

Ethnobotany and in vitro antimicrobial study of selected medicinal plants used by Magar community in Dhaubadi VDC, Nawalparasi district, Nepal

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ABSTRACT

Magars are one of the oldest tribes in Nepal having indigenous knowledge of health care practices. Dhaubadi VDC is homogenously inhabited with Magars. There are authentic healers and elder people having knowledge of traditional health care practices. Data of traditional knowledge about *Oroxylum indicum* (L.) Kurz, *Premna barbata* Wall ex Schaure and *Lagetroemia parviflora* Roxb. were collected with voucher specimens. Antimicrobial tests of these crude plant extracts were done against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Salmonella typhi*, *klebsiella pneumoniae* and *Escherichia coli*) bacteria. Aqueous extract of *L. parviflora* showed antimicrobial test against Gram positive and Gram negative bacteria. Extract of *O. indicum* showed antimicrobial property against tested bacteria. Leaf extract of *P. barbata* showed antimicrobial property against *S. typhi*, *S. aureus* and *E. coli* but not to *B. subtilis*. The result of the research scientifically validated the traditional use of these plants as medicines.

Keywords: antimicrobial tests, Magars, medicinal plants, traditional knowledge

INTRODUCTION

Ethnobotanical study is an important tool to record plant data along with traditional botanical knowledge. This knowledge has been gained due to relationship between plant and people (Martin, 1995) which has been started from the beginning of human civilization. Currently, the plants with traditional knowledge are used normally for therapeutic purpose. World Health Organization (WHO, 2002) has reported that 80% of the world population use traditional medicines for primary health care (Farnsworth and Soejarto, 1991). About 90% of the Nepalese people reside in villages where access of Government health care facilities is not available (Bhattarai, 1998; Manandhar, 2002). They have to rely on traditional medicines.

Biological properties of traditional medicine are due to active compounds which are produced during secondary plant metabolism. The biological properties of plant species traditionally used are confirmed scientifically by antimicrobial tests and other chemical tests. These active compounds are used throughout the globe for various purposes including treatment of infection by micro-organisms. Numerous studies have aimed to describe the chemical composition of these plant antimicrobials (Husain *et al.*, 1992; Sinhababu *et al.*, 1999; Gabbriella *et al.*, 2010;

Luitel *et al.*, 2010) and mechanisms involved in microbial growth inhibition. Now-a-days these compounds become great interest for pharmaceutical intermediates and chemical entities for synthetic drugs (Tiwari *et al.*, 2011). About 25% of prescribed drugs contain active principles derived from higher plants (Tiwari & Joshi, 1990).

Magars are first largest indigenous ethnic nationality of Nepal (CBS, 2011). They are the oldest tribe in Nepal (Gautam & Thapa-Magar, 1995). They are dominated in Nawalparasi district, Dhaubadi village of Dhaubadi VDC. Magars in this site are closely linked with nature and have rich knowledge, skill and technique on traditional utilization of natural resources especially local plant species for traditional healing purposes. They use *Lagestroemia parviflora* Roxb., *Oroxylum indicum* (L.) Kurz and *Premna barbata* Wall ex Schauer as traditional medicines. *O. indicum* is one of the ingredients in Dashmularistha and Chyavanprash in Ayurvedic medicine (Lawania *et al.*, 2010).

Dhaubadi VDC has no government facilitated hospital. The local people rely on traditional medicine for their primary health care. Documentation and scientific validation of their traditional medicine is necessary. This research is focussed on antimicrobial study for scientific validation of ethnomedicinal knowledge of three plants.

MATERIALS AND METHODS

Study area

Ethnobotanical study was carried out in ward no. 1, 2 and 9 of Dhauwadi VDC in Nawalparasi District. The study area extends from 150 to 1900m altitude. The tentative coordinates of the study area ranges from 27°36'N to 27°45'32"N Latitude and 84°05'E to 84°09'E Longitude. The climate is tropical to sub-tropical. The Magars reside in this VDC are ethnic group and have indigenous knowledge of medicinal plants.

