

ETHNOMEDICINAL STUDY AND ANTIBACTERIAL ACTIVITIES OF SELECTED PLANTS OF PALPA DISTRICT, NEPAL

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Abstract: Altogether 25 plant species of Palpa district, Nepal have been documented for their ethnomedicinal uses and screened for their antibacterial activity. The disk diffusion method was used to test the antibacterial activity. Four strains of bacteria employed in test were two-gram positive *Bacillus subtilis*, *Staphylococcus aureus* and two gram negative *Escherichia coli* and *Pseudomonas aeruginosa*. These 25 plant species belong to 20 families. Leguminosae includes the largest number of plants (3 species) followed by Compositae, Euphorbiaceae and Labiatae (2 species each). Out of the 25 plant species, the extracts of 13 species (52%) showed positive response against at least one of the tested bacteria, while the extracts of 11 species (44%) showed positive response against at least 2 bacteria. Similarly the extracts of 10 species (40%) showed positive response against three bacteria and 9 species (36%) showed positive response against all of the 4 tested bacteria. However the extracts from 12 plant species showed no such antibacterial activity against any of the 4 strains of tested bacteria. They constitute about 48% of the total tested plant species. The findings of the present investigation will be helpful for traditional healers, local community and all those involved in the study of ethnomedicine.

Key words: Antibacterial test; Disk diffusion; Ethnomedicinal plants; Palpa district; Nepal.

I. INTRODUCTION

Innumerable biologically active compounds that are found in plants (Alade and Irobi 1993, Clark and Hufford 1993, Samy *et al.* 1999) possess antibacterial properties (Brantner and Grein 1994, Samy and Ignacimuthu 1998). Evaluation of plants possessing antibacterial activity for various diseases is growing in recent years (Clark and Hufford 1993). Ethnobotanical plants have a greater 'hit rate' or number of positive results than randomly selected plants (Balick 1994, Balick and Cox 1996, Vanden Berghe *et al.* 1986, Slish *et al.* 1999). Ethnobotanical information from all over the world has led to the discovery of the approximately 120 plant derived drugs which account for about 25 percent of all prescription drugs consumed per year in North America (Cox and Balick 1994).

Palpa district is located in Lumbini zone in the western development region of Nepal. It is situated between 27°34' to 27°57' N latitudes and 83°15' to 84°22' E longitudes. The total land area of this district is about 1366 sq. km. The forest area is about 711 sq. km, which constitutes about 52.11% of the total land area. In this district, altitude ranges from 314m (tropical) to 1845m (subtropical region). The district enjoys a monsoon type of climate with wet summer and dry winter. The average maximum temperature is 32° C and minimum 4° C. The total annual rainfall is 1903.2 mm. The monsoon starts from June and most of the precipitation occurs during June to September.

The ethnomedicinal use of plant resources of Nepal have been documented by various researches (Manandhar 1985, 1986, 1987, 1993, 1994, Shrestha 1988, Joshi and Edington 1990, Bhattarai 1992, Mahato 1998, Joshi and Joshi 2001, Rajbhandari 2001, Chaudhary *et al.* 2002, Taylor *et al.* 2002, Panthi and Chaudhary 2003); but only a few species have been screened for biological activity (Taylor *et al.* 1995, 1996, Taylor and Towers 1998, Parajuli *et al.* 2001, Chaudhary *et al.* 2002). In present investigation ethnomedicinal uses of 25 useful plants species of Palpa district have been documented and screened for their antibacterial activity.

2. METHODOLOGY

2.1 Ethnomedicinal data

Ethnomedicinal data were collected by consulting the local healers and knowledgeable villagers. The study was conducted by covering three different seasons (spring, rain and winter) between the years 2000-2003 in the Palpa district. A standard questionnaire was used to collect data, which includes local name of plants, plant parts used, methods of preparation and approximate dosage of administration. The uses of the plant species were verified in other villages cross checking the information with other respondents showing plant species in natural habitat or a collected sample. The data were considered valid if at least five informants provided similar uses about the medicinal plants.

