IOF Discussion Paper

No. 8

ANTIBACTERIAL ACTIVITIES OF SOME TRADITIONALLY USED MEDICINAL PLANTS OF DAMAN, NEPAL

By
Prof. I.C. Dutta, Ph.D
&
Ajeet Kumar Karn, M.Sc.(Forestry)

2007






Tribhuvan University
Institute of Forestry
Hariyokharka
Pokhara, Nepal

ComForM
Community Based Natural Forest and Tree Management in the Himalaya

Thulo Okhati

Kharane Bark

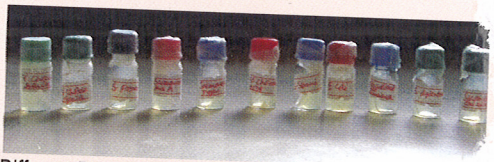
Katus Bark

Thulo Okhati in Powder Form

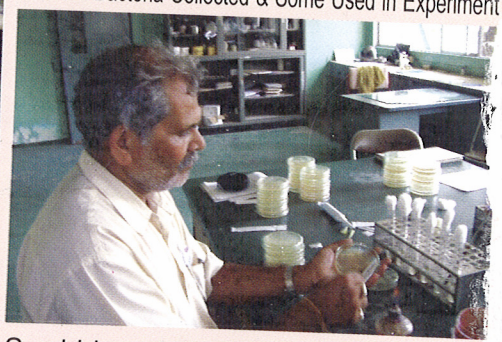
Kharane Bark in Powder Form

Katus Bark in Powder Form

Extraction Procedure of Different Medical Plants Parts



Different Bacteria Collected & Some Used in Experiment



Swabbing of Bacteria on MHA Plates

IOF Discussion Paper

No. 8

ANTIBACTERIAL ACTIVITIES OF SOME TRADITIONALLY USED MEDICINAL PLANTS OF DAMAN, NEPAL

By
Prof. I.C. Dutta, Ph.D
&
Ajeet Kumar Karn, M.Sc.(Forestry)

2007

Tribhuvan University
Institute of Forestry
Hariyokharka
Pokhara, Nepal

ComForM
Community Based Natural Forest and Tree Management in the Himalaya

Editors

Prof. Dr I.C.Dutta, Institute of Forestry, Tribhuvan University, Nepal

Dr. H.O.Larsen, Danish Center of Forest Landscape and Planning, Royal Veterinary and Agricultural University, Denmark

Printed at

Pashupati Printing House

Chipledhunga, Pokhara

Tel. 061-535289

Computer Graphics & Setting

Magan Computer Service

Chipledhunga, Pokhara

Mailing Address

Dean's Office

Institute of Forestry Pokhara

P.O. Box : 203

Hariyokharka, Pokhara, Nepal

E-mail : iofdean@fewanet.com.np

Sources of funding for this publication

IOF/ComForM Project

Cover Photo

Left to Right : Eklebir Root, Ban Lasun, Ainselu Munta, Eklebir Root in Powder Form, Ban Lasun in Powder Form, Ainselu Munta in Powder Form

Acknowledgements

The contribution of the local people of Karunabhumi Community forest User groups sharing their knowledge on medicinal plants and their uses are acknowledged. Forest department Makawanpur district and personnel from Daman range office are also acknowledged for helping and mobilizing the local people. The study was carried out with the financial support of the IOF/ComForM Project. Dr Helle O Larsen is thankful for editing the text. Regional Health Laboratory, Western Regional Hospital and IOF Chemistry laboratory Pokhara are dually acknowledged for the facilities and permission provided to carryout this research for microbial and Chemical extractsb respectively. Similarly Plant research department Thapathali is thankful for Phytochemical screening of the samples. Manipal college of Medical Science, Pokhara is also acknowledged for providing bacteria's.

ABSTRACT

Local healers in high altitude traditionally use some medicinal plants for curing different common diseases like food poisoning, diarrhea, dysentery, cholera, pneumonia and typhoid etc. Medicinal plants and their antibacterial activity also contribute to the livelihood According to an estimate 25% of all prescribed medicines in the developed world contain ingredients derived from plants. Most of the plant derived drugs were discovered through the study of traditional customs and folk knowledge of indigenous or ethnic peoples- the ethno-botanical approach[3]. WHO estimate around 80% of the world's 6 billion population in the developing world relies on herbal remedies for their primary health care needs. The present study aims to provide scientific evidence to the local practices.

In the present study, 33 plant extracts of 17 medicinal plants obtained by solvent extraction method at room temperature were investigated for activity against *E.Coli, S. typhi, S. aureus, Sh. Dysentriae, P. aeruginosa, and V. cholera* using disc diffusion method. Four most active extracts were assayed for the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) using two fold serial dilution method. They were also subjected to phytochemical screening.

The results obtained indicate that mixture of five plants (*Viburnum cylindricum*, *Rubus sps*, *Castanopsis indica*) showed the best zone of inhibition (ZOI) in all tested bacteria except *S. typhi*. In case of *S. aureus* the mixture of plant extracts showed same ZOI (inhibition diameter 28 mm) to that of the standard drug Clarithromycine (inhibition diameter 28 mm) but much more higher than the standard drug Ofloxacine (15mm). In case of *V. cholera* the extract showed slightly less ZOI (20mm) than the standard drug Tetracycline (22mm)while higher ZOI than standard drug Clarithromycine (14mm).

The gross MIC values of active extracts were determined. The MBC value of *Astible rivularis* extract against *P.aeruginosa* was found most effective at very low concentration 1.5 mg/mL Similarly, the extract of mixture of plants was found to be very effective against *V.cholera, S. aureus* at low conc^{n} of <5mg/mL. The MBC values of plant mixture extract against *P. aeruginosa* and *Sh. dysentriae* was also found very effective.

Phytochemical screening showed that the antibacterial activity was probably due to the presence of flavonoid, steroids, caumarine, reducing compounds, polyuronides and polyoses. These results support some of the traditional remedial uses of plants while in some cases not. In addition, some of the medicinal plants are effective against some other diseases about which traditional users are unknown Therefore the feedback should go to them for corrections and encouragement

KEY WORDS: Antibacterial Activity, Medicinal Plants, Traditional users

Authors contact email: icdutta@yahoo.com

Introduction

The Nepal Himalayas are famous for their rich plant biodiversity, especially the medicinal and aromatic plants. The local people in the remote areas are using these medicinal plants for the ailments of different diseases from their traditional and indigenous knowledge. Different types of bacterial and fungal infections such as infected wounds, cholera, pneumonia, typhoid, dysentery, diarrhea, vomiting, fever, cough, bleeding, etching of skin, food poisoning, fractures etc are treated by traditional medicine in various forms especially under the ayurvedic system, homeopathic system, the unani system etc. The present trend indicates that there is an increasing global demand for these medicinal plants for industrial purposes. Since the allopathic medicines have several side effects on the human health many people are interested to depend more on the use of herbal medicines, but due to lack of scientific evidence they are afraid of using it. Therefore, there is a need to provide the scientific evidence of the effects of medicinal plants.

Nepal is rich in its tribe composition, and consequently very rich in the ethno-medical knowledge, which are now-a-days explored and documented as field manuals to manage the medicinal plant resource base, and also to produce many drugs for the day to day usage. More than 75% of the Nepalese people still depend on herbal plants as a local source of medicine for their primary health care (Devkota and Dutta 2001). Moreover, there are more than 90 ailments cured by medicinal and aromatic plants (MAPs) by local healers. Local healers (*baidyas*) are few but there are some local people in different ethnic groups, like Gurung, Magar, Tharu, Brahmins, Chhetriya, Tamang and others, who have learned healing with medicinal plants either from their ancestors or from their own experience. It has been reported and experienced that in some case the disease is cured and in some cases not because of incomplete or lack of scientific knowledge, and diagnosis.

