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Socio-ecological Status and Antibacterial Activity of *Paris polyphylla* from Panchase Area of Kaski District

Meena S. Chhetri, Yajna P. Timilsina, Hari P. Tripathee and Krishna P. Devkota
Institute of Forestry, Tribhuvan University
Pokhara Campus, P. O. Box: 43, Pokhara, Nepal
e-mail: devkotakpd@yahoo.com

Abstract

The research conducted in Panchase area of Kaski district aimed to assess the ecological and social status of *Paris polyphylla* with its antibacterial activity and ethno-medicinal uses of available plant species. *P. polyphylla* shows relative density, frequency and coverage of 5.26/ha, 6.29% and 3.05%, respectively. Similarly, the importance value index of this plant was found to be 14.6. Rhizomes of this plant in the study site are mainly applied to wounds for rapid healing and antidote to the bite for poisonous insects and snakebite, fever, headache and stomach problem of both human and domestic animals. Different extracts of aerial parts of the plant are active against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aureginosa*, *Escherchia coli* and *Salmonella flexinerai* whereas, rhizome extract was active only against *S. aureus*. Petroleum ether extract from the rhizomes of *P. polyphylla* was most effective against *S. aereus* with minimal inhibitory concentration value of 62.5 mg/ml.

Key words: ecological, importance value, minimum inhibitory concentration, *paris polyphylla*, zone of inhibition, index and social status

Introduction

Panchase forest area is situated at the nexus of three districts, Kaski, Parbat and Syanja in the Western Region of Nepal. It covers an approximate area of 10-12 square kilometer situated in the altitude range of 1400 – 2517 m. It is one of the largest source of different NTFPs. The forest is mainly divided into two zones: sub-tropical zone which is dominated by *Schima wallichii- Castanopsis indica- Daphniphyllum himalense* and temperate zone that is dominated by *Quercus semicarpifolia-Quercus lamellose-Rhododendron arboretum*. The local people consider the Panchase forest and the mountain peak (2509 m) as a sacred landscape and it has special religious significance for both Hindu and Buddhists. Major ethnic groups are Brahmin, Chhetri, Gurung, Bishwokarma, Nepali, Pariyar, Magar and Thakali. The Panchase Forest is major source of water for agricultural and domestic use in the surrounding communities, as well as the primary source area for a number of local watersheds including Phewa Lake Watershed. Furthermore this forest PFE is rich in biodiversity, home to a variety of endangered wildlife such as black bear, leopard, etc. and rare and endemic plants including orchids and rhododendron. It also represents a critical linkage between Chitwan-Annapurna range and an important component of Greater Himalayan Landscape containing diverse medicinal plants.

Medicinal plants are the local heritage with global importance. The world is endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine and they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in concert with the body's own defenses (Manandhar 1993). Medicinal plants also play an important role in the lives of rural people particularly in remote parts of developing countries with few health facilities (Prajapati & Purohit 2003). The plants are the most valuable resources of Nepal, which provide wide range

of useful products: food, medicine, timber, fodder, fuel wood, fiber, condiments, etc. Among different plant resources, medicinal and aromatic plants (MAPs) have been considered as one of the most precious and important source of medicine for the local healers in the villages, as well as the basic raw materials for Ayurvedic, Tibetan, Homeopathethic and Allopathic medicines. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition which are found as secondary plant metabolites in one or more parts of these plants (Prajapati & Purohit 2003).

P. polyphylla Smith (Local name: Satuwa) is broad-leaved perennial plant that has a preference to woodlands, forests, bamboo forests, thickets, grassy or rocky slopes and streamside. It is distributed in Himalayan region from Kashmir to Bhutan and Western region in China. It grows naturally in sub-tropical and alpine zones. It can grow in full shade (deep woodland) or semi-shade (light woodland) moist habitat mostly on the exposed north facing slopes (Dutta 2007). In Nepal, *P. polyphylla* is distributed abundantly in Eastern, Mid and Western regions between 2000-3000 m elevation range with associated *Rhododendron* species, *Daphniphyllum himalense,* etc. The average market price of *P. polyphylla* is Rs. 260-350 per kg. The rhizomes have high demands in both national and international markets for its valuable rootstock to treat variety of ailments (Bhattarai & Ghimere 2006).