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2.2 Plant Collection and identification

The plant specimens were collected from different areas of Palpa district, Nepal and used for laboratory testing and voucher specimen preparation. Two sets of plant specimens for each species were collected from the natural habitats pressed and dried for voucher specimen. Identification of specimens was confirmed at National Herbarium and Plant Laboratories, Godawari (KATH) and Central Department of Botany, Tribhuvan University, Kirtipur (TUCH). Ethnomedicinal data obtained in the field were documented and compared with published literature such as Bhattarai (1992), Joshi and Edington (1990), Manandhar (1985, 1986, 1987, 1993, 1994), Shrestha (1985). The voucher specimens were deposited at Central Department of Botany, Tribhuvan University (TUCH), Kirtipur.

2.3 Disk Diffusion Assay: The disk diffusion assay (Taylor *et al.* 1995) was used to screen for antibacterial activity. This was accomplished by placing a known amount of the extract on a small paper disk. This disk was placed on an agar growth medium containing a confluent lawn of bacteria. The absence of bacterial growth around the disk containing the extracts, indicated the plant extract containing antimicrobial activity against that particular bacterium.

2.3 (a) Preparation of the Test Extracts: Plant parts used in medicine were dried in shade and stored in cotton bags for antibacterial test. Two grams of ground material was soaked in 25 ml methanol for 24 hours and filtered using Whatman filter paper. This process was repeated for 3 times to extract the chemical substances from plant material. The filtrate was then allowed to evaporate until completely dry. The extract was dissolved in 2 ml of methanol. The concentration of the final extract was 1 g dried plant material/1 ml.

2.3 (b) Microorganisms: Four species of bacteria employed in test were two gram positive *Bacillus subtilis*, *Staphylococcus aureus* and two gram negative *Escherichia coli* and *Pseudomonas aeruginosa*. These species were obtained from Teaching Hospital, Tribhuvan University and used to test antibacterial activities of collected medicinal plants. Bacterial species were cultured in nutrient broth for 24 hours and fresh inoculums were taken for the test.

2.3 (c) Preparation of the Test Disks: Sterile test disks were prepared by dipping and saturating filter paper disks (4 mm in diameter) in plant extracts solution using spirit flame sterilized forceps. These disks were dried in the sterilized petridishes. The name (code) of the plant extract was written underneath the petridish.

Positive Controls: Positive control disks were prepared by dipping sterilized filter paper disks into a solution of tetracycline (5 mg/ ml in methanol) and drying on a sterile glass petridish.

Negative Controls: Negative control disks were

prepared by dipping the sterilized filter paper disks into methanol and drying on a sterilized glass petridish.

2.3 (d) Culture Media and Inoculum: Solid media of nutrient agar was prepared by dissolving 2.8 g powder of agar in 100 ml water. About 25 ml of nutrient media was poured into a petridish. The inoculum for bacteria was prepared by culturing a large number of bacteria in a tube containing 10 ml liquid media of nutrient broth and incubating over night at 37°C. The agar plates for the assay were prepared by labeling them with the date, the name of the microorganism and the name (code) of the disks. The inoculums of bacteria were transferred into petridish containing solid nutrient media of agar using a sterile swab. The swab was used to spread the bacteria on the media in a confluent lawn. It was done by rotating the petridish at 90° and continuing the spread of bacteria. One swab was used for one species of bacteria.

2.3 (e) Placing Test Disks: Dried test disks were transferred on bacterial lawn under aseptic conditions using spirit-flame sterilized forceps each time. Each disk was placed gently on the agar surface, and patted with the forceps so that it sticks. The petridish was incubated upside down at 37°C for 24 hours. Resulting zones of inhibition were observed and recorded as +ve and -ve results. The inhibitory zone around test paper disks indicates absence of bacterial growth and that was recorded as positive test and absence of zone as negative. Tests were repeated three times to insure reliability of the results.

3. RESULTS

3.1 Ethnomedicinal Information

In the present study ethnomedicinal uses of 25 selected plant species of Palpa district, Nepal have been documented. These species were most commonly used in the treatment of diarrhoea and dysentery, cuts and wounds, pain and swelling and boil and burns. These 25 plant species belonging to 20 families; Leguminosae (3 species), followed by Compositae, Euphorbiaceae, Labiatae (2 species each and remaining other (1 species each).