Allopathic medicines, hospitals and doctors facilities are not available in the hilly remote areas of Nepal. So this study will help to fulfill to a greater extent the need of first aid and local remedy of the poor Nepalese who cannot afford costly medicine and treatment in the hospitals in urban area. Furthermore, the resistance to synthetic antibacterial is a worldwide public health problem responsible for a number of infections becoming untreatable in both hospital and community settings. Use of herbal based antibacterial medicine may contribute to achieve the goal of worldwide public health.

Recently, the study of antibacterial and antifungal activities of plants used for medicinal purpose is beginning to receive attention. Studies have included tests for activity against a variety of bacteria by many plants used by local healers (Sharma and Saxena 1996;

Sanabria et al. 1997; Sasidharas 1997; Hewage et al. 1998), and extracts exhibit activity against both Gram-positive and Gram-negative microorganisms, although Parajuli et al. (2001) found a relatively high degree of gram negative antibacterial activity among medicinal plants used for treatment of skin diseases. Generally, alcoholic extracts are found to be better than aqueous extracts (Sasidharas 1997; Giri 2000; Ramana and Krishna 2004) and hexane extracts (Ali et al. 1999), and the antibacterial activity has also been found to vary with season.

A few studies of antibacterial activity of locally used medicinal plants have been conducted in Nepal (e.g. Devkota et al. 2000; Giri 2000; Pokharel 2000). Tests have been conducted against *Bacillus subtilis, E. coli, P. aureginosa, Salmonella typhi*, *Shigella dysenteriae*, *S. aureus, V. cholarae*, *Candida albicans*, and *Micrococcus* species. Especially effective medicinal plants to be mentioned are e.g. Pakhanbed (*Bergenia ciliata*), Bojho (*Acorus calamus*), Kurilo (*Asparagus racemosus*), and Sugandhwal (*Valeriana jatamansi*) (Devkota and Dutta 2001), *Cinnamomum zeylanicum, Syzygium aromaticum* and *Zanthozylum alatum* (Sharma 2002), and *Parmelia nepalensis* (Karna and Dutta, 2003). However, the medicinal plant species richness of Nepal is vast, and the need for studies to verify assumed properties is great. This study consequently aims to evaluate antibacterial activity of medicinal plants used by rural people as traditional medicine. We hope that this work will be helpful for the planners, policy makers, conservationist, local healers, users, academicians, students and others who are concerned to this field. The study is focused on the following two objectives:

i) To find out traditionally used medicinal plants from a community forest for common diseases like diarrhea, dysentery, typhoid, fever, cough and cold, pneumonia, cholera, etc. based on preferential ranking.
ii) To see the effect of plants on six different common bacteria responsible for common diseases.

Methods

The research was undertaken from February to August 2005, involving the following steps:

(i) Identification of medicinal plants used by the local community and/or healers from the study area to treat common diseases caused by bacteria.
(ii) Collection of medicinal plant material used by local community/ healers.
(iii) Extraction of crude compounds from the plant materials.
(iv) Screening of antibacterial activity of crude extracts of six pathogenic microbials.
(v) Identification of some more active extracts.

Study Site

Karunabhumi Community forest at Daman in Makwanpur District was selected as the study site with the assistance of the District Forest Office, Makwanpur. It is located at a high altitude, having rich biological diversity, people using medicinal plants, and common diseases being prevalent in the area.

Karunabhumi Community forest User Group (FUG) lies at Daman in Makawanpur district of Narayani Anchal under Central Development Region, Nepal. To the East it borders to Kitbhanjyang, dry pine to Jurthumpani Haluwa bandel danda Fafarbari whereas to the West to Dabar ChowkTharokhola to kholaDhari, to the North: Ghattekhola ko bari to khola ko dui ghare chheu, and to the South: Parichowki-Mandu khola to old ChowkiPani Dhalle to Nange ko Chulidarchowk. The forest area is 988 ha (19760 Ropani). The FUG includes 617 households, with a total population of 3703 (Male: 1863, Female: 1840). The area has 15 toles, and the people belong to Tamang, Chhetri, Brahmin and Newar ethnic groups. The Tamangs use more forest products than other ethnic groups, and many of the forest users are illiterate (6% male and 50% female). People engage mainly in vegetable farming and livestock rearing; there is a livestock density of 4.34/ha. The climate of the area is Subtropical to Temperate, the forest has a general slope of 10-35^0, and major tree species found are Oak (*Quercus semicarpifolia*) and Blue Pine (*Pinus wallichiana*).

Study team

The study team was comprised of various disciplines, i.e. chemist, forester, laboratory technicians, microbiologists and local people. Prof. Dr I. C. Dutta from Institute of Forestry, Tribhuvan University, Pokhara, Nepal was the main investigator, who developed the proposal under ComForM Project to assist communities in the district of Makwanpur and for initiating viable commercial activities based on the sustainable use of the local natural resources (Medicinal Plants) in future. It was envisioned as a community-based approach for development that would support existing and encourage new community efforts to sustainably, utilize and conserve natural resources by providing economic return. Mr. Ajeet Kumar Karn, a forester, also assisted the work. Key informants/local healers (See Annex 1), ranger and administrative staff from the range office assisted throughout the fieldwork in Makwanpur district, Daman. Similarly, a laboratory technician (Microbiologist), Mr. Jagat Khadka of Regional Public Health Laboratory, Pokhara, also assisted the team for the antibacterial study. Assistance and cooperation was also obtained from Dr. K. P. Devkota for antibacterial tests, and Mr. Dev Muni Shakya from Plant Research Department, Thapathali for the study of Phyto-chemical Screening. Mr. Bharat Mahto, lecturer at IOF, was given practical training in the chemistry laboratory and microbiology lab in order to continue this kind of work in future, which will be helpful for him in carrying out Ph.D work at IOF.

Medicinal plants investigated

A PRA tool (interviews, focus group discussion, key-informant consultation, etc.) was used to explore the ethno-medical knowledge of the local healers. Listing of the medicinal plant diversity available in the community forest was done by mass meeting. The seventeen most used medicinal plants based on preference ranking were selected for study. *Acorus calamus*, *Berginea ciliata* and *Paris polyphylla* were mentioned by the local community as important, but as these species have already been explored (Dutta and Devkota 2001) they were not included.

The target plants were selected based on the discussions with local healers and their ranking of Medicinal plants for use. They are Ainsalu, Kharane bokra, Katus, Sunpati, Jaringo, Banlahsun, Lauth salla, Thulookhati, Gangadol, Eklebir, and Rudilo (Table 1). The medicinal plant part(s) was collected from the natural habitats, where it was collected by the local people. The plant parts were collected in the same way as by the local people. The selected plants were collected by the farmers for local uses, selling and their income generation. The collection time was May –June 2005. The roots and rhizomes of plants were collected by digging the soil with *kodalo* and cut with scissors. Barks were peeled by using axe. Arial parts were collected by cutting small branches through scissors. Whole plants (herbs) were collected by uprooting.

Table 1 - Medicinal plant and their parts used in this study

Plant code	Name of plant collected			Parts used for the study
	Scientific name	Family	Local name	
1	*Nyctanthes arbortristis*	Oleaceae	Rudilo	stem/branch
2	*Styrium nepalense*	Orchidaceae	Gangadol,	root
3	*Paris polyphylla*	Trilliaceae	Satuwa	root
4	*Rubus paniculatus*	Rosaceae	Kalo Ainsalu	rhizomes
5	*Allium wallichii*	Amnaryllidaceae	Banlahsun	bulb
6	*Viburnum cylindricum*	Caprifaliaceae	Kharane Bokra	bark
7	*Rubus ellipticus*	Rosaceae	Ainsalu Munta	whole plant
8	Unidentified		Ekle jhar	whole plant
9	*Taxus bacatta*	Taxaceae	Lauthsalla	
10	*Bergenia ciliata*	Saxifragaceae	Pakhanved	leaf
11	*Phytolacca acinosa*	Phytolaccaceae	Jaringo	root
12	*Rhododendron lepidotum*	Ericaceae	Sunpati	root
13	*Astilbe rivularis*	Saxifragaceae	Thulo Okhati	
14	*Valeriana jatamansi*	Valerianaceae	Sugandhwal	whole plant
15	*Acorus calamus*	Araceae	Bojho	root
16	*Lobelia pyramidalis*	Lobeliaceae	Eklebir,	
17	*Castanopsis indica*	Fagaceae	Katus	bark
			Kalo Ainselu+Ekle Jhar+Kharane Bokra+ Katus Bokra+Ainselu Munta	

All the collected parts of plant were first washed thoroughly with clear water at the spot, dried by pressing through blotting paper and finally placed in bag with blotting paper. The blotting paper was changed twice a day. Some species were easily identified on the spot by the collector's knowledge and according to the description given in different books (HMG/N 1970). One species could not be identified i.e. *Kharane bokra*. It may be either *Viburnum cylindricum* or *Lindera pulcherrima* tree.

