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Research Article

ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS FROM EAST AND CENTRAL PART OF NEPAL

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Abstract

To evaluate the antimicrobial activities of extract of eleven plants were examined against four common bacterials. The ethanolic extracts of various plants such as *Cissus repens*, *Hedyotis scandens*, *Jatropha curcas*, *Morus alba*, *Inula cappa*, *Equisetum ramosissimum*, *Osyris wightiana*, *Alternantheria sessilis* and *Hibiscus lampas* investigated individually for antimicrobial activity by disc diffusion method. These were investigated against selected species of *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae* to find the inhibitory activities of the microbes. The ethanolic extract of *C. repens* showed considerably high activity against *P. vulgaris*, *E. coli* and *S. aureus* than other extracts.

Keywords: antimicrobial; medicinal plants; zone of inhibition; multi- drug resistant

Introduction

In recent years, the number of multi –drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics is continuously increasing in alarming rate. The reason is due to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill.1988; Ng. 1994; Dean and Burchard. 1996; Gonzalez *et al.*, 1996). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. In this scenario, natural products from plants could be interesting alternatives (Lu *et al.*, 2007; Mbwambo *et al.*, 2007). Some plant extracts and photochemical are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine (Nascimento *et al.*, 2000; Rios and Recio. 2005). For over thousands of years now, natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents (Iwe *et al.*, 1999).

In this context, Nepal can be considered as a natural storehouse of medicinal plants as it is rich in biodiversity due to its geographical features. It has many plants with medicinal and aromatic values. Some of them are used in traditional medicine and some are still not explored scientifically for their medicinal values. Screening of plants for their antibicrobial activities is important for finding potential new compounds for therapeutic use. The present study was undertaken to investigate the antimicrobial properties of ethanolic extracts of *Cissus repens*, *Hedyotis scandens*, *Jatropha curcas*, *Morus alba*, *Inula cappa*, *Equisetum ramosissimum*, *Osyris wightiana*, *Alternantheria sessilis* and *Hibiscus lampas* against four bacteria (*Staphylococcus aureus* , *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*). Literature survey of these plants revealed not much information about their medicinal values; however some of these plants are used in folk medicine for the treatment of general aliments as mention in Table 1.

Experimental

Collection and processing of Plants

In this study medicinal plants were selected on the basis of their medicinal importance in literature and to people, especially in the Tanahun and Dhankuta districts of Nepal. The ethnobotanical data (Local name and medicinal uses) were collected through questionnaire, interviews and discussions among the traditional practitioners. All plants were authenticated by National Herbarium and Plant Laboratories, Godawari, Nepal.

Table 1: List of plants screened

Family	Scientific name	Local name	Traditional uses
Ericaceae	*Lyonia ovalifolia*	Angeri	Crushed leaves are used in Scabies.
Moraceae	*Morus alba*	Kambu	Bark of the plant is used as astringent, carminative and antiseptic. Decoction of bark is useful in asthma, lungs infection, chronic bronchitis, diarrhea and dysentery.
Compositae	*Inula cappa*	Tihare phool	No use reported.
Equisetaceae	*Equisetum rammossium*	Chasme jhar	Whole plant is used as cooling medicine and is given in gonorrhea.
Santalaceae	*Osyris wightiana*	Nundhiki	Infusion of bark is given to women after delivery to stop bleeding. Infusion of leaves is emetic. Leaves are used as tea.
Euphorbiaceae	*Jatropha curcas*	Sajiban	Plant juice is a well-known purgative and used in whitlow, convulsion, syphilis, neuralgia, dropsy, anasarca and pneumonia. Leaves are galactogogue, rubefacient, insecticidal and are used in tumors and scabies.
Polypodiaceae	*Aglaomorpha coronans*	Kamaru	Roots are used against diarrhea, constipation.
Vitaceae	*Cissus repens*	Jogilahara	Plant is used in boils and abscesses or maturation. It is applied in sloughing and foetid ulcers.
Amaranthaceae	*Alternanthera sessilis*	Bhiringe jhar	Plant is astringent, cooling, constipating, depurative, digestive, galactogouge, cholagouge, and febrifuge. Root juice is effective against blood dysentery.
Malvaceae	*Hibiscus lampas*	Bankapas	Root and fruits are used in gonorrhea and syphilis. Decoction of roots is taken in case of jaundice. Root paste is applied on boils.
Rubiaceae	*Hedyotis scandens*	Dudhelahara	Plant is used in eye diseases and in troubles following child birth. Root is used for sprain. Root paste is used in indigestion and plant paste in peptic ulcer.

Leaves of *L. ovalifolia* (from Dhankuta), root of *M. alba, I. cappa, H. lampas, J. curcas and O. wightiana* , Whole plant of *A. sessilis* and *E. ramosissium* , stem of *H. scandens* and *C. repens* and rhizome of *A. coronans* (from Tanahun) were collected during the winter of 2011, dried in shadow, and then powdered.