Methodology

Study area selection

Panchase forest is considered as a one of the large sources of non-timber forest products including medicinal plants. Altogether 335 species of flowering plants including 107 species of orchids together with some valuable medicinal and aromatic plants such as *P. polyphylla, Swertia chiraita, Taxus baccata, Asparagus racemosus, Rubia cordifolia,* etc. were reported from this forest area (MDO 2005) stimulated us to conduct a research on the selected site.

Data collection

Social Data Collection (Exploration of ethno-medical knowledge)

Participatory rural appraisal tools (structured questionnaire, household survey, key informant interview) were used to explore ethnomedicinal knowledge of the rural people of Bhadaure Tamagi VDC of Panchase area. At least 10% respondents were selected for the structured questionnaire household survey to understand their knowledge regarding ethnomedicinal uses of *P. polyphylla*. Key informants interview (comprising of the local collectors, middlemen, women and Dhami) were conducted to collect information on distribution status and utilization of *P. polyphylla*.

Biophysical data collection

Biophysical data were collected by following four methods:

- **Preliminary survey:** First of all, preliminary survey of the *P. polyphylla* habitat was done before starting the study to identify the appropriateness and adequacy of data and to ensure better results as well as to observe the habitat characteristics of the targeted species.
- **Participatory Resource Mapping (PRM):** PRM was drawn to identify the potential areas of *P. polyphylla* inside forest area.
- **Direct Field Observation**: It was done in and around the field site for the identification of MAPs species including *P. polyphylla*. It helped in the verification of the accuracy of participatory mapping. The species composition, their number and condition were observed in the forest during field observation.
- **Area Delineation:** After above study, the best *P. polyphylla* habitat of 58 ha was identified in Panchase protected forest possessing northern aspect and was surveyed by using GPS. Then, inventory was done within that total surveyed area according to plotless survey method (Rabindranath Premnath 1997).

Data analysis

Data collected during field visit and sampling were thoroughly analyzed both quantitatively and qualitatively using appropriate statistical tools depending upon nature of data. Both quantitative and qualitative data were analyzed as described by Dingle *et al.*1953, WHO 1991, Salie *et al.* 1996 and Rabindranath and Premnath 1997.

Laboratory test

Plant processing and extraction

The healthy plants of *P. polyphylla* were collected from the study site. The plants were processed according to the method of Salie *et al.* (1996) and

separated into aerial and underground parts. Each separated part was chopped into small pieces and shade dried. The dried plants were ground and extracted using different solvents such as petroleum ether, chloroform, methanol and water which was then concentrated to yield crude extract of respective solvents. Five hundred miligrams of each extract was transferred into a clean, dry and sterile test tube and 1 ml of ethanol was added to each tube to make 500 mg/ml dilutions which was used for the determination of the zone of inhibition (ZOI) of tested bacteria. Lower concentrations (250, 125, 62.5, 31.25 and 15.625 mg/ml) were also used to determine minimum inhibitory concentration (MIC).

Antibacterial activity test

Preparation of stock culture of bacteria

Five strains of bacteria such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aureginosa, Escherichia coli* and *Salmonella flexinarae* were used for the screening process. All of the tested bacteria were received from western regional hospitals (WRH) and Pokhara cultured collections.

Agar well diffusion technique (Cup plate technique)

In this technique, small cups (wells) were made by a 4 mm cork borer in petri-plates containing Muller Hinton Agar medium and inoculated test organism (Devkota & Dutta 2001). The diluted extract (test solution) was added to the wells. Which were then placed in an incubator at 37°C for 18-24 hours and the zone of inhibition was measured. This method was successfully used by Pepeljnjak *et al.* (1999) and Devkota *et al.* 2000. The antibiotics, ciprofloxacin and norfloxacin were used as standard drugs during the assay.

Two fold serial dilution technique

This is a technique used to determine MIC of bioactive compounds. This technique was recommended by WHO (1991) and used successively by Devkota *et al.* (2000) *et al.* and Devkota & Dutta (2001).

Results and Discussion

Ethno-medicinal knowledge of *P. polyphylla* and associated species

The ethno-medicinal uses of *P. polyphylla* and its associated species that are found in Panchase forest area of Kaski district are presented in Table 1. The findings indicated that together with *P. polyphylla*, there are several other medicinal plant species in the study site that are being used by local people to treat different diseases and also in their seasonal cookery. People have full faith with those medicinal plants and majority of the respondents wanted to continue this practice and sustain it by conserving those species in their natural habitat.