Bacteria

Six common bacteria that are the main cause of number of physiological disorders to human beings of different communities of Nepal were included in this study: *Pseudomonas aeruginosa*, *Staphylococus aureus, Salmonella typhi*, *Shigella dysenteriae*, *Escherchia coli*, and *Vibro cholerae* (Table 2).

Table 2. Bacterial species[1], their code, gram stain, source and type

S.No	Bacterial species	Bacterial code	Gram stain	Source	Type
1	*Shigella dysenteriae*	F	Gm -ve	MTH	MTH standardized
2	*Escherichia coli*	E	Gm - ve	MTH	ATCC 25922
3	*Staphylococcus aureus*	D	Gm + ve	MTH	ATCC 25923
4	*Pseudomonas aureginosa*	C	Gm -ve	MTH	MTH standardized
5	*Salmonella typhi*	B	Gm -ve	MTH	ATCC 27853
6	*Vibrio cholerae*	A	Gm –ve	MTH	MTH standardized

[1]Bacteria's are selected on the basis of high pathogenicity but not on the basis of gram stain

Shade drying of the plant materials

All the collected different parts of plants were shade dried in the blotting paper at laboratory room of Institute of Forestry, Pokhara, Nepal. The drying place was maintained dark in order to prevent the degradation of bioactive compounds of medicinal plants by interfering through light.

Grinding of the plant materials

After drying all the plant parts were ground into powder separately.

Extraction of crude compounds from natural products

There are several methods for extraction of crude compounds from natural products like steam distillation, batch extraction, soxhlet extraction, cold extraction etc. Among all, cold extraction method was found to be the best method. In this technique the powdered form of the plant material is submerged in a suitable solvent and kept at room temperature for a few days. The soluble constituents from the solvent are then filtered and concentrated to obtain the crude extract. In this method the chances of decomposition of compounds is very low. In the present study the medicinal plants were soaked in different solvents.

The solvents used in this extraction process of medicinal plants were Petroleum ether (P), Hexane (H), Ethyl alcohol (E), Chloroform (C), Ethyl acetate (EA), Methyl alcohol (M), and Water (W). The calculated amount of medicinal plant parts in the form of powder was taken in conical flasks and beakers and dissolved in calculated volume of different solvents which were covered with aluminium foil. It was left for 72 hours at room temperature. Then these were filtered with the help of filter paper. These plant extract were left for at least 48 hours in order to remove the solvents used (except water). After drying, they were weighed in order to know the amount of extract of each individual plant and percentage yield.

The % yield of crude plant extract was calculated by using following formula:

$$\% \text{ yield of crude plant extract} = \frac{\text{Wt. of beaker with extract} - \text{wt. of dry beaker} \times 100}{\text{Wt. of sample powder taken}}$$

Color of extract

The color of each extract was noted. The colour indicates the type of compound dissolved, e.g. flavenoid, chlorophyll or glycosidic.

Screening of antibacterial activity

The screening of antibacterial activity of plant species can be done by many techniques. Charpinella et al. (1999) recommended the methods for screening of antibacterial activity by determination of Zone of Inhibition (ZOI), Minimum Inhibitory Concentration (MIC) and/or Minimum Bacterial Concentration (MBC). MIC is defined as the lowest concentration of anti-microbial that prevents growth of organisms after over-night incubation (WHO 1991). MBC is the lowest concentration of antimicrobial required to produce a sterile culture (Cheesbrough 1993).

Preparation of stock solution

The calculated amount of each dried plant extract obtained from different solvents were dissolved in a calculated volume of ethanol to make stock solution and were used for determination of Zone of Inhibition (ZOI) of different bacteria.

General principle of antibacterial activity test

Antibacterial activity tests measure the ability of an antibacterial agent to inhibit bacterial growth in vitro. This kind of ability can be estimated by either the diffusion method (Bauer et al 1966) or by the dilution method (Miles and Amyes 1996). The followed method is diffusion method. There are two types i.e. agar well diffusion method or dingles cup method) and paper disc method. Agar well diffusion method proceeds over paper disc method due to its more accurate result.

Agar well diffusion method

In this technique, small cups (wells) are made by cork borer in the Petri-plate containing the required medium, already inoculated by test organism. The diluted extract (test solution) is added to the well. Dingle et al. (1953) first proposed this technique for the evaluation of enzymatic activity for degradation of pectin and other polysaccharides. But the technique is also used for evaluation of antimicrobial activities. This method is generally used (e.g. Pepeljnjak et al.1999; Devkota et al. 2000; Pokharel 2000; Karn and Dutta 2003).

Collection of test organism and preparation of stock culture

The test organisms (bacteria) were obtained from the Manipal Teaching Hospital (MTH), Fulbari, Pokhara. All the organisms were either standardized by the laboratory themselves or procured from American Type Culture Collection (ATCC) (Table 2). Table 2 also provides the gram stain of the organisms.

The stock culture of each organism was prepared by taking two nutrient agar slants and sub-culturing each confirmed test organism aseptically. One set slant was kept as stock culture and another as working set.

Preparation of Mc Farland (turbidity) Standards

This is a Barium Chloride standard against which the turbidity of the test and control inocula can be compared. The turbidity of standard is equivalent to 4 hrs to overnight broth culture. The turbidity standard was prepared by mixing 99 ml of 1% v/v solution of H_2SO_4 (1 ml conc. H_2SO_4 + 99 ml H_2O) with 0.5 ml of 1.175% w/v solution of $BaCl_2$. $2H_2O$ (2.35g of $BaCl_2$ $2H_2O$ in 200 ml distilled water) in a beaker and mixed well. Small volume (about 5ml) of such turbid solution was transferred to a screw cap bottle of the same type as used for preparing the test and control inocula. It was then well sealed to take in a dark at room temperature (20-28°c). This standard can be kept up to 6 months.

Laboratory proceedings

All the necessary equipment's/apparatus for the antibacterial activity testing like cotton swabs, culture media, micropipette tips, glassware etc were sterilized in an autoclave at 15 psi pressure and 121°c for 15-20 minutes. The antibacterial activity testing of plant extract was carried out inside the laminar flow hood (horizontal type) strictly by creating sterile environment through spirit lamp. The bacterial stock cultures, subcultures agar plates etc were stored in the refrigerator. The bacterial suspension in nutrient broth, the zone of inhibition testing plates, the MIC tubes, the MBC testing plates etc. were placed inside the incubator at 37°c for needed period. Sterile transport viscose swab with polypropylene stick of size

150 x 2.5 mm diameter for bacteriological culture work was used. When this viscose swab was not available in the market then Small piece of cotton wool was wrapped in one tip of small sticks for making cotton swab. They were then autoclaved by placing inside test tube with tight lid of cotton wool.

Nutrient Broth (NB) solution

Nutrient broth solution was prepared on the guideline of manufacturer's (13gm/ lit). The required amount of solution was prepared by dissolving calculated amount of NB powder on calculated volume of distilled water and homogenized by proper shaking. Calculated volume of NB solution was put in different test tubes (e.g. 5 ml for standard working inoculums and 1 ml for serial dilution techniques etc.) The mouth of all tubes was tightly cotton plugged and sterilized on autoclave.

