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Biological Activities of Some Nepalese Medicinal Plants Used in Treating Bacterial Infections in Human Beings

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Abstract

In continuation of Nepalese medicinal plant screening research, we have examined the antibacterial property of 40 methanol crude extracts *in vitro* using disc diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Overall, 95 % of the extracts showed antibacterial activity against at least one Gram-negative bacteria and 90 % of the extracts against at least one Gram-positive bacteria examined. Twenty-one extracts including *Artemisia caruifolia*, *Dicranostigma lactucoides*, *Rauvolfia serpentina*, and other plants showed antibacterial activity with all the tested bacterial species. The laboratory results are promising because several plants showed broad-spectrum antibacterial property. It is hoped that this scientific research will aware the related partners in Nepal to search for safe, efficacious, cheap alternative antibacterials of plant origin and may create an opportunity for biological conservation of the medicinal plants and cultural diversity conservation.

Key words: antibacterial activity, *in vitro*, ethnomedicinal plants

Introduction

Approximately 85,000 plant species world-wide are reported as being medicinally useful (Liu & Wang 2008). Medicinal plants are a highly valued resource - the natural plant product and organic sector is considered as the fastest growing in the entire agribusiness industry (Makunga *et al*. 2008). Historically, the development of novel drugs was primarily through the extraction of biologically active compounds from plants which were identified through medicinal use or a variety of bioactivity screening programs (Hunter 2001). Therefore this research aims to examine the *in vitro* antibacterial activity of some selected ethnomedicinal plants from the Nepal Himalaya and Tarai in order to identify potential antibacterials.

Many studies have investigated traditional uses of medicinal plants, but only a few studies have followed these ethnobotanical investigations with laboratory work to verify the actual antimicrobial property of these plants (Bhattarai *et al*. 2008a, 2008b, Shakya *et al*. 2008, Vaidya *et al*. 2006, Pokhrel *et al*. 2003; Page *et al*. 2003; Sharma *et al*. 2002; Prajuli *et al*. 2001, Taylor & Towers 1998, Taylor *et al*. 2002, 1995, 1996a, 1996b, 1996c, 1996d). The scientific investigation of traditional knowledge of medicinal plants may eventually reveal new medicines, which in turn could be sold and possibly exported. In this way, plant screening research has the potential to provide economic benefit nationwide along with biodiversity conservation.

In the present study, 40 extracts from 38 ethnomedicinal plants used to treat at least one ailment that may be caused by pathogenic bacteria were collected from Manang, Mustang and Nawalparasi districts of Nepal. Plants for antibacterial screening were chosen after consultation with traditional healers and knowledgeable villagers. To screen for biological activity, methanol extracts were prepared and tested against four different bacterial species. The aim of screening was to correlate any antibacterial activity with the indigenous uses/knowledge. This can be seen as the first step in the search for primary health care

products that are socially acceptable and scientifically valuable.

Materials and Methods

Collection of samples with voucher specimens for antibacterial testing

This research project was approved by the Central Department of Botany Research Committee of Tribhuvan University, Kirtipur, Kathmandu, Nepal and University Grant Commission, Nepal. The samples for antibacterial testing were collected during 2004 to 2007. Several field visits to the study area (Manang, Mustang and Nawalparasi districts) was conducted for the collection of samples for antibacterial testing. Samples were collected and selected because of their use among the local people. Only species that were consistently used to treat the same illness by several healers, villagers and traders were selected.

The herbarium specimens were identified with the help of standard literatures (Malla *et al*. 1986, Polunin & Stainton 1984, Stainton 1988, Grierson & Long 1983-2000) and nomenclature of the species by Hara *et al*. (1978, 1982); Hara & Williams (1979) and Press *et al*. (2000). The herbarium of the specimens was prepared following the standard technique (Martin 1995). Voucher herbarium specimens were made and their numbers are listed in Table 1. These vouchers will be deposited at the Tribhuvan University Central Herbarium (TUCH), Nepal, after the first author completes his doctoral research.

Information regarding the ethnomedicinal uses of the plants was gathered by interviewing knowledgeable villagers and local healers (Bhattarai *et al*. 2006). For comparison, literature was searched for previously recorded uses of the same plants.