Table 1. List of medicinal plants with their ethno-medicinal uses found in Panchase forest

S N.	Botanical name (Family)	Local Name	Parts used	Ethnomedicinal uses
1.	*P. polyphylla* (Liliaceae)	Satuwa	Rhizomes	• Root paste is applied to wounds for rapid healing and antidote to the bite for poisonous insects and snakebite. • Rhizome powder is used for fever, headache and stomach problem of both human and domestic animals. • Chewing a piece of rhizome is believed to alleviate drunkardness and throat problem.
2.	*Swertia chiraita* (Gentianaceae)	Chiraito	Whole plant	Juice of plant boiled in water is used to get relief from fever, body ache, cough, stomach pairing, gastric problem, malaria, typhoid, high blood pressure, diabetes and joint problem.
3.	*Asparagus racemosus* (Liliaceae)	Kurilo	Tender shoots, Roots	Root paste is taken to treat fever and as a tonic to lactating women and livestock. Tender shoots are cooked as vegetable.

4.	*Berberis aristata* (Berberidaceae)	Chutro	Fruit	Fruits are edible and used for making alcohol.
5.	*Rubus elluoticus* (Rosaceae)	Ainselu	Roots, Fruit, Tender shoot	Ripe fruits are eaten. Tender shoots are used to treat fever. Root juice is used to treat typhoid and indigestion.
6.	*Bergenia ciliate* (Saxifragaceae)	Pakhan-ved	Rhizomes	Juice from rhizome is useful in joint problem. Rhizome is also used as an antidote.
7.	*Lindera neesiana* (Lauraceae)	Sil timur	Seed	Seeds are used in treating stomach paining and diarrhea.
8.	*Acorus calamus* (Araceae)	Bojho	Rhizomes	Rhizomes are used to treat cough, cold, throat infection, diarrhea.
9.	*Girardiana diversifolia* (Urticaceae)	Allo	Roots, Tender Parts	Tender parts are boiled and eaten as vegetables. Root juice is used for cooling purpose and fever.
10.	*Cissampelos pareira* (Menispermaceae)	Batulpate lahara	Whole plant	Plant paste is given to livestock to stop bleeding and counteract the loss of blood.
11.	*Ampelocissus divaricata* (Vitaceae)	Pureni	Whole plant	Plant juice is used to treat inflammation of eye.
12.	*Rhus javanica* (Anacardiaceae)	Bhakimlo	Seeds	Power of seed is used to treat stomach paining.
13.	*Cheilanthes anceps* (Pteridaceae)	Rani jhar	Whole plant	Plant paste is used in wound after operation as an analgesic.
14.	*Urtica dioca* (Urticaceae)	Sisnu	Roots, tender shoots	Tender shoots are used as vegetable. Root juice is used for cooling purpose.
15.	*Allium blansum* (Amaryllidaceae)	Ban lasun	Rhizome	Rhizome is used to treat uric acid and stomach problem.
16.	*Daphne bholua* (Thymelaceae)	Lokta	Bark, Roots	Roots are used for parasites. Bark of the stem is used for making ropes.
17.	*Adiantum caudatum* (Adiantaceae)	Fern	Whole plant	Plant paste is used to control bleeding.
18.	*Tinospora sinensis* (Menispermaceae)	Gurjo	Whole plant	Plant is used for urinary problem. Root juice is used to treat diarrhea and dysentery.
19.	*Combretum Roxburghii* (Combretaceae)	Thakailo	Root	Root juice is used to treat fever and cooling purpose.
20.	*Centella asiatica* (Umbelliferae)	Ghotapre	Leaves	Leaves are used as a vegetable. Plant is used for treatment of skin diseases and for cooling purpose.
21.	*Costus speciosus* (Zingiberaceae)	Betlauri	Roots	Root paste is used for throat problem.
22.	*Cissampelis Pareira* (Menispermaceae)	Gujar-gano	Whole plant	Roots are used in cough, diarrhea, snake-bite, etc. Leaves are externally applied for itch.