Extraction

Extraction was carried out by soaking 150 g of dried powdered samples in about 600 mL of ethanol (analar grade) for 3 days. The extracts were filtered first through cotton wool, then through Whatman filter paper no. 42 (125 mm).The collected extract was dried using a rotary evaporator.

Antibacterial Screening

Inhibition of bacterial growth was tested by using the paper disc diffusion method with slight modification (Bauer *et al.*, 1966).

Micro organism

The microorganisms used in this study were identified strains obtained from Central Department of Microbiology, T.U. Among bacteria taken in this study, one was gram-positive and three were gram-negative as given below.

Gram positive bacteria: *Staphylococcus aureus*

Gram negative bacteria: *Escherichia coli, Proteus vulgaris* and *Klebsiella pneumoniae*

Antimicrobial activity

The anti-microbial activity of the plant extracts were carried by disc diffusion method (Bauer *et al.*, 1966). A suspension of tested microorganisms was spread on Muller-Hilton Agar (MHA) medium. The sterile filter paper discs (6 mm in diameter) were individually impregnated with different concentration of plant extract prepared in ethanol and then placed into the agar plates which had previously been inoculated with the tested microorganisms. The plates were subsequently incubated overnight at 37 ^{o}C. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. For control ethanol discs were used. All tests were performed in triplicate.

Results and Discussion

The paper describes the antimicrobial screening of eleven indigenous plants of Nepal. The effects of two different concentrations of eleven plant extracts on the growth of test microorganisms by the paper disc diffusion method were

observed. The results of antimicrobial screening are show in Table 2.

All the extracts exhibited activity against at least one organism tested (Fig.1) Among 11 extracts examined, 10 (90%) extracts showed antibacterial property with *S. aureus* and *P. vulgaris* and 11 *(*100%) extracts with *K. pneumoniae* and *E.coli.* Among these plants the strongest antimicrobial activity was obtained for extract of *C. repens* (20 mm) followed by *A. sessilis* (18 mm) and *H. lampas* (18 mm) against *P. vulgaris. C. repens* showed the highest activity at both the concentration against *P. vulgaris*. It has been reported as the highly potent ethnomedicinal plant against malaria, high blood pressure and diabetes (Neli lokho *et al.*, 2005). Former studies of the methanol extract of *C. repens* revealed antimicrobial activity against *E.coli, Pseudomonas aeruginosa, S. aureus* and *Bacillus subtilis* (Bhatarai *et al.*, 2009b).

The strong antimicrobial activities was obtained for extract *H. scandens* (16 mm) followed by *C. repens* (16 mm) and *A. sessilis* (12 mm) against *K. pneumoniae* in concentration dependent manner. Former studies of the ethanolic extract of *A. sessilis* also revealed antimicrobial activity against *E. coli* but no activity was observed against *K. pneumoniae*. Saravanakumar and Venkateshwaran found that the methanolic extract of the flowers of *H. lampas* is active against *S. flexneri, Rhodococcus terrae, E. coli, S. faecalis and K .pneumoniae* (Sarawanakumar *et al.*, 2009).

S. aureus was found more susceptible towards the extract of *C. repens* (13 mm) followed by *H. scandens* (13 mm). Previous study found that the ethanolic extract of the *H. scandens* plant have maximum activity against *Sarcina lutea, K. pneumoniae and S. aureus* (Tamanna and Rashid *et al.*, 2010). The present study also correlates with the result.

Similarly *E.coli* was found more susceptible towards the extract of *C. repens* (13 mm) followed *I. cappa* (12 mm). Previous study found that extract of *I. cappa* shows activity against *S. aureus* and *S. paratyphi* (Liu ***et al.,*** 2010). The result shows ethanolic extract of the root of *J. curcus* is effective against all the tested bacteria. Also Igbinosa *et al.*, found that the ethanolic extract of the stem bark of *J. curcus* showed broad spectrum activity against *S. aureus, P. aeruginosa, E.coli, P. vulgaris, B. cereus and S. faecalis but* ineffective against *K. pneumoniae* (Igbinosa *et al.*, 2009

Table 2: Antimicrobial activity of ethanol extracts of the medicinal plants.