The parts of the plant most commonly used in the treatment of disease were leaves (8 species), stem bark (8) and root (8). These were mainly used in fresh condition.

Nomenclature of the plant species followed Hara *et al.* (1982); Hara and Willians (1979); Press *et al.* (2000). Botanical names are given in bold letters and arranged alphabetically, followed by family in parentheses, local vernacular name in inverted comma, common name (if any), and collection number (italic) in parentheses. It also includes plant parts used, quantity of plant parts, details of preparation method and mode of use

Ageratum conyzoides L. (Compositae) 'Gane jhar, Ganhaune Jhar, Ilame Jhar'; Bad smelling plant (*RBM*

47). Five to ten leaves are squeezed between the palms of hands and juice is dropped on the cuts or wounds to stop bleeding.

Amaranthus spinosus L. (Amaranthaceae) 'Ban lunde, Kande lunde'; Spiny amaranth, prickly amaranth. (*RBM 30*). Depending upon the needs about 10-100 heated leaves are applied locally three times a day for 5 days to cure boils and burns.

Asparagus racemosus Willd. (Liliaceae) 'Kurilo, Satawari'; Asparagus (*RBM 378*): About 5 teaspoonfuls of powder of dry root is taken three times a day for a week to cure urinary troubles. About a cup of root decoction (A tuberous root boiled in water) is taken by women thrice a day for 15 days after delivery as a tonic.

Berberis asiatica Roxb. ex DC. (Berberidaceae) 'Chutro, Chautari'; Berberry (*RBM 36*). Paste of root is applied externally on wounds and inflammations twice a day for about 4-5 days to cure them. Thick decoction of stem bark is taken to cure fever.

Butea minor Buch.-Ham. ex Baker (Leguminosae) 'Palabi' (*RBM 209*). About 2 teaspoonfuls powder of seed is given twice a day for 3 days to eliminate or destroy intestinal worms.

Centella asiatica (L.) Urban (Umbelliferae) 'Ghod Tapre'; Indian pennywort, water pennywort (*RBM 279*). About 4 teaspoonfuls of leaf juice (juice is obtained by squeezing 50 leaves between palms of hands) is taken orally in the morning for 2-3 weeks for its alleged cooling property to body and stomach.

Cinnamomum tamala (Buch.-Ham.) Nees & Eberm. (Lauraceae) 'Tejpat, Dalchini'; cinnamon leaf (*RBM 204*). Leaf infusion (about 5 leaves boiled in a cup of water) is given twice a day for 5 days to control diarrhoea and colic pain.

Clerodendrum viscosum Vent. (Verbenaceae) 'Bhait' (*RBM 287*). One teaspoonful of root paste (about 5cm long 10 pieces of root ground by mortar and pestle in water) is given orally twice a day for 7 days to cure blood dysentery.

Colebrookea oppositifolia Sm. (Labiatae) 'Dhasure' Dosul (*RBM 78*). Two teaspoonfuls of paste of root (about 5 cm long 5 pieces of root ground by mortar and pestle) is used twice a day for 7 days to cure epilepsy. Leaf paste is applied locally twice a day for 3-4 days to cure wounds.

Ficus racemosa L. (Moraceae) 'Dumri, Udumber'; Country fig tree (*RBM 381*). Paste of bark is applied twice a day for 2-3 days to cure swellings of foot and hands. Bark decoction (about 100g boiled in two cups of water) is gargled to cure mouth ulcer.

Flemingia strobilifera (L.) W.T.Aiton (Leguminosae) 'Bhatwasi' (*RBM 91*). Root juice (about 5 cm long five pieces of fresh root is crushed in a cup of water by mortar and pestle) is taken twice a day for 7 days to cure

diarrhoea and dysentery.

Justicia adhatoda L. (Acanthaceae) 'asuro'; Malbur nut (*RBM 19*). Warm decoction of the leaves (about 5 leaves crushed and boiled in a cup of water) is given twice a day for a month to treat asthma. Two or three fresh leaves are ground using mortar & pestle to obtain the juice. About two teaspoonfuls of leaf juice is taken with honey twice a day for a week as expectorant.