Standard working inoculums

One-two colonies of pure culture of different organism from stock culture were transferred through sterile loop to the respective sterile test tubes (A, B, C, D, E. and F organism, table 2) containing 5 ml of nutrient broth by taking out cotton plug. For transferring each organism the loop was sterilized over oxidizing flame of spirit lamp. It was then incubated for 4 hours and the turbidity of NB was observed which was matched with McFarland (turbidity) standard. The NB tubes having less or high turbidity (if) than the McFarland standard were made same by adding little organism or by adding fresh sterile nutrient broth. In such standard working inoculums, the bacterial cell density is 1.5 $x10^8$ CFU/ml.

Muellar Hinton Agar (MHA) plates and Nutrient Agar slant

The Muellar hinton agar medium was prepared according to the manufacturer's recommendations (38 gm/lit respectively). On the basis of needs of plates, the calculated amount of media was dissolved in calculated volume of distilled water in a conical flask by warming above burner to homogenize them. The conical flasks were then tightly cotton plugged and sterilized in autoclave at 121°c (15 lbs) for 15-20 minutes. Sterilized media was then cooled to about 50°c. It was then poured to 90mm diameter Petri-plates aseptically in the amount of about 25 ml per plate (approximately 4 mm thickness) and labeled as MHA plates. The plates were left at room temperature for 15-20 minutes for solidification and stored in refrigerator packed up in sterilized polythene. For the preparation of nutrient agar slant, the solution of NA media were sterilized in appropriate size of screw cap test tubes and cooled in tilt condition.

Determination of zone of inhibition (ZOI)

The zone of inhibition was determined as follows:

i. Inoculation of MHA plates

Fifty four number of MHA plate prepared as described above were taken and dried in an incubator at 37°c for 30 minutes. The standard working inoculums of six different organisms were also taken. A sterile cotton swab was dipped into the standard working inoculums (equivalent with McFarland turbidity standard, cell density 1.5 $*10^8$ CFU/ml) of code An organism tube (e.g. *V. Cholera*). The swab was then pressed to the wall of tube above liquid with rotating to remove excess inoculums (WHO, 1991). The swab was streaked all over the MHA plates in the angle of 60° for three times with rotating the plates. Finally the swab was rotated on the edge of the MHA plate. All fifty four plates were inoculated by this method, but each nine plates by one organism only (i.e. 9 plates for *V. Cholera*, 9 plates for *Sh. dysenteriae* and so on). All the inoculated plates were left to dry at room temperature for about 10 minutes with closed lid. The used cotton swab was discarded in a beaker containing Lysol solution.

ii. Preparation of wells and transfer of diluted plant extract

The cork borer having 6 mm diameter was sterilized and used to prepare wells in the MHA plates. On each plate, four wells were prepared. Each of nine plates containing similar organism (e.g. *V. cholera*) have altogether thirty six wells. Thirty three of such wells were for thirty three different diluted plant extracts, one well for control (redistilled ethanol), and two wells for standard drugs any two out of (*O-floxacine*, *Tetracycline* and *Clarithromycine*).

iii. Incubation of the MHA plates

All the twelve MHA plates prepared by the above process were incubated for 18 hrs in an incubator at 37°c. After 18 hrs the result of zone of inhibition was measured with the help of a ruler and tabulated.

Determination of MIC and MBC values of potent extracts

From the result of ZOI the plant extracts having code 12M (against *V. cholera* and *S. aureus*), 1M (against *V. cholera, P. aureginosa, S. aureus, and S. dysentrie*), 6C (against *S. aureus*) and 13H (against *P. aureginosa*) showed excellent antimicrobial activity. So, these extracts were chosen for determination of MIC and MBC up to maximum dilution by two-fold serial dilution method (Table 5). 56 numbers of tubes were taken and 1 ml of such NB solution was aseptically transferred to each tube having codes as shown in Table 5 and placed properly in test tube racks. Five test tubes for each bacterium were labeled as no 1^{st} to 5^{th}. In the same way, other two test tubes were taken for positive and negative control. The mouth of each test tube was tightly plugged by cotton and sterilized for 15-20 minutes at 21°c (15 lbs). After sterilization, each tube was left to cool for sometime. To the positive

control (+ve) test tubes 1 ml of nutrient broth and 50μL of bacteria suspension were added. In the same way 1 ml of nutrient broth and diluted plant extract (Table 5) were added to the negative control (-ve) tube. To the remaining test tubes containing 1 ml of Nutrient broth, the diluted plant extracts were added on the basis of two fold serial dilution techniques.

After the two fold dilution techniques, 50μL of standard working inoculums of each bacterium were added aseptically to each test tube except in negative control for all set. All these test tubes of seven sets were incubated at 37°c for 18 hrs. One of the principles is that, after 18 hours incubation, some of serial number of tubes will not show the turbidity due to inhibition of bacteria by plant extract and such last tube having less concentration gives the reading of MIC value. But, in this study, the extracts itself has color and once it serially diluted in NB solution as described above, there was color and turbidity of diluted plant extract. The tube shows turbidity before and after addition of bacteria. So MIC value couldn't be determined due to presence of color and turbidity in most of the tubes. So the exact MIC value is uncertain but the rough MIC value and exact MBC values were determined by following method.

Eight number of fresh and dry MHA media plates were taken. Each plate was divided into 7 fractions out of which five diluted plant extracts and one for + ve and one for - ve control. All tubes of one set (+ ve control, – ve control 1st, 2nd...etc) were sub cultured in MHA medium plates on the basis of respective labeling on the plates. Similarly other sets were also sub-cultured in respective plates and incubated at 37°c for 18 hrs. After18 hrs, the growth of subculture was noted to determine MIC and MBC values. The last diluted plant extract tube which do not grow bacteria in MHA plate is MBC value and MIC value was considered as less than the MBC, value.

Purification of natural products (Plant extracts)

On extracting plant by using solvent, the extract may contain number of different compounds. So, the purification of natural products plays an important role. In the case of antibacterial activity testing, *even though the crude extract shows a positive effect, the pure compound may not show a positive effect or vice versa.* Purification of natural products can generally be done either by solvent extraction techniques or by chromatography techniques (Sanabria et al. 1997). Giri (2000) used solvents of different polarities method to fractionate methanolic extract of *Lycopodium japonicum*. In chromatography techniques, the thin layer chromatography (Formi, 1980) and column chromatography (Verma et al., 1994) were considered the cheapest, easiest and fastest methods for isolation of pure compound from natural products.

Limitations

(i) Some of the plants are used in the mixture form by local healers so instrumental analysis like IR, UV, NMR, Mass spectroscopy etc is not advisable

(ii) Difficulties were experienced due to non-availability of all the laboratory and chemical facilities and some equipment.

(iii) It was very difficult to break the ice to gather information about medicinal herbs from a few local people.