Test organisms		Zone of inhibition in mm							
		S. aureus		*K. pneumoniae*		*P. vulgaris*		*E.coli*	
Plants	Concentration	0.5 mg/mL	1 mg/mL	0.5 mg/mL	1 mg/mL	0.5 mg/mL	1 mg/mL	0.5 mg/mL	1 mg/mL
Cissus repens		11	17	9	16	20	20	10	13
Alternanthera sessilis		9	10	9	12	17	18	8	8
Hibiscus lampas		8	9	8	8	10	18	8	8
Hedyotis scandens		10	13	9	16	9	13	8	9
Jatropha curcas		9	9	14	14	9	9	8	8
Inula cappa		10	10	13	14	9	13	10	12
Osyris wightiana		8	10	10	10	8	12	10	11
Lyonia ovalifolia		10	11	8	10	8	11	8	11
Equisetum ramosissium		8	10	9	9	8	10	8	11
Morus alba		10	10	9	10	9	9	7	10
Aglaomorpha coronans		none	none	8	10	none	none	8	8

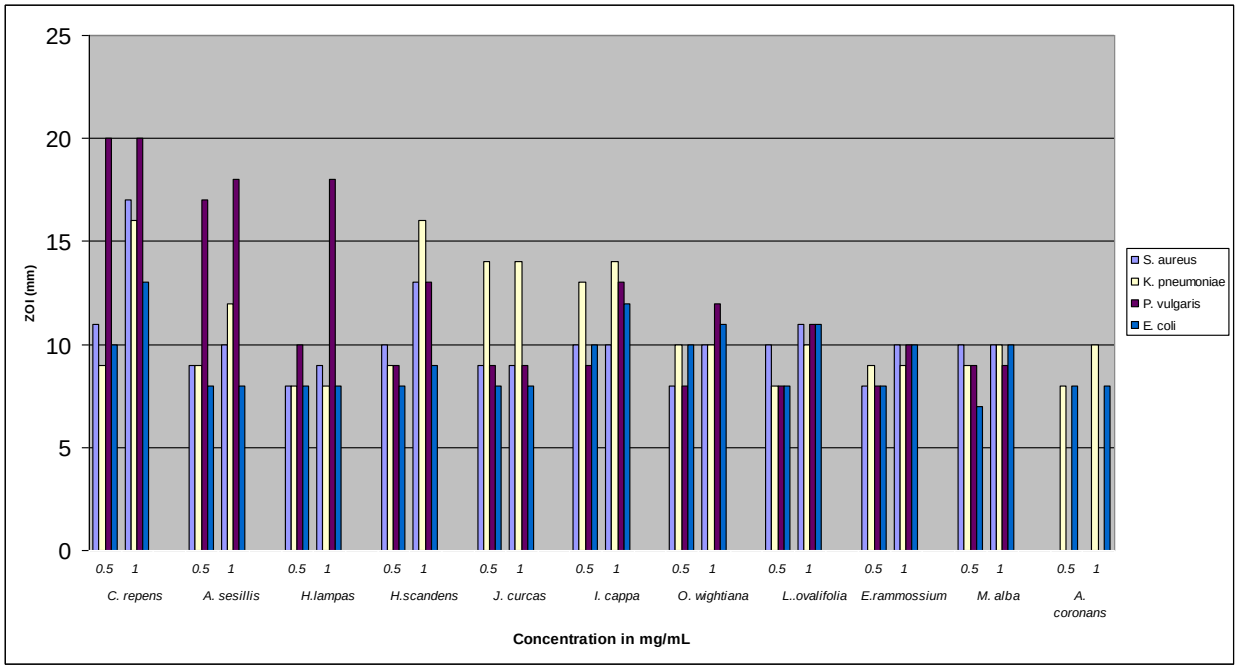

Fig 1: Plot of antibacterial activity of the ethanol extract of medicinal plants against various bacteria.

M. alba shows good activity against all the tested bacteria with maximum against *S. aureus.* This extract showed the higher ZOI with *S. aureus, K. pneumoniae* and *E. coli* (10 mm) whereas at lower concentration shows low activity against *E.coli* only. This result was well supported (Moorthy *et al.*, 2011).

Lyonia ovalifolia, Equisetum ramosissium, Osyris wightiana showed the comparatively moderate ZOI ranges from 8 mm to 11 mm against all the tested bacteria. Former studies of (Negi and Singh *et al.*, 2011) the ethanolic extract of the leaves of *L. ovalifolia* revealed antimicrobial activity against *S. aureus, S. epidermis, K. pneumoniae.* On the other hand, it was found that *L. ovalifolia* show negative effect against *E. coli* and positive against *P. aeruginosa* and *shigela boydii* (Panthi and Chaudhary 2006). The present study showed that the ethanolic extract of the leaves of *Lyonia* show broad spectrum activity against all the tested bacteria but against *E. coli* it is rather less active.

The antibacterial study of *O. wightiana* and *A. coronans* in the past has not been reported. In the present study *A. coronans* show the least activity. It did not show any activity against *S. aureus* and *P. vulgaris.* It shows little effect against *K. pneumonia* (10 mm) and *E.coli* (8 mm).