Mallotus philippensis (Lam.) Muell. (Euphorbiaceae) 'Rohini, Sindure'; Kamala tree (*RBM 67*). About 3-4 teaspoonfuls of bark juice (about 50g bark crushed in 10 teaspoonful water) is taken 3 times a day for 7 days to treat diarrhoea and dysentery.

Maesa chisia Buch.-Ham. ex D. Don (Myrsinaceae) 'Bilaune' (*RBM 103*). Plant juice (about 100g plant boiled in 1 liter) is used as insecticidal.

Melastoma normale D. Don 'Chulesi' (Melastomataceae) (*RBM 101*). About two teaspoonfuls of paste of plant (a 20 cm long branch ground by mortar and pestle) is taken twice a day for 7 days to cure diarrhoea and dysentery.

Melia azedarach L. (Meliaceae) 'Bakaino'; Persian lilac (*RBM 349*). Thick decoction (*Bakainu ko khoto*) prepared by boiling 100g crushed stem-bark in a cup of water is tied around the sprained part and kept for 3-4 days to relieve pain and swelling.

Mentha spicata L. (Labiatae) 'Pudina'; Spearmint (*RBM 356*). Leaves decoction (about 100 leaves boiled in a cup of water) is taken twice a day for a week to cure throat infection and indigestion. Its decoction with cinnamon is given to women for easy childbirth at delivery.

Mimosa rubicaulis Lam. subsp. **himalayana** (Gamble) H. Ohashi (Leguminosae) 'Boksi ghans' (*RBM 223*). Infusion of leaves (about 100 leaves boiled in half a cup of water) is given twice a day for 15 days to cure piles. About 2 teaspoonfuls of powdered root is given twice a day for two days to cure vomiting caused by weakness.

Myrica esculenta Buch.-Ham. ex D. Don (Myricaceae) 'Kaphal'; Box myrtle, Bayberry, Wax myrtle (*RBM 102*). Bark decoction (about 50g bark boiled in a cup of water) is given twice a day for 7 days to cure diarrhoea, dysentery and chronic bronchitis.

Rhus javanica Thunb. (Anacardiaceae) 'Bhaki Amilo' (*RBM 646*). About two teaspoonfuls of powder of dry fruit is taken orally twice a day for 5 days to cure dysentery. Paste of the bark is applied externally to the affected part once a day for 3 days to cure muscular swelling.

Schima wallichii (DC.) Korth. (Theaceae) 'Chilaune' (*RBM 116*). Two teaspoonfuls of pounded stem bark (about 50g fresh bark is ground in 5 teaspoonfuls of water by mortar and pestle) is given twice a day for 3-4 days to cure fever and stomach pain.

Table 1: Result of Antibacterial Test

Plant	Parts used in test	P. a.	S. a.	B. s.	E. c.
<i>Ageratum conyzoides</i>	Leaves	+ve	+ve	+ve	+ve
<i>Amaranthus spinosus</i>	Leaves	-ve	-ve	-ve	-ve
<i>Asparagus racemosus</i>	Tuberous root	+ve	+ve	+ve	+ve
<i>Berberis asiatica</i>	Stem bark	+ve	+ve	+ve	+ve
<i>Butea minor</i>	Flower buds	-ve	-ve	-ve	-ve
<i>Centella asiatica</i>	Leaves	-ve	-ve	-ve	-ve
<i>Cinamomum tamala</i>	Leaves	-ve	-ve	-ve	-ve
<i>Clerodendrum viscosum</i>	Root	+ve	+ve	+ve	+ve
<i>Colebrookea oppositifolia</i>	Leaves	-ve	-ve	-ve	-ve
<i>Ficus racemosa</i>	Stem bark	-ve	-ve	+ve	-ve
<i>Flemingia strobilifera</i>	Root	-ve	+ve	-ve	+ve
<i>Justicia adhatoda</i>	Leaves	+ve	+ve	+ve	-ve
<i>Maesa chisia</i>	Whole plant	-ve	-ve	-ve	-ve
<i>Mallotus philippensis</i>	Stem bark	+ve	+ve	+ve	+ve
<i>Melastoma normale</i>	Whole plant	-ve	-ve	-ve	+ve
<i>Melia azedarach</i>	Stem bark	-ve	-ve	-ve	-ve
<i>Mentha spicata</i>	Leaves	-ve	-ve	-ve	-ve
<i>Mimosa rubicaulis</i>	Leaves	-ve	-ve	-ve	-ve
<i>Myrica esculenta</i>	Stem bark	+ve	+ve	+ve	+ve
<i>Rhus javanica</i>	Fruit	+ve	+ve	+ve	+ve
<i>Schima wallichii</i>	Stem bark	-ve	-ve	-ve	-ve
<i>Solanum virginianum</i>	Root	-ve	-ve	-ve	-ve
<i>Terminalia alata</i>	Stem bark	+ve	+ve	+ve	+ve
<i>Thysanolaena maxima</i>	Root	+ve	+ve	+ve	+ve
<i>Tridax procumbens</i>	Whole plant	+ve	-ve	-ve	-ve
Tetracycline - Positive control		+	+	+	+
Methanol - Negative control		-	-	-	-