Phyto-chemical screening of medicinal plant extract

The phyto-chemical screening of active samples was carried in the Botany Department Natural Product Research Laboratory, Thapathali Kathmandu. The detailed test of sample No 12 M, 1M, 17W, 6C and 13H, regarding their phyto-chemical screening was carried out. The method was based on the procedure given by Prof. I. Ciulei. In this method the plant material was extracted on different solvent on the basis of their increasing polarity and the natural chemical constituents in different extract solution were analyzed by using respective reagents.

i. Test for Gallic Tannins

0.5 ml of solution was mixed with 1 ml of water and 2-3 drops of dilute ferric chloride (1% w/v) solution was added. The occurrence of blue-blackish precipitate shows the presence of Gallic tannins

ii. Test for Catecholic tannins

All the process is same as above. The occurrence of green-blackish colour indicates the presence of catecholic tannins.

iii. Test for Reducing Compounds

0.5 ml of solution was diluted with 1 ml of water and 0.5 ml of Fehling's reagent (Fehling's reagent A and B in 1:1) was added. It was warmed over water bath for 5 minutes. A brick red precipitate indicates the presence of reducing sugar.

iv. Test for Alkaloid salts

10 ml of ethanolic solution was concentrated above water bath to yield residue. To that residue 4ml of 2% (v/v) hydrochloric acid was added and was vigorously shaked. It was then filtered and filtrate was treated with 10% (v/v) ammonium hydroxide solution till solution becomes alkaline. The solution was extracted thrice with 10 ml of chloroform. The upper alkaline layer was discarded. The lower chloroform layer was concentrated over water bath and 5 ml of 2% (v/v) hydrochloric acid was added. It was then divided into 2 parts.

v. **Maeyer's Test:** The first part of solution was treated with 2-3 drop of Maeyer's reagent. Appearance of white precipitate indicates the presence of alkaloid salts.

vi. **Dragendroff's test:** The second part of solution was treated with 2-3 drops of Dragendroff's reagent. The appearance of white precipitate indicates the presence of alkaloid salts.

vii. **Test for Anthracene glycoside**
If the acid solution is red and turns neither to violet at neutral nor to blue at basic medium indicates the presence of anthracene glycoside.

viii. **Test for Coumarin**
4 ml of etheric solution was placed on water bath to obtain residue, which was dissolved in 4 ml of hot water. After cooling, the solution was divided in 2 parts. First part was used as control. To the second part 10% v/v ammonium hydroxide was added drop by drop till it becomes alkaline. Both parts were then observed on u.v light. The blank tube gives yellow florescence and next tube greenish yellow fluorescence indicates the presence of coumarin

ix. **Test for Flavonic glycoside:**
10 ml of solution was evaporated to yield residue and was dissolved in 4 ml of methanol. This solution was divided into two parts

x. **Shinoda's test:** One small spatula of magnesium powder was added to first part and 4-5 drops of conc. hydrochloric acid was added. An orange yellow colour indicates the presence of flavonic glycoside.

xi. **Shibata's test:** One small spatula of zinc dust and 4-5 drops of conc. hydrochloric acid was added to the second part. Yellow colour indicates the presence of flavonic glycoside.

xii. **Test for tri-terpenoid glycosides**
4 ml solution was evaporated to yield residue. Then 0.5 ml acetic anhydride, 0.5 ml of chloroform and 1-2 drops of conc. $BaS0_4$ was added. The presence of red violet color indicates triterpenoid gloycoside.

xiii. **Test for Steroidal glycosides.**
The process is same as test for triterpenoid gloycoside. Green bluish colour indicates the presence of flavonoid glycosides.

xiv. **Test for Anthracynadin glycosides**
4 ml of solution was concentrated to 2ml and 1-2 ml of 25% (v/v) NH_4OH was added with shaking. A cherrish red colour indicates the presence of Anthracynadin glycosides.

Results

Traditional uses of medicinal plants

1M: Mixture of 5 plants Ekle jhar (unidentified), Katus bokra (*Castanopsis* sp.), Kalo ainsalu (*Rubus paniculatus*), Kharane bokra (*Viburnum cylindricum*) and Ainsalu munta (*Rubus ellipticus*) are crushed and used in typhoid, fever. Or they are dried and powdered. One tea spoon of powder is mixed in water and left, after some time when colour is observed in the glass this water is taken.

Thulo Okhati (*Astilbe rivularis*) (13): The root is powdered and kept in water. After some time color of the mixture becomes red which is taken to cure dysentery. A single dose is good enough to control dysentery. The mixture of Pakhanved and Thulo okhati powder of root is also used in gastric. The powder is mixed in mild warm water and taken

Katus bokra (*Castanopsis* sp.) (17): The bark is powdered and used in diarrhea, dysentery indigestion stomach burning sensation due to hard liquor drinking.

Ban lasun (*Allium wallichii*) (5): It is used in cough and cold

Eklebir (*Lobelia pyramidalis*) (8): Root about 1 inch is powdered and kept in water. This water is taken 3-4 times a day to cure gastric problem, Stomach pain, dysentery and in typhoid fever.

Sunpati (*Rhododendron lepidotum*) (12): Leaf, stem is powdered; a teaspoon of powder is given to cure diarrhea, dysentery to both animal and man. It is used in cholera as well

Jaringo (*Phytolacca atinosa*) (11): Root is used in food poisoning, constipation, diarrhea, stomach trouble. Leaf is used as vegetable. Care should be taken not to use it in iron vessel.

Rudilo (*Nyctanthes arbortristis*) (18): Branches and stem is ground and used in cough and cold. It is also rubbed on the body.

Percentage yield, color and concentration of extract solutions

The plant code, color of extract, percentage yield and concentration of extract is provided in Table 3. Jaringo (11M) has high percentage yield (30.2%) and Thulookhati (13H) has low percentage yield (1.2%) when extracted by cold extraction method. The greater the percentage yield, the greater will be the quantity of the respective solvent soluble compounds in that plant species and vice versa. Most of the extracts have green coloration and rest is pink, yellow and colorless. The colour of extracts depends on the type of compound dissolved on it. For example green color might be due to excess of chlorophyll, while yellow color might be due to flavenoid compounds and colorless might be due to presence of glycosidic compounds.

Table 3. Percentage yield, colour and concentration of extract.

S.N.	Plant Code and dissolvent[1]	Colour of extract solution	Percentage yield	Concentration of extract (mg/mL)
1	1M (Mixture of 5 plants)	Green	16	160
2	2E (Gangadol)	Brownish pink	14	7
3	5H (Ban Lausn)	V. L. yellow	1.4	14
4	5EA (Ban Lausn)	colorless	2	20
5	5M (Ban Lasun)	L. yellowish	7.5	7.5
6	5W (Ban Lasun)	L. yellowish	-	-
7	6P (Kharane)	Colorless	1.5	15
8	6C (Kharane)	Colorless	2.5	25
9	7P (Ainsalu)	Yellow	1.5	15
10	7C (Ainsalu)	G. yellow	3	30
11	9EA (Lauth Salla)	V.D.Green	11.2	110
12	9M (Lauth Salla)	V.D.Green	13.2	132
13	9H (Lauth Salla)	Y. Green	2.8	28
14	9W (Lauth Salla)	Green	-	-
15	11E (Jaringo)	Colorless	23.4	234
16	11M(Jaringo)	Colorless	30.2	302
17	11EA(Jaringo)	Colorless	3	60
18	11P (Jaringo)	Colorless	3	60
19	11W (Jaringo)	Colorless	-	-
20	12C (Sunpati)	Deep green	13.4	138
21	12P (Sunpati)	Green	10.6	106
22	12M (Sunpati)	D.Green	17	170
23	12W (Sunpati)	D.Green	-	-
24	13EA (Thulo Okthi)	Colorless	1.8	16
25	13E (Thulo Okthi)	Pink	27.4	274
26	13H (Thulo Okthi)	Colorless	1.2	12
27	13W (Thulo Okthi)	Colorless	-	-
28	17C (Katus Bokra)	Light yellow	1.5	15
29	17P (Katus Bokra)	Colorless	1.5	15
30	17W (Katus Bokra)	Colorless	-	-
31	18EA (Rudilo)	Green	3.75	35
32	18H (Rudilo)	Colorless	2.5	25
33	18M (Rudhilo)	Green	3	30

[1]C = Chloroform, P = Pertoleum ether, H = Hexane, EA = Ethyl Acetate, E = Ethanol, M = Methanol, W = Water.

ZOI, MIC and MBC

Table 4 provides the measured ZOI against the six bacteria of the medicinal plant extract and two standard drugs, O-floxacine and Tetracycline. The mixture of five plants (Kalo Ainsalu, Ainsalu Munta, Kharane Bokra, Eaklejhar and Katus Bokra) showed the best ZOI in all tested bacterias except in *S. typhi*. In the case of *S. aureus*, the mixture of plants extract showed same ZOI (28 mm) to that of the standard drug Clarithromycine (28 mm), but much higher than the another standard drug O-floxcacine (15 mm). Similarly, in the case of *V. cholera*, the extract showed slightly less ZOI (20 mm) than standard drug Tetracycline (22 mm), while higher ZOI than standard drug Clarithromycine (14 mm). Similarly, the mixture of plant extract showed almost similar ZOI to that of the standard drugs O-floxacine and Clarithromycine with *P. aureginosa*, but higher ZOI with *E. coli* and *S. dysenteriae*.