Extract and disc preparation

Plant materials were air dried and ground in an electric grinder. A 2 g sample of each ground plant material was soaked in 25 ml methanol (MeOH) for a minimum of 24 h. The sample was then suction-filtered through Whatman number 1 filter paper and the residue was again soaked with another 25 ml MeoH for another 24 h. This process was repeated until the extracts became colourless. The filtrates were then dried with the help of an electric table fan. After being taken to complete dryness, the extract was resuspended in 2 ml of MeOH. This gave an extract concentration equivalent to approximately 1g of dried plant material per ml, which varied slightly depending on the plant material. Paper disks of 6 mm were impregnated with that extracts and allowed to dry at room temperature. Positive control disks were prepared by using the following antibiotics: ciprofloxacin, erythromycine, tetracycline (paper disks dipped in 0.25 mg/ml ciprofloxacin, erythromycine, tetracycline, solution with MeOH) and negative control disks with MeOH (paper disks dipped in MeOH).

Microorganisms used

Four different strains of bacteria were used in the screening process: Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. These bacteria were supplied by the Department of Clinical Microbiology, Teaching Hospital, Maharajgunj, Kathmandu and the Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu. Inoculum of each bacterial strain was suspended in 5 ml of nutrient broth and incubated overnight at 37ºC. The overnight cultures were diluted 1/10 with nutrient broth before use.

Antibacterial assay

The bioassay used was the standard disc diffusion assay, adapted from Taylor *et al*. (1995, 2002) to take into consideration the equipment available on site in Nepal. Standard sterile filter paper disks of 6 mm were saturated with plant extracts (known to have a saturation point of 20 µl). Overnight cultures were prepared by suspending 3-4 isolated colonies in 5 ml of nutrient broth and incubating overnight at 37ºC. This overnight culture was used to inoculate the nutrient agar test plates. The test plates were inoculated with the appropriate bacterial overnight culture on a sterile cotton swab. Once inoculated, test disks and controls were added. Plates were incubated upside down for 18-24 h at 37ºC. Results were recorded as presence or absence of zone of inhibition, and testing was repeated three times to confirm and ensure the reliability of results.

Results and Discussion

A total of 40 extracts (2 extracts each from *Ephedra gerardiana* and *Crateva unilocularis*), were examined for antibacterial property against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. Among 40 extracts examined,

30 (75 %) extracts showed antibacterial property with *S. aureus*, 31 (78 %) extracts with *B. subtilis*, 32 (80 %) extracts with *E. coli*, and 33 (83 %) extracts with *P. aeruginosa* (Table 1).

In this study, a total of 21 species including *Artemisia caruifolia*, *Dicranostigma lactucoides*, *Hyoscyamus niger*, *Rauvolfia serpentina*, and other plants showed the broad spectrum of activity (showed antibacterial property with all the tested bacterial species), which verified the antibacterial property of the plant parts used in this study. This study is the preliminary evaluation of antibacterial activity of the plants which demonstrated that several plants have the potential to generate novel metabolites. It is expected that the plants demonstrating broad spectra of activity, may help to discover new chemical antibacterial antibiotics that could serve as selective agents for the maintenance of human health (Srinivasan *et al*. 2001).

Although the nature and number of active antibacterial principles involved in each extract are not clear, but the broad spectra of activity of several plant extracts are promising. Several species showed promising antibacterial property which demonstrates their importance in traditional remedies in remote populations of the study areas where western medicines are not readily available. Antibacterial activity of 40 extracts against two Gram-positive and two Gram-negative bacteria along with their traditional uses were given below in Table 1.

Table 1: Antibacterial activity of some Nepalese medicinal plants against two Gram-positive and two Gram-negative bacteria

SN	Scientific name (Family), Voucher number	Part used	Traditional uses	***S.a.***	***B.s.***	***P.a.***	***E.c.***
1	*Achyranthes aspera* L. (Amaranthaceae), V 2401.	Rt	Diarrhoea, dysentery, stomachache, fever, skin diseases, cholera, boils, astringent, food poisoning, diuretic, hemorrhoids, emetic, hysteria, toothache, stomach troubles, (Muller-Boker 1999, Manandhar 2002; Bhattarai 2007).	-	+	+	+
2	*Aconitum spicatum* (Bruhl) Stapf (Ranunculaceae), V 3457.	Rt	Infected wounds, boils, fever, cuts, to counteract the effects of poison, allergy, edema (Rajbhandari *et al*. 1995, Manandhar 2002).	-	-	-	-
3	*Arnebia benthamii* (Wall. ex G. Don) I.M. Johnst. (Boraginaceae), V 3102.	Wp	Chronic fever, blood fever, lung disease, swelling (Bista & Bista 2005).	+	+	+	+
4	*Artemisia caruifolia* Buch.-Ham. (Asteraceae), V 4003.	Wp	Cough, asthma, cold, fever, allergies of the skin (Manandhar 2002).	+	+	+	+
5	*Artemesia gmelinii* Weber ex Stechm. (Asteraceae), V 4004.	Wp	Fever, cough, cold, sore throat, ear pain, skin wounds, allergies (Bhattarai *et al*. 2006).	+	-	+	+
6	*Artemisia indica* Willd. (Asteraceae), V 4005.	Wp	Diarrhea, dysentery, conjunctivitis, wounds, cuts, cough, sinusitis anthelmintic, abdominal pains, asthma, diuretic, ringworm (Manandhar 2002).	+	+	-	+

