

Antibacterial activities of medicinal plants of Nepal

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Background: Medicinal plants of Nepal are still remaining to be assessed for their antimicrobial properties, which may lead to discovery of broad-spectrum antimicrobial compounds.

Methods: Various parts of medicinal plants, viz. *Acorus calamus*, *Curcuma longa*, *Emblica officinalis*, *Glycyrrhiza glabra* (a non-indigenous to Nepal), *Justicia adhatoda* and *Xanthoxylum armatum*, were collected from hilly regions of Nepal. The plant parts were chopped into fine pieces, air-dried at 37°C for several hours, surface sterilized and grinded to powder. The antibacterial compounds were extracted by Soxhlet Reflux method and tested against common bacterial pathogens by agar well diffusion test.

Result: All the crude extracts were found to be effective against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Among tested ones *E. officinalis* was found to be the best antibacterial plant. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values of the crude extract of *E. officinalis* were found to be 8mg/ml and 16mg/ml respectively. Similarly, MIC value of *A. calamus* was 128mg/ml.

Conclusion: Being highly effective against both Gram-positive and Gram-negative common bacterial pathogens, the antibacterial compounds from the plants can be exploited to commercial values provided in vivo assessment of the compounds are studied.

Keywords: Antibacterial agents; medicinal plants; minimum inhibitory concentration; Nepal

Introduction

Since the dawn of human civilization, various plants are being used as traditional medicine. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because, better compatibility with the human body and fewer side effects.¹ The complex chemical compounds found in plants aid the synergistic effect of healing process. Proved are the medicinal uses of plants curing several ailments from infectious diseases like malaria, tuberculosis to cancer. But with the rising incidences of multidrug resistant diseases and established side effects of some common drugs, it has

accentuated the importance of quest for alternatives in medicine.

Nepal has rich plant diversity with thousands of species possessing medicinal values. The presence of extreme ranges of altitude, climate and soil within the small geographical area has seeded natural diversity in vegetation and flora. It is estimated that about 7000 species of flowering plants exist in Nepal and 5% of flowering plants are endemic. Flowering plants are common sources of herbal medicines.^{2,3} In recent years, a large number of plant products have been investigated for their antimicrobial properties against bacteria and fungi. There is vast diversity

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among medicinal plants. Different chemotypes of the same species may grow in the same place and produce different oils with different activity. The current work presents an evaluation of antibacterial activity of *Acorus calamus*, *Curcuma longa*, *Embllica officinalis*, *Glycyrrhiza glabra*, *Justicia adhatoda* and *Xanthoxylum armatum* of hilly regions of Nepal against some common bacterial pathogens.

Materials and Methods

Materials: The plant samples were collected from different sites of Dhading and Gulmi districts of Nepal, and processed at Microbiology Laboratory, Lord Buddha Education Foundation, Maitidevi, Kathmandu. The taxonomic identification of plant materials was confirmed at the National Herbarium and Plant Laboratories, Godawari, Lalitpur. The test bacteria were obtained from the same laboratory. The medicinal plants were air dried separately and ground to fine powder by using Mortar and Pistol. The plating media viz. nutrient agar, Nutrient broth, Muller Hinton agar used in this study were purchased commercially from local suppliers as dehydrated media of Hi-Media Laboratories Limited, India. Other chemicals and reagents used were analytical grade.

Extraction of antibacterial compounds in methanol: Thirty gram dried powder of each medicinal plants was wrapped with filter paper and loaded into a clean and dried thimble of Soxhlet-Reflux extractor⁴, followed by heating at 70°C until colored solvent disappeared from the cylinder containing plants powder. Further, concentration of the extract was done after detaching the soxhlet assembly by heating in the water bath set at 37°C. Then the extracts were made solution at a concentration of 0.5mg/ml. The extracts were labeled and kept in the refrigerator at 4°C until used.

Removing solvent: The flask containing extract was set with condenser and heated with water bath. Solvent was completely removed and collected in a separate round bottom flask. The crude extract was then transferred in sterile tubes, labeled and kept in a refrigerator at 4°C.

Agar Well Diffusion Method⁵: Sterile Mueller Hinton Agar (pH 7.3 ± 0.1) plates were used for the sensitivity testing. The test cultures were incubated at 37°C for a few hours to obtain turbidity equivalent to 0.5 McFarland's standard and swabbed onto the MHA plate. After 10 minutes, wells were prepared with a sterile cork borer (diameter, 6mm) in a distance of 15mm from each other. Various concentrations (1X, 0.5X and 0.25X) of the extracts in 0.1M phosphate buffer of pH 7 (100µl each) were dispensed aseptically using a micropipette. The plates were allowed to stand at a room temperature for 30 minutes and incubated in upright position

at 37°C for 24 hours. The diameters of the zone of inhibition (ZOI) were measured with a ruler. All test systems included respective controls.

Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC):

Stock solutions of the crude extract were prepared to 0.5mg/ml each. Corresponding crude extract was serially diluted in sterile nutrient broth (0.5gm/l Peptone and 0.3gm/l Yeast extract in each tube). Serial dilution was done in 5ml broth followed by subsequent transfer of 2.5ml into next tube in the series. Standard inoculum (0.5 McFarland Standard) was prepared and 100µl of the culture was inoculated into each tube to challenge about 5X10⁵ CFU/ml of test bacteria and incubated for overnight at 37°C.⁵ The tubes were observed for growth. For the assessment of growth finely, 50µl of the antimicrobial treated culture broth was subcultured onto fresh nutrient agar plates and the results were interpreted.

Result

All extracts were effective against the test bacteria, viz. *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (Fig. 1–5). *E. officinalis*, *A. calamus*, and *G. glabra* were most effective among the plant extracts. MIC and MBC values of the crude extract of *E. officinalis* were found to be 8mg/ml and 16mg/ml respectively (Table 1). Similarly, MIC value of *A. calamus* was 128mg/ml (Table 2).

Table 1: Determination of MIC and MBC of *E. officinalis* crude extract (5mg/ml) against *S. typhi*^a

Tube No.	Average concentration of the antimicrobial agent (mg/ml)	Relative growth on nutrient agar plate ^b	Inference
T1	128	-	
T2	64	-	
T3	32	-	
T4	16	-	MBC
T5	8	+	MIC
T6	4	2+	
T7	2	4+	
T8	0	4+	+ Control
T9	0	-	- Control

^a = 100 µl of inoculum (0.5 McFarland Standard) in each broth tube containing extract except control (T9).

^b = Growth after inoculation of 50µl of inoculum.

Table.2: Determination of MIC of *A. calamus* crude extract (5mg/ml) against *S. typhi*^a

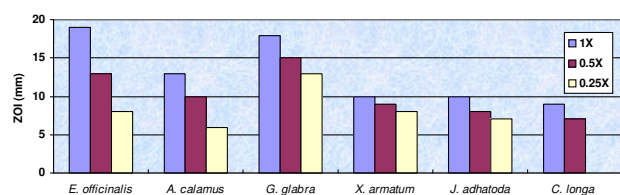
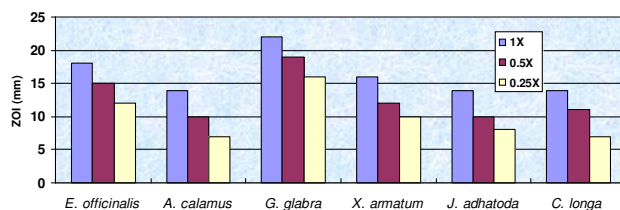
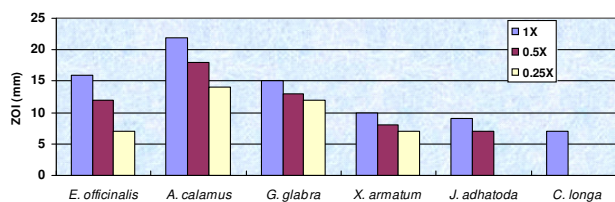
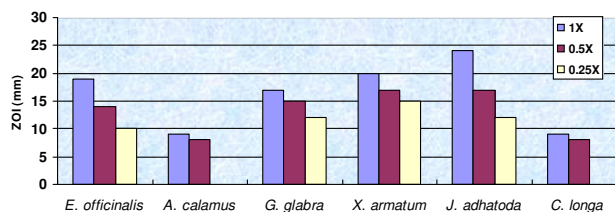
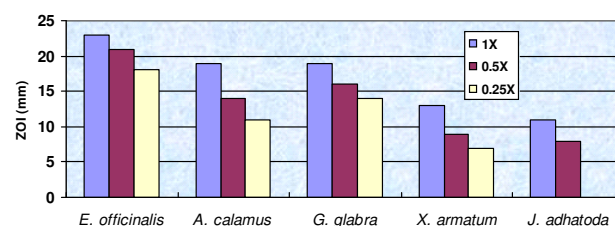
Tube No.	Average concentration Of the antimicrobial agent (mg/ml)	Relative growth on nutrient agar plate ^b	Inference
T1	128	+	MIC
T2	64	2+	
T3	32	3+	
T4	16	4+	
T5	0	4+	
T6	0	-	+ Control
			- Control

^a = 100 µl of inoculum (0.5 McFarland Standard) in each broth tube containing extract except control (T6).

^b = growth after inoculation of 50µl of inoculum.

Discussion

The widespread use of herbal remedies and healthcare preparations has been traced to the occurrence of natural products with medicinal properties. Plant materials remain an important resource to combat serious diseases in the world. Pharmacognostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents. Among the more than 250,000 species of higher plants, only about 5–10% are chemically investigated.⁶ Long before mankind discovered the existence of microbes the idea that certain plants had healing potential was well accepted.⁷ Since antiquity, man has used plants to

**Fig 1:** Effects of different concentrations of plant extracts against *E. coli***Fig 2:** Effects of different concentrations of plant extracts against *S. typhi***Fig 3:** Effects of different concentrations of plant extracts against *P. aeruginosa***Fig 4:** Effects of different concentrations of plant extracts against *K. pneumoniae***Fig 5:** Effects of different concentrations of plant extracts against *S. aureus*

treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies.^{8,9} Over 50% of all modern clinical drugs are of natural product origin¹⁰ and natural products play an important role in drug development among pharmaceutical industries¹¹. These days, herbal medicines are being popular due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new of bioactive compounds.⁹ Hence, plants are the basic source of knowledge of modern medicine.