Extracts 12M, 1M, 6C and 13H showed good antibacterial activity and were therefore selected for MIC and MBC. Table 5 shows the dilution and Table 6 the results. The MBC values of different extracts against different pathogenic organism were different. The MBC value at low concentration (i.e. high dilution) is said to be more effective than the MBC value at high concentration (i.e. low dilution). The MBC value of Thulo Okhati (13H) against *P. aureginosa* (C) was found most effective at very low concentration (i.e. 1.5 mg/mL). Similarly, the extract of mixture of plants (1M) was found to be effective against *V. cholera* and *S. aureus* at the concentration of <5 mg/mL. The MBC results of 1M against *P. aureginosa* and *S. dysenteriae* were also found to be effective.

Table 4. Measurements of ZOI of different plant extracts[1].

Sample code	Bacteria code (Measurement of ZOI in mm)					
	A	B	C	D	E	F
Std. (O-floxacine for **B-F** and Tetracycline for **A**)	**22**	**20**	6	**15**	6	**18**
Solvent	6	6	6	6	6	6
17C	**15**	14	13	9	**15**	9
17P	14	**15**	11	13	12	11
7P	14	9	**16**	**15**	11	10
7C	12	6	14	10	**15**	8
6P	8	13	10	12	7	8
6C	14	9	**15**	**18**	10	10
5EA	**17**	9	11	**16**	**15**	**16**
18EA	13	6	9	14	6	10
18H	**15**	11	8	10	8	9
13EA	14	14	**15**	11	13	7
13H	12	7	9	9	10	7
12C	13	6	10	**16**	11	9
9EA	11	10	7	7	8	9
11E	9	8	11	11	9	11
9H	10	10	**21**	11	9	8
12P	**15**	**18**	14	**18**	9	11
9W	8	10	7	6	6	6
13W	6	10	7	6	6	6
17W	**20**	**15**	**20**	8	9	12
12W	6	10	12	9	8	6
5W	6	8	14	6	6	6
11W	6	12	9	6	6	6
Std. *Clarithromycine*	14	5	**26**	**28**	8	8
11M	6	10	9	8	6	8
9M	11	8	8	13	10	7
18M	12	10	6	8	7	8
13E	13	9	11	11	6	8
5M	11	10	6	10	6	10
12M	**22**	11	11	**22**	10	8
1M	**20**	6	**24**	**28**	**17**	**22**
11E	8	**15**	6	10	6	6
2E	13	10	11	**16**	6	6
16E	12	6	7	12	6	6
5H	14	10	12	9	6	8

[1]Diameter of well = 6mm; Depth of well = 4mm, ZOI more than 20mm = best, 15mm to 19 mm ZOI = better, 10mm to 14mm ZOI = good, 7-10mm = less active (The activity is categorized on the basis of ZOI of standard drugs used in the experiments)

Table 5 Dilution methods:

Extract Code	Bacteria code	Dilution of extracts (mg/mL) 1st	2nd	3rd	4th	5th	6th (-ve)	7th (+ve)
12M	A	85	42.5	21.25	10.62	5.31	1mlNB+2.65	1mlNB+50mcl bacteria
12M	D	85	42.5	21.25	10.62	5.31	1mlNB+2.65	do
1M	A	80	40	20	10	5	1mlNB+2.5	do
1M	C	80	40	20	10	5	1mlNB+2.5	do
1M	D	80	40	20	10	5	1mlNB+2.5	do
1M	F	80	40	20	10	5	1mlNB+2.5	do
6C	D	12.5	6.25	3.12	1.5	0.78	1mlNB+0.39	do
13H	C	6	3	1.5	0.75	0.35	1mlNB+0.18	do

Table 6 MIC and MBC values of active extracts

S.No	Code of Bacteria/plant extracts	MIC value (mg/ml)	MBC value (mg/ml)
1	12M for Bacteria A	<5.31	<5.31
2	12M for Bacteria D	<5.31	<5.31
3	1M for Bacteria A	<5	<5
4	1M for Bacteria C	<20	20
5	1M for Bacteria D	<5	<5
6	1M for Bacteria F	<20	20
7	6C for bacteria D	<12.5	12.5
8	13H for Bacteria C	<1.5	1.5

Phytochemicals

The phytochemical screening of active extracts clearly indicates that the presence of catecholic tanin, reducing compounds and caumarins were the major constituents which might show the antibacterial activities (Table 7). The activity of 12M might also be due to presence of steroid and flavoniod, whereas the activity of 17W might be due to additional constituents' polyuronoide, polyoses and flavonoid. In the case of hexane extract (13H), the antibacterial activity could be due to presence of some volatile oils. The results indicated that less polar solvent extracts less polar compounds such as volatile oils, where as high polar solvent (water) extracts more polar and polyhydroxyl compounds such as polyuronoide and polyoses.

Table 7. Results of Phyto-chemical screening[1]

S.N	Test	12M	1M	17W	13H
1	Gallic Tannin	-	-	-	-
2	Catecholic Tannin	+++	+++	+++	-
3	Basic Alkaloid	-	-	-	-
4	Reducing Compounds	+++	+++	+++	-
5	Anthracine Glycoside	-	-	-	-
6	Coumarine	+++	+++	+++	-
7	Steroid	+++	+	-	-
8	Triterpenoid	-	-	-	-
9	Flavonoid	++	-	++	-
10	Anthracinadine Glycoside	-	-	-	-
11	Polyuronoide	-	-	+++	-
12	Saponin	-	-	-	-
13	Polyoses	-	-	+++	-
14	Alkaloid Salts	-	-	-	-
15	Volatile oil	-	-	-	+
16	Fatty acid	-	-	-	-
17	Emodine	-	-	-	-
18	Carotenoide	-	-	-	-

[1]The phytochemical screening to **6C** couldn't be determined due to its low quantity.
+++ = Presence in high concentration, ++ = Presence in moderate concentration, + = Presence in low concentration, - = Absence.

Discussion

The exact MIC value couldn't be determined by two fold dilution process due to the color and turbidity of nutrient broth due to the plant extract in the two-fold dilution process. Although the MIC value is not exact, this gives an approximate idea about the MIC value.

Conclusion

All seventeen tested medicinal plants with their 33 extracts in different solvents showed different antibacterial activity. The mixture of five plants (Kalo Aisalu, Aisalu Munta, Kharane Bokra, Eakle Jhar and Katus Bokra) showed the best ZOI in all tested bacteria except in *S. typhi*. Most of the extracts showed good ZOI, whereas a few were found to be inactive against the tested bacteria.

On comparison of pathogenicity of bacteria, the use of medicinal plants as explained in traditional uses of medicinal plants and the results that we obtained revealed that some of the medicinal plant extract, such as the mixture of five plants (1M), Kharane Bokra (6C), Sunpati (12M), Thulo Okhati (13H) and Katus Bokra (17W) were responsible to inhibit tested bacteria. For example, On the basis of our investigation, dysentery caused by *E. coli, Sh. dysenteries* can be treated by Ban Lasun (5EA) and the mixture of five plants (1M). Food poisoning caused by *S. aureus* can be treated by Ainsalu Munta (7P), Kharane Bokra (6C), Ban Lasun (5EA), Sunpati (12C, 12P, 12M), mixture of five plants (1M), and Gangadole (2E) extracts. Diarrhea caused by *V. cholera* can be treated by using Katus Bokra (17C, 17 W), Ban Lasun (5EA), Rudilo (18H), Sunpati (12 P, 12M), and mixture of five plants (1M) extracts. Similarly, typhoid caused by *S. typhi* can be treated by using Katus Bokra (17P, 17 W), Sunpati (12 P), and Jaringo (11E).