Overall, the finding results here are quite interesting as the result show that gram-negative bacteria were more sensitive than gram positive bacteria. It has been reported that greater numbers of extracts were active against Gram-positive bacteria than Gram-negative bacteria (Bhattarai *et al.*, 2008a). This is because of difference in cell wall of Gram-positive and Gram-negative bacteria. The antimicrobial activities of the extracts of the plants varied in relation to the test organisms. The activity against Gram-negative bacteria was surprise finding. These finding results are very interesting as the microorganism *E.coli,* which is already known to be multi-resistant to drugs, was also resistant to the plant extracts tested.

Conclusion

The results of this research are encouraging, as most of the selected plants appeared to contain antibacterial substances. These plants could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity. This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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References

Bauer AW, Kirby MDK, Sherris JC and Turck M (1966) Antibiotic susceptibility testing by standard single disc diffusion method. *Am. J. Clin.Pathol.***45:**493- 496.

Bhattarai S, Chaudhary RP and Taylor RSL (2008a) Antibacterial activity of selected ethnomedicinal plants of Manang district, central Nepal. *Journal of Theoretical and Experimental Biology.* **5**(1 & 2): 1-9.

Bhattarai S, Chaudhary RP, Taylor RSL and Ghimire SK (2009b) Biological Activities of Some Nepalese Medicinal Plants Used in Treating Bacterial Infections in Human Beings. *Nepal J. of Sci. Technol.* **10**:83-90.

Dean DA and Burchard KW (1996) Fungal infection in surgical patients. *Am J Surg.* **171**: 374-382. DOI: 10.1016/S0002-9610(97)89647-X

Gonzalez CE, Venzon D, Lee S, Mueller BU, Pizzo PA and Walsh TJ (1996) Risk factors for fungemia in children infected with human immunodeficiency virus: a case-control study. *Clin Infect Dis*. **23:** 515-521. DOI: 10.1093/clinids/23.3.515

Graybill JR (1988) Systemic fungal infections: diagnosis and treatment. I. Therapeutic agents. *Infect Dis Clin North Am***. 7:** 805-825.

Igbinosa OO, Igbinosa EO and Aiyegoro OA (2009) Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr. J. of Pharmacy and Pharmacology.* **3**: 058-062.

Iwu MW, Duncan AR and Okunji CO (1999) New antimicrobials of plant origin. In: Janick J, editor. Perspectives on new crops and new uses. Alexandria, VA: ASHS Press 457–462.

Liu S. *et al.* (2010) Antimicrobial Dynamics of *Inula cappa* Extracts, *J. of Anhui Agricultural Science.*26.

Lu Y, Zhao YP, Wang ZC, Chen SY and Fu CX (2007) Composition and Antimicrobial Activity of the Essential Oil of *Actinidia macrosperma* from China. *Nat. Prod.Res.* **21:** 227-233. DOI: 10.1080/14786410601132311

Mbwambo ZH, Moshi MJ, Masimba PJ, Kapingu MC and Nondo RS (2007) Antimicrobial Activity and Brine Shrimp Toxicity of Extracts of Terminalia brownii Roots and Stem. *BMC Complem Altern M*. **7: 1**-5. DOI: 10.1186/1472-6882-7-9

Moorthy V and Boominathan M (2011) Comparative antimicrobial activities of *Morus alba* crude extract and fraction against *S. aureus. Intl. J. of Institutional Pharm. and Life Sciences* 1(2).

Nascimento Gislene GF, Juliana L, Paulo CF and Giuliana LS (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria**.** *Braz. J. Microbiol.* **31**: 247 – 256.

Negi R, Negi YK, Uniyal V, Saxena and Bisht S (2012) Phytochemical Analysis and Antibacterial Activity of Three Indigenous Plants of Garhwal Himalaya against some Pathogenic Microorganisms. *J. of Pharm. Res*, **5**: 1583-1586.

Neli LP and Ngolo CD (2005) *Dicentra scandens*- A highly potent ethnomedicinal plant against malaria, high blood pressure and diabetes. *Indian J. of Tradl. Knowledge.* **5**(2): 268-270.

Ng PC (1994) Systemic fungal infections in neonates. *Arch Dis of Childhood*. **71:** F130-F135. DOI: 10.1136/fn.71.2.F130

Panthi MP and Chaudhary RP (2006) Antimicrobial activity of some selected folklore medicinal plants from west Nepal. *Scientific World*. **4**(4): 16-21.

Rios JL and Recio MC (2005) Medicinal plants and antimicrobial activity. *J. Ethnopharmacol*. **100:** 80-84. DOI: 10.1016/j.jep.2005.04.025

Sarawanakumar A, Venkateshwaran K, Vanitha J , Ganesh M (2009) Evaluation of antibacterial activity, phenol and flavonoid contents of thespesia populnea flower extracts.*Pak. J. Pharm. Sci.* **22** (3): 82-286.