7	*Asparagus racemosus* Willd. (Liliaceae), V 2406.	Rt	Dysentery, diarrhoea, gonorrhaea, painful urination, urinary tract infection, diuretic, stomachic, epilepsy, inflammations, eye diseases, throat complaints, worms, (Rajbhandari *et al*. 1995, Muller-Boker 1999, Manandhar 2002, Bhattarai 2007).	+	+	+	+
8	*Azadirachta indica* A. Juss. (Meliaceae), V 2481.	L	Diarrhoea, stomachache, wounds, fever, cough, bleeding from gums, burns, dysentery, antidotal, diuretic, skin diseases, urinary complaints, swelling, hemorrhoids (Rajbhandari *et al*. 1995, Muller-Boker 1999, Manandhar 2002, Bhattarai 2007).	+	-	+	+
9	*Berberis aristata* DC. (Berberidaceae), V 4567.	Br	Diarrhoea, dysentery, edema, eyes diseases, jaundice, skin diseases, malarial fever (Rajbhandari *et al*. 1995, Manandhar 2002, Bhattarai *et al*. 2006).	+	+	+	+
10	*Bistorta affinis* (D. Don) Greene (Polygonaceae), V 3083.	Wp	Cough, tonsillitis, fever, diarrhoea, dysentery, cold (Kletter & Krieckbaum 2001, Bhattarai *et al*. 2006).	+	+	+	+
11	*Caragana gerardiana* Royle (Fabaceae), V 4006.	Fl	Fever	+	-	+	-
12	*Cinnamomum camphora* (L.) J. Presl (Lauraceae), V 2425.	L	Cough, cold, anthelmintic, inflammations, sedative effect in hysteria, (Rajbhandari *et al*. 1995, Manandhar 2002, Bhattarai 2007).	+	+	-	+
13	*Cissampelos pareira* L. (Menispermaceae), V 2435.	Rt & L	Stomachache, diarrhoea, cough, fever, swelling of gums, painful urination, wounds, diuretic, urinary troubles, skin diseases (Rajbhandari *et al*. 1995, Manandhar 2002, Bhattarai 2007).	+	+	+	+
14	*Cissus repens* Lam. (Vitaceae), V 2433.	L & St	Cough, bronchitis (Bhattarai 2007).	+	+	+	+
15	*Crateva unilocularis* Buch.-Ham. (Capparaceae), V 2437.	Br	Sinusitis, stomachache, cuts, wounds, boils, fever, urinary complaints (Manandhar 2002, Bhattarai 2007).	+	-	-	+
16	*Crateva unilocularis* Buch.-Ham. (Capparaceae), V 2437.	L	Sinusitis, stomachache, cuts, wounds, boils, fever, urinary complaints (Manandhar 2002, Bhattarai 2007).	-	+	+	+
17	*Cupressus torulosa* D. Don (Cupressaceae), V 4017.	Sd	Sinusitis, gingivitis	+	+	+	+
18	*Ephedra gerardiana* Wall. ex Stapf (Ephedraceae), V 4008.	Wp	Syphilis, bronchitis, cuts, wounds, respiratory diseases, kidney fever, cough, skin diseases, asthma, hay fever (Rajbhandari *et al*. 1995, Manandhar 2002, Bista & Bista 2005, Bhattarai *et al*. 2006).	+	+	+	+