The information obtained from the local healers shows that mixture of five plants is used against typhoid, fever, and dysenteries. Our results showed that the mixture can be used against diarrhea, dysenteries and food poisoning but not in typhoid. It is concluded that healers' use of these plants against diarrhea and dysentery is proved scientifically but the use against typhoid cannot be verified by this study. The local healers used Katus Bokra in heart disease (due to excess alcohol consumption) and typhoid. Our investigation showed that this plant can also be used against diarrhea (due to *V. cholera*), besides typhoid. In our observation, Sunpati can be used against diarrhea, typhoid, and food poisoning, but the local healers used it against diarrhea and dysentery only in both human and animals and not in food poisoning and typhoid.

Modern medicine is relatively costly and practitioners and facilities are fewer in number and concentrated in the urban areas. Medicine men are unable to share knowledge about the use and the availability of medicinal plants in the rural area. They know little about the cultivation of medicinal plants for commercial purposes. This study therefore calls for collaboration of central decision makers, through local organizations, NGO's, INGO's and Government officials to collectors on management of the medicinal plant resource.

Recommendations

- **Screening:** Anti microbial screening of all the other medicinal plants should be done against the common pathogens and thus a data –base should be established based on the findings.
- **Conservation:** Ex-situ and or insitu conservation of screened medicinal plant resources should be encouraged in the community forests, national forests and private forests. Here, in the present study we recommend at least Katus, Sunpati, and Thulo okhati, Jaringo, Ban lasun, Kharane, Ainsalu, and Eklebir should be planted, conserved and cultivated.
- **Local health:** In some remote areas, allopathic medicinal facilities are either extremely limited or not –existent. Local ayurvedic knowledge through **inexperienced** healers can be unreliable or unavailable. Basic knowledge of the uses of local herbal medicine (*jadibuti*) to give relief from common local ailments could be wider spread amongst villagers.
- **Data base:** Gathering base-line data on what medicinal plant are being used and growing in which areas of each district. This information can then be included in the FUG operation plan, either as an annex to future reference or as a direct influence on silvicultural treatments, to encourage and manage the growth of medicinal herbs.
- **Value addition:** Add value to the forest: Encourage people for potential Medicinal plant –sensitive forest management.
- **Income generation:** Create opportunities for income generating activities (IGA) and Ethno pharmacology and alternative medicine. For many rural families in the different areas of Nepal, there are few opportunities for earning money. The market for herbal medicine and other NTFP can provide an opening for individuals, small groups and FUGs. The seasonality of agricultural work can provide opportunities for alternative income generation during what may be up to 6 months of reduced farming activity.
- **Documentation and networking:** Establish a Rural Herbal Medicinal Practioner's Network for documentation of traditional use of medicinal plants and sustainable harvesting practices of medicinal plants to chalk-out clear -cut conservation threats and strategies to be adhered to by their members of their communities within the area.
- **State level:** There is a need to recognize the important role of traditional medicine and to create a separate department of Nepalese Systems of Medicines.

Acknowledgement

The contribution of the local people sharing their knowledge on medicinal plants and their uses are acknowledged. The study was carried out with the financial support of the IOF/ComForM Project.

References

Ali MS, Azhar I, Amtul Z, Ahmad VU, Usnmanghani K. 1999. Antibacterial Screening of some Casesalpeniaceae. *Fitoterapia,* 703: 299-304.

Bauer AW, Kirby WMM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of clinical pathology* 45: 493-496.

Charpinella MC, Herrero GG, Alonso RA, Palacios SM. 1999. Antifungal Activity of Melia azedarach Fruit Extract. *Fitoterapia* 70: 296-298.

Cheesbrough M. 1993. *Medical Laboratory Manual for Tropical Countries.* Reprinted Edition. ELBS, London.

Devkota KP, Acharya R, Baral MP, Pokherel RP. 2000. *Antimicrobial Activity of Some Herbal Plants Used in Traditional Medicine in Nepal.* Proceedings, Third National Conference on Science & Technology. RONAST, Kathmandu. Vol. II p 1311-1317.

Devkota, KP, Dutta IC. 2000. Antibacterial Activities of some commercially traded species of Doti District of Nepal. IUCN Seti Project, Nepal.

Dingle J, Reed WW, Solomons GI. 1953. The Enzymatic Degradation of Pectin and other polysaccharide II. Application of the Cup Plate Assay to the Estimation of Enzymes. *J. Sci. Food. Agric.* 4:149-153.

Formi GP. 1980. Thin layer Chromatography and High Pressure Liquid Chromatography in the Analysis of Extracts. *Phytohterapia* 15:13.

Giri R. 2000. *Antibacterial activity of Lygodium japonicum.* Unpublished M.Sc. Thesis, Central Department of Chemistry, Tribhuvan University. Kathmandu.

Hewage CM, Bandara BMR, Karunaratna V, Wannigama GP, Pinto MRM, Wijesundara DSA. 1998. Antibacterial Activity of some Medicinal plants of Sri Lanka. *Journal of National Science Council of Sri Lanka* 26: 27-34.

HMG/Nepal. 1970. *Medicinal plants of Nepal,* Ministry of Forest, Department of Medicinal plants. Kathmandu.

Karn A. 2003. Relationship between Contribution to Local Livelihood and antibacterial Activity of selected medicinal Plants. Unpublished M.Sc. Thesis. Institute of Forestry, Tribhuvan University. Pokhara.

Miles RS, Amyes SGB. 1996. Mackie and Mc cartney *Practical medicinal microbiology*, International student edition. 4th ed. Churchill Livingstone, London. pp. 151-177.

Pepeljnjak S, Stanik G, Potochi P. 1999. Antimicrobial Activity of the Ethanolic Extract of Saturej montana spp. *Acta Pharmaceutica,* 49: 65-69.

Pokharel NR. 2000. *Screening and Evaluation of Antibacterial Activity of some Medicinal Plants of Nepal and Isolation of Pure Antibacterial compound from Bauhinia variegata linn.* Unpublished M. Sc. Theses. Central Dept. of Microbiology, Tribhuvan University, Kathmandu.

Ramanna P, Krishna A. 2004. Antimicrobial evaluation of Tectona grandis leaves. *Journal of Non-Timber Forest Products* 11, No 4

Sanabria A, Garcia J, Quinteroal L. 1997. Polygodial: The main Antimicrobial Compound from Drymis graradensis. *International Joint Symposium Chemistry, Biological and Pharmacological Properties of Medicinal Plant* A-16: 23-26

Sasidharas VK. 1997. Search for Antimicrobial and Antifungal Activity of some plants of Kerala. Acta. *Pharmaceutica* 47: 47-51.

Sharma RN, Saxena VK. 1996. In vitro Antimicrobial Efficacy of Leaves Extract of B. variegata. *Asian Journal of Chemistry* 8: 811-812.

Sharma 2002. Antimicrobial Activities of essential oils of some common spices. *Nepal journal of science and technology* 4 : 95-100.

Parajuli S, Chaudhary RP, and Taylor RSI. 2001. Antibacterial activity of medicinal plants used to treat skin ailments in Kaski district, *Nepal*. In: Environment and agriculture biodiversity, Agriculture and pollution in South Asia, ECOS, Kathmandu. pp 230-237.

Verma DK, Singh SK, Nath G, Tripathi V. 1994. Antimicrobial Active Triteroenenoids from Lantana species. *Indian Journal of Pharmacology* 26: 194.

WHO. 1991. *Basic Laboratory Procedure in Clinical Bacteriology*. World Health Organization, Geneva.