Plant collection

The study site, Dhaubadi VDC of Nawalparasi district was visited on January 2014, April 2015 and March 2016. The dominant community in these sites is Magars who are ethnic, indigenous of Nepal and second largest population after Chhetri and Bramin. Ethnomedicinal data were collected by PRA and semi-structural interview methods with authentic healers and respondents having knowledge of herbal medicines. The plants were collected during transect walk with local informants having knowledge of vegetation. Herbaria were prepared and identified by standard botanical procedure, referring Hara *et al.* (1978), Press *et al.* (2000), and website (www.theplantlist.org). Final identification was done in, Department of plant Resources, National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal. The plant materials for antimicrobial study were dried in shade at room temperature.

Extract preparation

The air dried plant materials were ground in electric grinder and extracted in Soxhlet apparatus successively with hexane and methanol and concentrated under vacuum at 40°C for hexane and 45°C for methanol by using rotary evaporator. The dried extract was stored in refrigerator at 4°C. Dried extract was dissolved in hexane and methanol separately to make 0.1 g/ml concentration for antimicrobial study. For aqueous extract plant material was boiled on water bath.

Micro-organism

Clinical isolates of the micro-organisms already collected in biology section, Department of Plant Research (DPR), Thapathali, Kathmandu were used along with the standard strains. *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae* were used in susceptibility test. These micro-organisms were sub cultured in sterile agar nutrient media. The isolate colonies were diluted in nutrient broth and compared with 0.5 McFarland standards.

Inoculum preparation

It was prepared by suspending the separate colonies in broth (Mueller Hinton) and standardize with 0.5 McFarland standards. Inoculum was prepared for each test bacterium just before use.

Antimicrobial screening

The antibacterial screening of three medicinal plant species extracts were evaluated by using the agar well diffusion technique. The 20 ml of sterilized nutrient agar (Hi media) was poured into sterile petri dishes. After solidification the prepared inoculums were swabbed on the respective plates. The wells were punched on the agar gel using sterile borer of 6 mm diameter. The wells were filled with 50 μ l of the prepared plant extract. Chloramphenicol of 0.03mg/ml concentration was used as standard reference (Cavalieri *et al.*, 2005). The plates were incubated at 37°C \pm 2°C for 24 hours. Tests were performed in duplicate. Same tests were repeated once again. Presence of zone of inhibition was measured in millimeter (mm).

RESULTS AND DISCUSSION

Ethnobotanical data were collected by semi-structural interview during the field survey. Three plant species belonging to three families are arranged in table 1 giving scientific name, family; voucher no, local name, common name, part used, form and diseases. The result of antimicrobial tests is given in table 2. Photographs of antimicrobial tests showing zone of inhibition are presented below (fig. 1).

TABLE 1. Ethnomedicinal plants used by Magar community, Dhaubadi VDC, Nawalparasi, Nepal.

S.N.	Scientific name (Family, voucher number)	Local name (Common name)	Part used	Form	Diseases
1.	<i>Lagestroemia parviflora</i> Roxb. (Syn. <i>Fatima napaulensis</i> DC.) {Lythraceae, 365}	Botdhayero, rangsing (Crape myrtle)	Stem, bark and Leaves	Decoction	Decoction use for irregular menstruation. Decoction of leaves, stem bark use for pneumonia, typhoid.
2.	<i>Oroxylum indicum</i> (L.) Kurz. {Bignoniaceae, 103 }	Sawane, Total (Broken bone tree)	Seed, stem and bark	Seed and ash	Seed is eaten for typhoid, fever, Ash of bark apply on infective wound.
3.	<i>Premna barbata</i> Wall.ex. Schauer {Lamiaceae, 329}	Gindari (Agni matha)	Leaf, stem bark	Juice	Juice is eaten for typhoid, paste apply on forehead for headache.

TABLE 2. Antimicrobial activity on Zone of Inhibition (mm) of three ethnomedicinal plants.