19	*Ephedra gerardiana* Wall. ex Stapf (Ephedraceae), V 4008.	L (St)	Syphilis, bronchitis, cuts, wounds, skin infections, kidney fever, hay fever, cough, respiratory diseases (Rajbhandari *et al*. 1995, Manandhar 2002; Bista & Bista 2005, Bhattarai *et al*. 2006).	+	+	+	-
20	*Equisetum debile* Roxb. ex Vaucher (Equisetaceae), V 2486.	Wp	Burns, scabies, malarial fever (Manandhar 2002, Bhattarai 2007).	-	+	+	-
21	*Euphorbia royleana* Boiss. (Euphorbiaceae), V 2515.	L & Lt	Diarrhoea, dysentery, wounds inside the ear, ear pain, cuts, fever, boils, cough, stomach disorders, asthma, anthelmintic (Manandhar 2002, Bhattarai 2007).	-	+	+	-
22	*Gentiana robusta* King ex Hook. f. (Gentianaceae), V 170.	Wp	Stomachache, fever, cuts, wounds, boils (Kletter & Kriechbaum 2001; Bhattarai *et al*. 2006).	+	+	+	+
23	*Hyoscyamus niger* L. (Solanaceae), V 2236.	Sd	Whooping cough, gingivitis, asthma, tooth pain (Manandhar 2002, Bista & Bista 2005, Bhattarai *et al*. 2006).	+	+	+	+
24	*Lawsonia inermis* L. (Lythraceae), V 2508.	L	Wounds, burns, toothaches, skin diseases, menstrual problems and disorders (Rajbhandari *et al*. 1995, Bhattarai 2007).	+	+	+	+
25	*Mangifera indica* L. (Anacardiaceae), V 2529.	Br	Cough, bloody dysentery, burns, gingivitis, diarrhoea, bronchitis, urinary tract infection, diuretic, toothaches, scabies, skin diseases, asthma, anthelmintic (Rajbhandari *et al*. 1995, Manandhar 2002, Bhattarai 2007).	+	+	+	+
26	*Malva verticillata* L. (Malvaceae), V 156.	Rt	Cough, tonsillitis, diarrhoea, wound, urinary diseases, kidney diseases, kidney fever, (Bista & Bista 2005, Bhattarai *et al*. 2006).	-	+	-	+
27	*Nerium indicum* L. (Apocynaceae), V 1145.	Fl	Skin diseases (ringworm, herpes) (Manandhar 2002).	-	-	-	+
28	*Phyllanthus urinaria* L. (Euphorbiaceae), V 2563.	Wp	Asthma, bronchitis, urinary tract infection (Bhattarai 2007).	+	+	+	+
29	*Pogostemon benghalensis* (Burm.f.) Kuntze (Lamiaceae), V 2571.	L	Cough, diarrhoea, fever (Bhattarai 2007, Manandhar 2002).	-	-	+	+
30	*Pterocephalus hookeri* (C.B. Clarke) Diels (Dipsacaceae), V. 4089.	Wp	Chronic diseases, fever, cough, poison fever (Kletter & Kriechbaum 2001, Manandhar 2002, Bista & Bista 2005).	-	-	+	+

31	*Ranunculus laetus* Wall.ex D. Don (Ranunculaceae), V 4001.	Wp	Cuts, wounds, stomachache, sinusitis (Manandhar 2002).	+	+	+	-
32	*Rheum australe* D. Don (Polygonaceae), V 3085.	Rt	Stomachic, malarial fever (Manandhar 2002).	+	+	+	+
33	*Rauvolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae), V 2592.	Rt	Dysentery, fever, cut, wounds, boils, stomachache, menstrual problems and disorders (Rajbhandari *et al*. 1995, Manandhar 2002, Bhattarai 2007).	+	+	+	+
34	*Rhododendron lepidotum* Wall. ex G. Don (Ericaceae), V 2122.	L, & Fl	Fever, cough, cold, tonsillitis (Lama *et al*. 2001, Manandhar 2002, Bhattarai *et al*. 2006).	+	+	+	+
35	*Selinum wallichianum* (DC.) Raizada & Saxena (Apiaceae), V 435.	Rt	Stomachache, cuts, wounds, cough, cold, fever (Lama *et al*. 2001; Manandhar 2002, Devkota & Karmacharya 2003, IUCN-Nepal 2004, Bhattarai *et al*. 2006).	+	+	+	+
36	*Stellera chamaejasme* L. (Thymelaeaceae), V 3072.	Rt	Skin diseases, swelling, wounds (GoN 2007, Kletter & Krieckbaum 2001, Manandhar 2002).	+	+	+	+
37	*Tinospora sinensis* (Lour.) Merr. (Menispermaceae), V 2711.	Rt, St & L	Chronic diarrhoea, chronic dysentery, cough, stomachache, urinary troubles, diuretic (Rajbhandari *et al*. 1995, Manandhar 2002, Bhattarai 2007).	+	+	+	+
38	*Urtica dioica* L. (Urticaceae), V 3553.	L & St	Cough, dental caries, cuts, wounds, fever, boils, diarrhoea, dysentery,asthma, anthelmintic, menstrualdisorders (Rajbhandari *et al*. 1995).	+	-	+	-
39	*Woodfordia fruticosa* (L.) Kurz (Lythraceae), V 2509.	Fl	Fever, diarrhoea, dysentery (Manandhar 2002, Bhattarai 2007).	+	+	+	+
40	*Xeromphis spinosa* (Thunb.) Keay (Rubiaceae), V 2613.	Br	Stomachache (Bhattarai 2007).	-	+	-	-

