent skin from sun burning, aromatic, headache, dizziness, tobacco substitute ^[17]
<i>Piptanthus nepalensis</i> (Hook.) D. Don	Leaf, Stem, Flower	Gastritis ^[18] , invertebrate poison, toxic, medicinal
<i>Senecio raphanifolius</i> Wall. ex DC.	Leaf, Stem, Flower	Medicinal
<i>Thermopsis barbata</i> Benth.	Whole plant	Medicinal, pain killer, reduce any kind of swelling ^[19] , cuts, wounds, dog bites, digestive, tonic ^[20-21] , fever, to throw out any types of ingested stomach poison through vomiting
<i>Thermopsis inflata</i> Cambess.	Whole plant	Medicinal, pain killer, reduce any kind of swelling ^[19] , fever, to throw out any types of ingested stomach poison through vomiting

replicated three times to insure the reliability of the results.

RESULTS AND DISCUSSION

For this research, *in-vitro* antibacterial assays of 15 extracts from five ethnomedicinal plants belongs to four genera (Anaphalis, Piptanthus, Senecio and Thermopsis) and two families (Asteraceae, Fabaceae) which were used to treat diseases potentially caused by bacteria were examined. The traditional usage of plants obtained from the field visit by interviewing the healers, knowledgeable villagers and also the uses from the literatures is compiled in table 1.

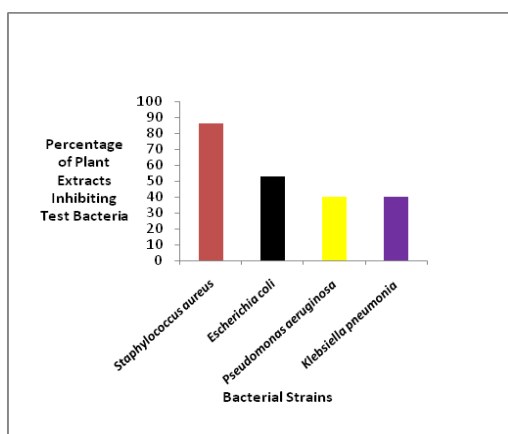


Figure 1: Activities of medicinal plant extracts against the bacterial strains tested during *in-vitro* screening of medicinal plants of Sagarmatha

Among 15 extracts examined, 13 (86 %) extracts showed antibacterial property against *Staphylococcus aureus* followed by 8 (53 %) extracts against *Escherichia coli* and 6 (40 %) extracts each against *Pseudomonas aeruginosa*

and *Klebsiella pneumonia* (figure1 & table 2).

The present study was undertaken to determine the therapeutic properties of some plants used in traditional medicine. The results showed that the antibacterial activity of the extracts varied according to the species of bacteria tested^[22] (table 2).

Plant extracts were more likely to inhibit Gram-positive bacteria, *Staphylococcus aureus*. With respect to Gram-negative bacteria, it was more common for plant extracts to inhibit *Escherichia coli* than *Pseudomonas aeruginosa* or *Klebsiella pneumonia*. Overall, 86 % of plant extracts showed activity against Gram-positive test bacteria, and 45 % showed activity against Gram-negative test

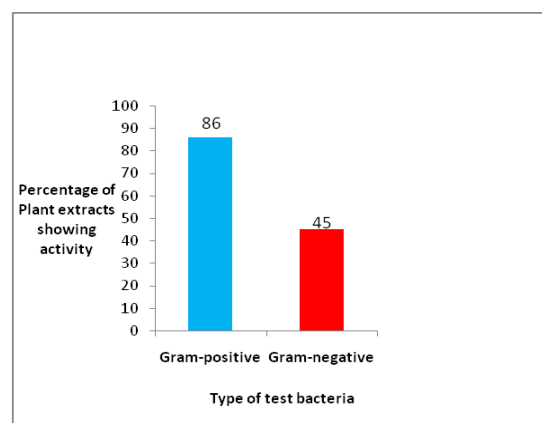


Figure 2: Percentage of plant extracts showing inhibitory activity against Gram-positive and Gram-negative bacteria

bacteria (figure 2).

There may be several reasons for the inactivity of the plant species against the test bacteria. Some

Table 2: Activity of medicinal plant species with methanol, dichloromethane and hexane extracts against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa*

Plant scientific name	Staphylococcus aureus			Klebsiella pneumonia			Escherichia coli			Pseudomonas aeruginosa		
	M	D	H	M	D	H	M	D	H	M	D	H
Anaphalis nepalensis (Spreng.) Hand.-Mazz.	+	+	+	-	-	+	-	-	-	-	+	-
Piptanthus nepalensis (Hook.) D. Don	+	+	+	-	-	-	+	+	-	-	-	-
Senecio raphanifolius Wall. ex DC.	+	-	-	-	-	-	-	-	-	-	+	-
Thermopsis barbata Benth.	+	+	+	+	+	+	+	+	+	-	+	+
Thermopsis inflata Cambess.	+	+	+	-	+	+	+	+	+	+	-	+
Positive control:												
Erythromycin/Chloramphenicol/Ampicilline	+	+	+	+	+	+	+	+	+	+	+	+
Negative control: MeOH/CH ₂ Cl ₂ /hexane	-	-	-	-	-	-	-	-	-	-	-	-

Key: '-' indicated no zone of inhibition; '+' indicated zone of inhibition; Solvent used for extraction: M: methanol; D: dichloromethane; H: hexane; All solvent controls (MeOH, CH₂Cl₂ and hexane) were negative, producing no zone of inhibition. Positive controls were erythromycin/chloramphenicol/ampicilline for Gram-positive and Gram-negative bacteria

of the possible reasons may be: the tested plant species may not contain antibacterial compounds, or the plants may have other medicinal uses, such as analgesia and others. Otherwise, the lack of activity may be because of degradation of active chemicals during the drying process and the extraction process. In this screening, we use a small number of microorganisms (only one Gram-positive bacteria) for testing. It is possible that these plants contain antibacterial compounds against pathogenic bacteria other than those tested^[23].

In general, the activity of more plant extracts against Gram-positive bacteria compared to Gram-negative was not a astonish result because earlier learnings showed that larger number of extracts were active against Gram-positive bacteria than Gram-negative bacteria^[22-23]. This is possibly explained by the more complex cell wall/membrane structure of Gram-negative bacteria.

CONCLUSION

In recent years, antibacterial activities of ethnomedicinal plants are increasingly being reported. The extraction with MeOH, CH₂Cl₂ and hexane crudely separated the components into groups of varying polarity. The activity of these extracts gives insight into the chemical nature of the biologically active constituents^[23]. It is hoped that this study will promote the concerned authority to search for alternative, inexpensive and simply available antibacterial of plant origin.

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