P. a.: *Pseudomonas aeruginosa*; S. a.: *Staphylococcus aureus*; B. s.: *Bacillus subtilis*; E. c.: *Escherichia coli*

Solanum virginianum L. (Solanaceae) 'Kantkari'; Indian salamin (RBM 277). Decoction of root (about 5cm long, 10 pieces of root boiled in a cup of water) is taken twice a day for seven days to cure cough, asthma and chest pain.

Terminalia alata Heyne ex Roth (Combretaceae) 'Saj'; (RBM 166). About 3-4 teaspoonfuls of fresh bark juice (about 50g bark crushed in 10 teaspoonfuls of water and filtered to obtain juice) is taken 3 times a day for 6 days to cure diarrhoea and dysentery

Thysanolaena maxima (Roxb.) Kuntze (Gramineae) 'Amriso' (RBM 335). Two teaspoonfuls root juice (5 pieces of root about 10 cm long, crushed by mortar and pestle) is given twice a day for 2-3 days as anthelmintic.

Tridax procumbens L. (Compositae) 'Kurkure' (RBM 180). A whole plant is squeezed between the palms of hands to obtain juice. Fresh plant juice is applied twice a day for 3-4 days to cure cuts and wounds.

3.2 Antibacterial test

Twenty-five species of ethnomedicinal plants were

screened for their antibacterial activity (Table-1). Out of the 25 tested plant species extracts, 13 (52%) showed positive response against at least one of the tested bacteria. These were *Ageratum conyzoides*, *Asparagus racemosus*, *Berberis asiatica*, *Clerodendrum viscosum*, *Ficus racemosa*, *Flemingia strobilifera*, *Justicia adhatoda*, *Mallotus philippensis*, *Myrica esculenta*, *Rhus javanica*, *Terminalia alata*, *Thysanolaena maxima*, *Tridax procumbens*. Similarly, 11 (44%) plant species extracts showed positive response against at least 2 bacteria, followed by 10 (40%) of the plant species showed positive response against three bacteria and nine (36%) of the plant species showed positive response against all of the tested bacteria. These 9 species were *Ageratum conyzoides*, *Asparagus racemosus*, *Berberis asiatica*, *Clerodendrum viscosum*, *Mallotus philippensis*, *Myrica esculenta*, *Rhus javanica*, *Terminalia alata*, *Thysanolaena maxima*.

Ten species of plant showed positive response against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and nine species of plants showed positive response against *Escherichia coli*.

4. DISCUSSION

The above documented plant species are widely used as ethnomedicine in Palpa district. Majority of species used in diarrhoea and dysentery, cuts and wounds, boils and burns, and pain and swelling. Therefore, these species are selected for ethnomedicinal use and antibacterial test.