Bacterial strains	<i>Lagestroemia parviflora</i>			<i>Oroxylum indicum</i>						<i>Premna barbata</i>		Chlo
	Stem bark			Stem bark		Leaf		Seed		Leaf		
	Hex	Met	Aqu	Hex	Met	Hex	Met	Hex	Met	Hex	Met	
<i>B. subtilis</i>	-ve	-ve	-ve	12.00	7.0	14.00	9.00	-ve	-ve	-ve	-ve	11.00
<i>E. coli</i>	8.00	-ve	13.00	15.00	-ve	20.00	23.00	-ve	-ve	10.00	-ve	13.00
<i>K. pneumoniae</i>	8.00	10.00	8.00	9.00	-ve	-ve	9.00	8.00	8.00	-ve	-ve	-ve
<i>S. aureus</i>	-ve	15.00	11.00	15.00	8.00	15.00	18.00	-ve	-ve	-ve	13.00	-ve
<i>S. typhi</i>	-ve	-ve	16.00	8.00	7.0	10.00	9.00	v	-ve	7.00	12.00	12.00

Note: negative= no inhibition zone

Hex: Hexane, Met: Methanol, Aqu: Aqueous, Chlo: Chloramphenicol

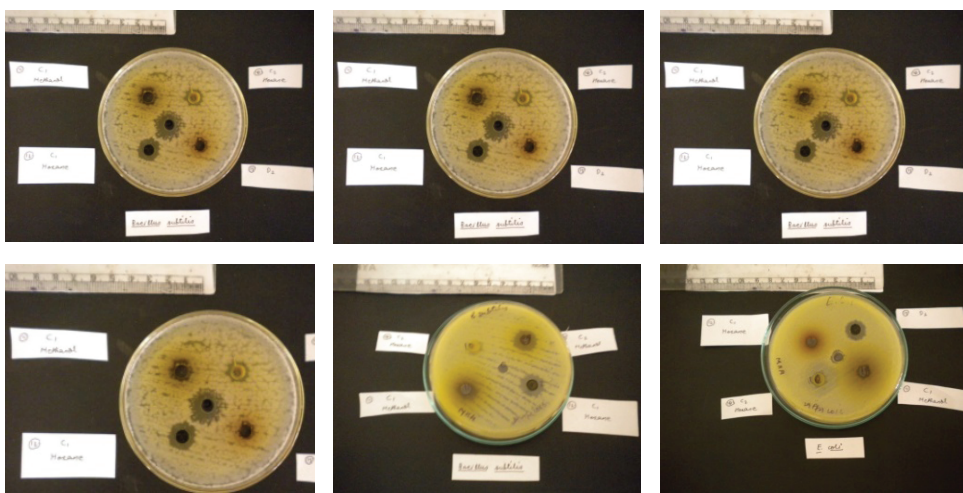


FIG. 1. Antimicrobial screening with inhibition zone in positive test and without inhibition zone in negative test.

The present study revealed that crude extract of each medicinal plant have different degree of inhibition against different micro-organisms. The maximum zone of inhibition was observed in methanolic extract of *O. indicum* leaf against *E. Coli* (23 mm) followed by hexane extract of *O. indicum* leaf against *E. coli* (20 mm) and then methanolic extract against *S. aureus* (18 mm). Inhibition zone in hexane extract of *O. indicum* leaf against *S. aureus* was equal to methanolic extract of stem bark of *L. parviflora* (15mm). Aqueous stem bark extract of *L. parviflora* exhibited antimicrobial activity against *S. typhi*, *K. pneumoniae*, *E. coli* and *S. aureus* except *B. subtilis*. Antibacterial activity of *L. parviflora* was previously reported as leaf extract of it was very active against Gram negative bacteria (*E. coli*) at low concentration than Gram positive (*S. aureus*, *B. subtilis*) (Mazumder *et al.*, 2002). Phytochemical studies of methanolic extract of

L. parviflora reveal the presence of tannin, triterpenoid, steroid and flavonoid (Mazumder *et al.*, 2005). Whole plants of *L. parviflora* is source of triterpenoid known as lageflorin (Sinhbabu *et al.*, 1999). These compounds are supposed to be responsible for antimicrobial activity. The result of this antimicrobial tests revealed that ethnobotanical knowledge of the Magars of the VDC has scientific value for *L. parviflora* Roxb.. This extract showed antimicrobial activity to *E. coli* which can cause bloody diarrhea. This study revealed out more knowledge about the other biological properties of the plant besides traditional use at the VDC.