Appendix 1: List of Respondents at Karu

1. Ganesh Bahadur Balla - Chairman FUGC
2. Bishnu Raimanjhi- Forest guard (Rishi Peshwar CF)
3. Dhan Bahadur Pandey- Baidya Simbhanjyang
4. Smti Hira maya Balla- Local healer Simbhanjyang
5. Gopal Lama –Local healer Daman 6
6. Ran Bahadur Thing- Key Informant
7. Man Bahadur Thing- Key informant, Daman 8
8. Angrej Bahadur Balla- Key informant, Daman 6
9. Akal Bahadur Balla- Key informant
10. Forest Range office Staff- Ranger, Khardar etc.

Appendix 2: Jadibuti of commercial importance in the study area:

Bojho (*Acorus calamus*), Lali gurans (*Rhododendron sps)*, Timur (*Xanthozylum armatum*), Jethimadhu (*Glycirrhiza glabra*), Bish (*Aconitum sps*), Khirailo (*Lilium nepalense*), Banmula (*Euphorbia fusiformis*), Banlahsun (*Allium wallichii*), Chiraito (*Swertia chiraita*), Sugandhwal (*Valeriana Jatamansii)* Jhyau (*Parmelia sps*), Pakhanved (*Berginea ciliate*), Lokta (*Daphane spps*), Dhasingre (*Gaultheria frgrantissima*), Siltimur (*Lindera neesiana*), Eklebhir (*Lobelia pyramidalis*), Chyau, Sunpati (*Rhododendron lepidotum*

Other NTFPs: Majhitho (*Rubia cordifolia*), Ram Tulsi (*Occimum baccilus*), Chutro (*Berberis spps)*, Kagati phool (*Citrus spps*), Ainsalu (*Rubus ellipticus*), Kafal (*Myrca esculenta*), Bhuinkafal, Sisnu (*Artica daoica*), Nundhiki (Osyris wightiana), Sadiko, Bantarul, Bhyakur (*Dioscorea deltoidea*), Rupsi, Githa (*Dioscorea bulbifera*), Bhakyanto, Ratopate (*Ajuga bracteosa*), Kalo ainsalu (*Rubus paniculatus*), Malagiri (*Pratia nummularia*), Chilaune (Schima wallichi), Nigalo (*Drepanostachyum intermedium*)

Appendix 3: A general description of bacteria of this study and their pathogenicity

Escherchia coli:
They are gram-negative mobile rods belong to the family Enterobacteriaceae. Their normal habitat is intestinal tract of humans and animals. They can also be found in water, soil and vegetation.

Pathogenicity (Cheesbrough, 1993)
It causes

- Urinary tract infections including cystitis, pyelitis and pyelonephiritis. This is commonest pathogen isolated from patients with cystitis. Recurrent infections are common in women.
- Wound infections, appendicitis, peritonitis and infection of the gall bladder.
- Bacteraemia and meningitis especially of the newborn.
- Diarrhoeal disease especially in infants but also in adults.

Shigella dysenteriae:
They are gram negative, rods belongs to the family Enterobacteriaceae. It is non-spore former, non capsulated and non-motile. Their normal habitat is only the human intestinal tract.

Pathogenicity (Cheesbrough, 1993)
It causes

- Bacillary dysentery (shigellosis) in developing countries, shigellosis has a high death rate especially among young children
- The infection includes toxaemia, sometimes bacteraemia and severe dysentery leading to marked dehydration and protein loss, inflammation and ulceration of the large intestine, hemorrhage, abdominal pain and high fever. Death can occur from circulatory collapse or kidney failure.

Staphylococcus aureus:
This is gram positive, non-motile cocci occur singly and in pairs. They are widely distributed in the environment. They form part of the normal microbial flora of the skin, upper respiratory tract and interstitial tract.

Pathogenicity (Cheesbrough, 1993)
It causes

- Abscesses, boils, styes and impetigo. It may also cause secondary infections of insect bites, ulcers, burns, wounds and skin disorders
- Conjunctivitis, especially of the newborn.
- Cross-infections in hospitals.
- Septicemia, endocarditic and osteomyelitis.
- Pneumonia and empyema.

- Mastitis (inflammation of the breast).
- Antibiotic associated enteritis.
- Food poisoning.
- Scalded skin syndrome in young children due to the toxin exfoliation.
- Toxic shock syndrome due to a colonization of *S. aureus* especially in the vagina.

Pseudomomas aureginasa

This is a gram negative, motile, slightly curved rod. They can be found in water, sewage, and vegetation and also in intestinal tract. They are frequently present in hospital environments, especially in moist, cleaning buckets and humidifiers

Pathogenicity (Cheesbrough, 1993)

It causes

- Skin infections especially at burn sites, wounds, pressure sores and ulcers.
- Urinary infection, usually following catheterization or associated with chronic urinary infection.
- Respiratory infection especially in patients with cystic fibrosis or conditions that cause immuno-suppression.
- External ear infection and eye infection.
- Septicemia especially in persons already in poor health.

Salmonella typhi

This is gram negative, motile, rod, non- capsulated and non spore former. It is mostly found in humans and rarely found in other animals

Pathogenicity (Cheesbrough, 1993)

It causes

- Typhoid (enteric) fever. The symptoms of infection include fever with low pulse rate, headache, toxaemia, and enlargement of spleen and apathy or mental confusion.
- Nephrotyphoid in those with urinary schistosomiasis specially in children with sickle cell disease and thalassaemia
- Abscess of the spleen and elsewhere.
- Meningitis and rarely pneumonia and endocarditis.

Vibrio cholera

This is gram negative; motile (by flagella) slightly curved rod and non-spore former. It is mostly found in fresh and brackish (slightly salty) water, shellfish and other seafood

Pathogenicity (Cheesbrough, 1993)

It mainly causes severe cholera with watery diarrhea containing vibrios; epithelial cells and mucus are passed. Urine is reduced in volume, dark in colour and usually contains albumin and casts.

Brief Curriculum Vitae

Name : ISHWAR CHANDRA DUTTA.
Date of Birth : 20-4-1950.
Designation : Professor.
Address : Institute of forestry, P.O. Box # 369; Pokhara, Nepal
Tel # 977-61-431179, Fax: 977-61-431563
Email: icdutta@yahoo.com

Academic Qualification:

Post Graduate Diploma in Forestry Teaching & Industry, Kuru College of Forestry, Finland.
M.Sc. in Forestry & Allied Subjects. Indian forest College, FRI, Dehradun, India
M.Sc. in Chemistry, Tribhuvan University, Nepal.
Ph,D, in Chemistry, Patna University, Patna , India.

Working Experience:

The author has 30 years of teaching and research experience in the field of chemistry and forestry. At present it includes Non timber forest product and soil conservation and watershed management. He is teaching in different levels – M.Sc and B.Sc at Institute of Forestry.He has supervised B.Sc, M.Sc students for their dissertation (Thesis works). He is supervisor of Ph.D students as well.

His research area is forestry in general and non timber forest products in particular. Besides teaching and research author has administrative and academic experiences by holding several posts time to time. He has worked as a **Project coordinator** of different research projects, as an **editor** of journal of forestry, as a **President** of Environment and Resources Development Center (ERDC), as **a Dean** Institute of Forestry, Tribhuvan University, as a **Chief Research Officer** IDRC supported "Farm Forestry Project" and others like **member secretary** research committee, chairman subject committee, **Head** of the Department, **member of faulty Board , Academic council , and member of examination Committee etc.** Currently he is working as a **Project manager/coordinator** Asia Link Quality Assurance and Sustainable Livelihood Project.

Publications and Research Reports:

More than 40 publications in various National and International Journals and seminar Proceedings on forestry, NTFP, Chemistry and soil.

Interest :

He loves teaching and keenly interested in various research on individual and collaborative basis in the field of forest science and NTFPs..

Merit Award:

i. MAHENDRA VIDHYA BHUSAN AWARD 2003
ii. NATIONAL EDUCATION AWARD 1994
iii Award From RECAST for scientific paper publication, T.U

International Travel:

Asia: India, China, Japan, Hong Kong, Thailand, Pakistan, Bangla desh, Sri-lanka,
Europe: Austria, Finland, Sweden, Denmark, England, Netherlands
Africa: Zimbabwe