Of these 25 tested plant species, 25 extracts were made from different plant parts used in medicine. These included 8 extracts from leaves, seven from stem bark, five from root, three from whole plant, one from fruit and one from flower buds. Majority of the root and the stem bark extracts showed positive response against all four strains of bacteria. Root extracts of *Asparagus racemosus*, *Clerodendrum viscosum*, *Thysanolaena maxima* showed positive response against all strains of bacterial species, while *Flemingia strobilifera* showed positive response only in two bacterial species. Similarly, the stem bark extracts of *Berberis asiatica*, *Mallotus philippensis*, *Myrica esculenta*, *Terminalia alata* showed positive response against all four strains of bacteria but *Ficus racemosa* showed active response only in *Bacillus subtilis*. The result was also verified by the work of Parajuli *et al.* (2001). Further out of eight extracts made from leaves of different plant species only two extracts made from *Ageratum conyzoides* and *Justicia adhatoda* showed positive response to bacterial strains and remaining six showed no such response. Extract made from fruit showed positive response against all four strains of bacteria tested and the extract made from flower buds showed no such response at all.

Out of 25 screened plant species seven species (*Cinamomum tamala*, *Clerodendrum viscosum*, *Flemingia strobilifera*, *Mallotus philippensis*, *Melastoma normale*, *Myrica esculenta*, *Terminalia alata*) were used to treat diarrhoea and dysentery, but not these seven plant species showed antibacterial activity against all tested bacteria. Stem bark of *Mallotus philippensis*, *Myrica esculenta*, *Terminalia alata*, and fruit of *Rhus javanica* showed antibacterial activity against all tested bacteria. The root extract of *Flemingia strobilifera* showed activity against *Staphylococcus aureus* and *Escherichia coli*. The extract of whole plant part of *Melastoma normale* showed antibacterial activity only against *Escherichia coli*, while leaves extract of *Cinamomum tamala* which was also used as spice showed no antibacterial activity against all the tested bacteria.

Three plant species (*Ageratum conyzoides*, *Colebrookea oppositifolia*, *Tridax procumbens*) were used to treat cuts and wounds. The extract of whole plant of *Tridax procumbens* showed antibacterial activity only against *Pseudomonas aeruginosa*, while the leaf extract of *Colebrookea oppositifolia* showed no antibacterial activity against all tested bacteria. Similarly, *Schima wallichii*, *Mentha spicata* used to treat various diseases showed only negative response against all tested bacteria.

Antibacterial test of the extracts of following 13 plant species tested showed positive response against all 4 bacteria strains to at least one of the bacteria. These species were *Ageratum conyzoides*, *Asparagus racemosus*, *Berberis asiatica*, *Clerodendrum viscosum*, *Ficus racemosa*, *Flemingia strobilifera*, *Justicia adhatoda*, *Mallotus philippensis*, *Myrica esculenta*, *Rhus javanica*, *Terminalia alata*, *Thysanolaena maxima* and *Tridax procumbens*.

Asparagus racemosus is a most important medicinal plant, and is used widely among different ethnic groups in Nepal (Chaudhary and Singh 1998). Its root powder is used in the treatment of urinary troubles and this part of the plant showed antibacterial activity against all four strains of bacteria tested. Another most widely used and easily available medicinal plant is *Berberis asiatica*. Thick decoction of stem bark of *Berberis asiatica* was used to cure fever and it was determined to have antibacterial activity against all four strains of bacteria used in test.

Root paste of *Clerodendrum viscosum* was used by different ethnic groups in treatment of blood dysentery. It showed antibacterial activity against all tested bacteria.

Warm decoction of the leaves of *Justicia adhatoda* was used to treat asthma. The extract of leaves showed activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Paste of stem bark of *Ficus racemosa* was used to cure swelling of foot and hand and showed activity against *Bacillus subtilis*.

The extracts made from 12 species of plant (48%) did not showed any antibacterial activity against all four strains of tested bacteria. These were *Amaranthus spinosus*, *Butea minor*, *Centella asiatica*, *Cinamomum tamala*, *Colebrookea oppositifolia*, *Ficus racemosa*, *Maesa chisia*, *Melastoma normale*, *Melia azedarach*, *Mimosa rubicaulis*, *Mentha spicata*, *Schima wallichii*, *Solanum virginianum*. The lack of activity, however, does not signify a plant as being 'useless' as a herbal medicine.

In conclusion, findings of the present investigation will be helpful in future ethnobotanical and ethnopharmacological studies in Nepal.

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