Antibacterial activity of *O. indicum* (Das & Chaudhary, 2010; Luitel *et al.*, 2010; Radhika *et al.*, 2011; Samatha *et al.*, 2013;) and *P. barbata* (Tanta *et al.*, 2012) were previously reported. Alcoholic extract of stem of *O. indicum* has shown antimicrobial activity against *E. coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas aeruginosa* and *E. coli*. Alcoholic extract of stem has more antimicrobial activity than alcoholic extract of root of *O. indicum*. There are differences in bioactive components between these two parts (Radhika *et al.*, 2011) which may be the cause of difference in degree of antimicrobial activities between different parts. *Oroxylum indicum* has broad range of bioactive compounds (Deka *et al.*, 2013). Flavones namely baicalein and oroxylin were claimed to be new compounds in *O. indicum* (Luitel *et al.*, 2010). The antimicrobial tests of seed extract did not show antimicrobial property to the test bacteria except *K. pneumoniae* (table 3). Only mild inhibition zone was formed by the seed extract against *K. pneumoniae*. But it did not mean absence of antimicrobial property because there were so many infectious bacteria remain to be tested. The Leaf extract of it showed encouraging inhibition zone (above 14 mm) against *S. aureus* and *E. coli* but mild inhibition zone against *K. pneumoniae*, *S. typhi* and *S aureus*. *S. aureus* causes skin infection. This result matches with the ethnobotanical knowledge of Magars in the VDC as *O. indicum* is used in wound infection. The plant extract has strong antimicrobial activity against *E. coli*. This finding proves use of the plant in diarrhea and dysentery. The stem bark extract of *O. indicum* showed moderate antimicrobial property than leaf. The degree of antimicrobial properties between stem bark and leaf are different.

P. barbata Wall ex Schauer leaf extract showed antimicrobial activity to *S. typhi*, *E. coli* and *S. aureus*.. This plant extract showed antimicrobial activity against Gram positive as well as Gram negative bacteria. The alcoholic extract of root bark of *P. integrifolia* was active against Gram positive whereas *P. oligotricha* was weak activity against Gram positive bacteria (Rekha *et al.*, 2015). Although more than hundred secondary metabolites are separated from different species of *Premna* by phytochemical work only one compound prenosidic acid was reported from leaves of *P. barbata* (Negi *et al.*, 2004). So, more work has to be done on *P. barbata*.