Key: Parts used refers to the part of the plant extracted and tested: L, leaves; Fl, flowers; Wp, whole plants; Rt, roots; St, Stems; Br, bark; Lx, latex; Sd, seeds; V, Voucher number; +: testing resulted in a zone of inhibition, indicating the presence of antibacterial activity, -: testing resulted in no zone of inhibition, indicating the absence of antibacterial activity, Test organisms: *S.a.*: *Staphylococcus aureus, P.a.*: *Pseudomonas aeruginosa*, *E.c.*: *Escherichia coli*, *B.s.*: *Bacillus subtilis*.

Three extracts i.e., from *Asparagus racemosus*, *Mangifera indica* and *Phyllanthus urinaria* used to treat urinary tract infection showed antibacterial activity against at least one Gram-positive and Gram-negative bacteria along with *Escherichia coli*. Similarly, *Artemisia indica* which is used to treat conjunctivitis showed antibacterial activity with *Staphylococcus aureus*. Of 15 extracts tested against diarrhoea and dysentery, 14 showed antibacterial activity against at least one Gram-positive and one Gram-negative bacteria.

Among 23 extracts tested, against skin infections (boils, infected wounds, wounds), five extracts did not show antibacterial activity with *Pseudomonas*

aeruginosa and seven extracts with *Staphylococcus aureus*. Among 26 extracts tested used to treat fever, 23 showed antibacterial activity with at least one Gram-positive and 25 with at least one Gram-negative bacteria.

Aconitum spicatum, used by the *amchis* (Tibetan Doctors) with different confindential mixtures was unable to show positive activity towards any bacterial species tested (Table 1). There may be many possible reasons for the negative activity of the extracts in this study. Firstly, the tested plant extract may not contain antibacterial compounds. The plants may have other medicinal uses, such as analgesic and others. Many antimicrobial screening studies use a relatively small number of microorganisms for testing. It is possible that these plants contain antibacterial compounds against pathogenic bacteria other than those tested in this study. In addition, the lack of activity may be because of degradation of active chemicals during the drying process, the extraction process, or perhaps methanol was not an appropriate solvent to extract the active constituents of these plant species.

Comparing the result shown by Gram-positive and Gram-negative bacteria, *S. aureus* and *B. subtilis* were found to be less sensitive then Gram-negative *P. aeruginosa* and *E. coli*. Overall, 95 % of the extracts showed antibacterial activity against at least one Gram-negative bacteria and 90 % of the extracts against at least one Gram-positive bacteria examined. The activity against Gram-negative bacteria was a surprise finding. It has been expected that greater number of extracts were active against Gram-positive bacteria than Gram-negative bacteria (McCutcheon *et al*. 1992, Taylor *et al*. 1995). This has been explained by the difference in the cell wall make up of Gram-negative and positive bacteria, with Gram-negative having a more complex, and therefore possibly harder to combat the cell wall (Bhattarai *et al*. 2008b).

In this study, the findings of more plant species with inhibitory activity against Gram-negative bacteria may be because of selection bias towards illnesses more commonly caused by Gram-negative bacteria (such as fever and diarrhoea). The results of this research are encouraging, as most of the selected plants appeared to contain antibacterial substances. It is hoped that the Nepalese medicinal plant screening research will aware the related partners in Nepal to search for safe, efficacious, cheap alternative antibacterials of plant origin and may create an opportunity for biological conservation of the medicinal plants and cultural diversity conservation.

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