Various plants were selected on the basis of their well-known medicinal values in local communities. Different parts of plants were used to study antimicrobial activity against common bacterial pathogens. Methanol was used to extract antibacterial agents as it has low boiling point and most of the organic and inorganic chemicals dissolve in methanol. Methanol in the extract was removed subsequently by distillation. As the extraction process involved treatment of

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the plant materials at 70°C, the antibacterials seemed to be relatively thermoresistant. This property may be valuable in commercialization of efficient extraction process.

Pokhrel¹² studied antibacterial properties of *Rhododendron arboretum* (Laliguras), collected from Himalayan region of Nepal, and found to possess selective toxicity against Gram-negative bacteria (GNB) but Gram-positive bacteria (GPB). Similar results were also reported independently by Caceres *et al.*¹³ and Sindambiwe *et al.*¹⁴. Pepeljnjak *et al.*¹⁵ determined antimicrobial activity of ethanolic extract of the *Satureja montana montana* against 11 species of bacteria and five species of fungi and found the strongest activity against Gram-positive bacteria. However, the compounds extracted from plants of hilly region of Nepal were found to be highly effective against both GNB (Fig 6) and GPB (Fig 7), indicating their broader spectra. The test bacteria included most common enterobacteria and pseudomonads. Alongwith staphylococci, these pathogens are the major burden in these days.

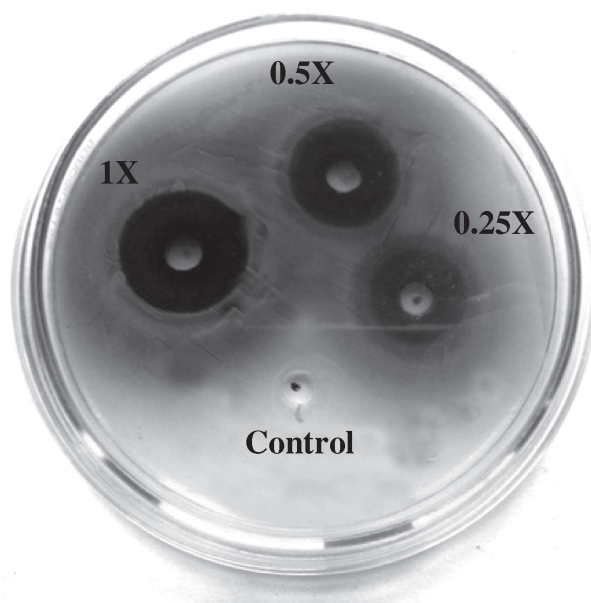


Fig 6: Antibacterial activity of *E. officinalis* against *P. aeruginosa*

The crude extracts from *E. officinalis* and *A. calamus* were further processed for their MIC and MBC values against *S. typhi* (typical GNB) and found to comparatively low than that of *R. arboretum* as reported by Pokhrel¹² against *S. aureus* (typical GPB). The finding indicates that the plants studied in this study are better sources of antibacterial compounds. The concentrations of the plants extracts were very comparable to the findings made by Samie *et al.*⁹

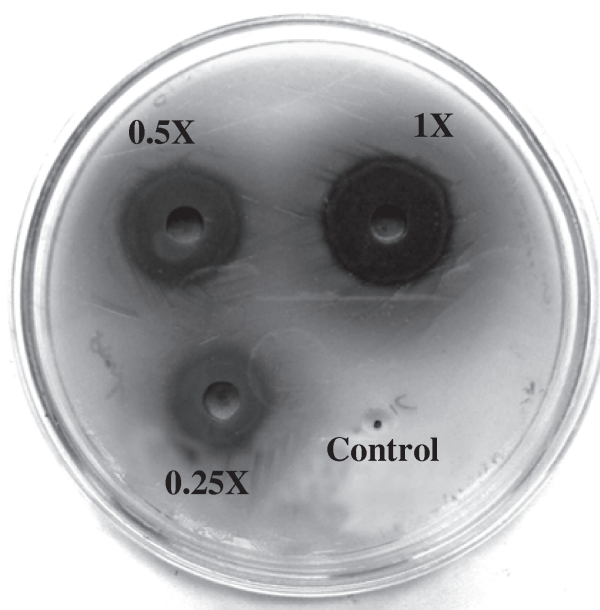


Fig 7: Antibacterial activity of *X. armatum* against *S. aureus*

Conclusion

Being highly effective against both Gram-positive and Gram-negative common bacterial pathogens, the antibacterial compounds from the plants can be exploited to commercial values. The fact that the plant extracts exhibits activity is of interest, but it is only a preliminary piece of data and should be followed by the identification of the active compounds by means of a bio-guided assay. Research should be kept up in order to uncover toxicity against animal or human cells, mechanisms of action, effects in vivo, positive and negative interactions with common antibiotics and so forth.

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