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REFERENCES

- BHATTARAI, N K (1998) Traditional medicine, medicinal plants and biodiversity conservation in global and Nepalese context. *Plant Research* 1(1): 22–31.
- CAVALIERI, S J; HARBECK, R J; MACARTER, Y S; ORTEZ, J H; RANKIN, I D; SAUTTER, R I; SPIEGEL, C; A (2005) *Manual of Antimicrobial Susceptibility Testing*. Seattle, University of Washington, Washington, USA.
- CBS (2011) *Statistical pocket book of Nepal*. Kathmandu: Central Bureau of statistics, Government of Nepal.
- DAS, S; CHAUDHARY, M D (2010) Antimicrobial activity of stem bark extracts from the plant *Oroxylum indicum* Vent. *Asian University Journal of Science and Technology* 15(1): 95–99.
- DEKA, D C; KUMAR, V; PRASAD, C; KUMAR, K; GOGOI, B J; SINGH, L; SRIVASTAVA, R B (2013) *Oroxylum indicum*– a medicinal plant of north east India: an overview of its nutritional, remedial, and prophylactic properties. *Journal of Applied Pharmaceutical Science* 3 (1): 104–112.
- FARNSWORTH, N R; SOEJARTO, D D (1991) Global importance of medicinal plants. In AKEVELEV, O; HEYWOOD, V; SYNGE, H (eds) *The conservation of medicinal plants*. Cambridge University Press; Cambridge, UK; pp 25–51.
- GABBRIELLA, I.; SEFAO, D; GIDITTA, S; ELENA, B; SILVIO, S; GURUNG, K; BARBERA, M; CARRARA, M (2010) Chemical composition and biological properties of *Rhododendron anthopogon* essential oil. *Molecules* 15: 2326–2338.
- GAUTAM, R; THAPA-MAGAR, K (1995) *Tribal ethnography of Nepal*. Books Faith, India.
- HARA, H; STEARN, W T; WILLIAMS, L H J (1978) *An enumeration of the flowering plants of Nepal*. The George Press, England, UK.
- USAIN, A; VIRMANP, O P; POPALI, S P; MISHRA, L N; GUPTA, M M; SRIVASTAVA, G N; ABRAHAM, Z; SINGH, A K (1992) *Dictionary of Indian medicinal plants*. Central Institute of Medicinal and Aromatic Plants, Lucknow, India.
- LAWANIA, R D; MISHRA, A; GUPTA, R (2010) *Oroxylum indicum*: a review. *J pharmacognosy* 2 (9): 304–310.
- LUITEL, H N; RAJBHANDARI, M ; KALAUNI, S K ; AWALE, S ; MASUDA, K ; GEWALI, M B (2010) Chemical constituents from *Oroxylum indicum* (L.) Kurz of Nepalese origin. *Scientific World* 8 (8): 66–68.
- MANANDHAR, N P (2002) *Plants and people of Nepal*. Timber Press, Portland, Oregon, USA.
- Martin, G J (1995) *Ethnobotany: a method of manual*. Chapman and Hall, London, UK.
- MAZUMDER, A; SINGN, S K; MAZUMDER, R; BASU, S P; SAHA, B P (2002) Antimicrobial action of the leaf extract of *Lagerstroemia parviflora* Roxb. *Ancient Science of Life* XXI (3): 198–201.
- MAZUMDER, A; SAHA, B P; BASU, S P; MAZUMDER, R (2005) In vitro antimicrobial efficacy of *Lagestromia parviflora* Roxb. flowers. *Pakistan Journal of Biological Science* 8(8): 1157–1159.
- NAGI, S ; SHUKLA, V ; RAWAT, M S ; PANT, G ; NAGATSU, A (2004) Premnosidic acid, a new iridoid glycoside from *Premna barbata*. *Indian Journal of Chemistry* 43b (8): 1805–1806.
- PRESS, J R; SHRESTHA, K K; SUTTON, D A (2000) *Annotated checklist of the flowering plants of Nepal*. Natural History Museum, London.
- RADHIKA, LG; MEENA, C V; PETER, S; RAJESH, K S; ROSAMMA, M P (2011) Phytochemical and

antimicrobial study of *Oroxylum indicum*. *Ancient Science of Life* 30 (4): 114–120.

REKHA, K; RICHA, P; SURESH B K; RAO, J M (2015) A Phytochemistry of the Genus *Premna*: a review. *International Journal of Pharmaceutical and Chemical Sciences* 4 (3): 317–325.

SAMATHA, T; SAMPATH, A; SUJATHA, K; RAMA, S N (2013) Antibacterial activity of stem bark extracts of *Oroxylum indicum*: an endangered ethnomedicinal forest tree. *Journal of Pharmacy and Biological sciences* 7(1): 24–28.

SINHABABU, A; LASKAR, S; THAKUR, S; BASAK, B K (1999) A brief resume on the genus *Lagerstroemia*. *Asian Journal of Chemistry* 11(4): 1089–1106.

TAMTA, M; KUMAR, A; SHUKLA, N; NEGI, D (2012) Effects of crude extracts of *Premna barbata* Wall. and *Clerodendrum viscosum* Vent. (Verbenaceae) on different pathogenic bacteria. *Asian journal of Traditional Medicine* 7(1): 1–7.

TIWARI, N N ; JOSHI, M P (1990) Medicinal plants of Nepal volumes I, II and III. *Journal of Nepal Medical Association* 28: 181–190, 221–232, 266–279.

TIWARI, P; KUMAR, B; KAUR, M; KAUR, G; KAUR, H (2011) Phytochemical screening and extraction: a review. *International Pharmaceutical Science* 1(1): 98–106.

WHO (2002). *WHO traditional medicine strategy 2002-2005*. World Health Organization, Geneva, Switzerland.