23.	*Artemisia vulgaris* (Compositae)	Titepati	Leaves	Leaves paste is used in wound.
24.	*Imperata cylindrical* (Gramineae)	Siru	Roots	Roots paste is used as an anti-parasite.
25.	*Mahonia napaulensis* (Berberidaceae)	Jamanem-andre	Bark	Bark paste is used to treat Jaraikhatira and inflammation of eye.
26.	*Polypodiodes amoena* (Polypodiaceae)	Bishphej	Rhizome	Powdered rhizome is used for relief of backache.
27.	*Smilax ovalifolia* (Liliaceae)	Kukurdi-ano	Roots, fruit, Tender shoots	Roots are used for dysentery. Fruit are edible and tender shoots are cooked as vegetable.
28.	*Arum esculentum* (Araceae)	Ban karkalo	Leaves	Leaf juice is used in internal haemorrhages(discharge of blood from blood vessels)
29.	*Rubia cordifolia* (Rubiaceae)	Majitho	Roots	Root is used to cure cough, jaundice and dysentery.
30.	*Arisaema intermedium* (Araceae)	Sarpa ko makai	Roots Leaves	Root paste is applied in case of ulcer. Leaf decoction is used to treat fever.

Ecological distribution of *P. polyphylla* and its associated species

The bio-physical data of *P. polyphylla* and its associated medicinal plants were studied by measuring frequency, relative frequency, density, relative density, relative coverage and important value index.

In the study, it was found that the frequency of *A. intermedium* was highest and *G. diversifolia* was lowest with values of 91% and 12%, respectively (Figure 1). Frequency depends upon the occurrence of species and *P. polyphylla* shows frequency of only 26%. It might be due to inappropriate schedule of our field work. Its proper availability time period is from April-May which was one and half month before our field work.

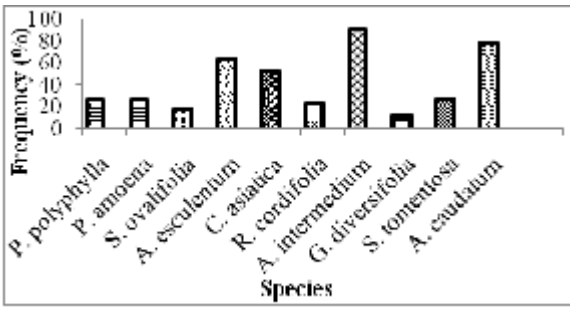

Fig. 1. Frequency of *P. polyphylla* and associated herbs

Fig. 2. indicated that *A. intermedium* has highest relative frequency of 21.68 followed by *A. caudatum, A. esculentum, C. asiatica, S. tomentosa, R. cordifolia, P. polyphylla, P. amoena, S. ovalifolia* and *G. diversifolia. P. polyphylla* shows an average relative frequency when compared with its associated species.

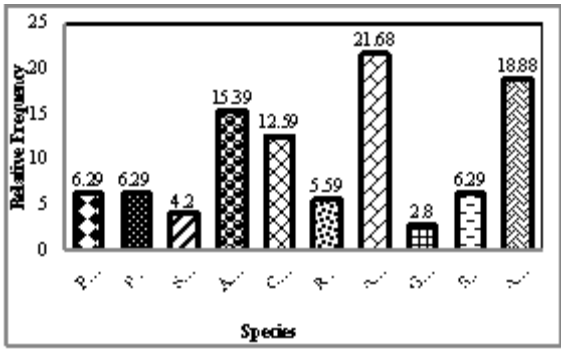

Fig. 2. Relative frequency of *P. polyphylla* and associated herbs

The study showed that *A. caudatum* has high density, thus is the dominant species and *S. ovalifolia* is a rare species among associated herbs species among which *P. polyphylla* has density of 1470.59/ha which is below the average density (Fig. 3).

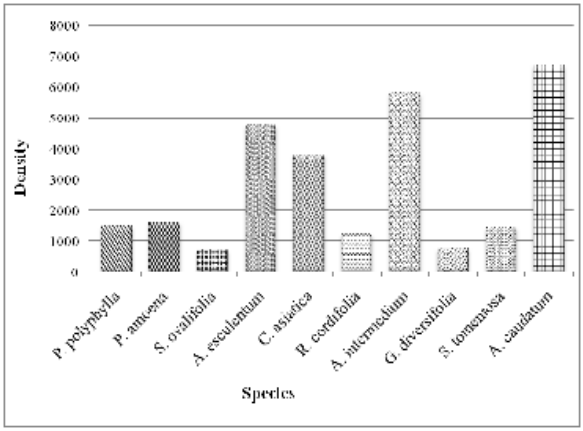

Fig. 3. Density of *P. polyphylla* and associated herbs

Fig. 4 represents relative density of *P. polyphylla* and associated species. The relative density of species depends upon the occurrence of individual species. In our study, *A. caudatum* shows highest relative density due to its high availability whereas *S. ovalifolia* shows lowest relative density and *P. polyphylla* has a moderate relative density of 5.26/ha.

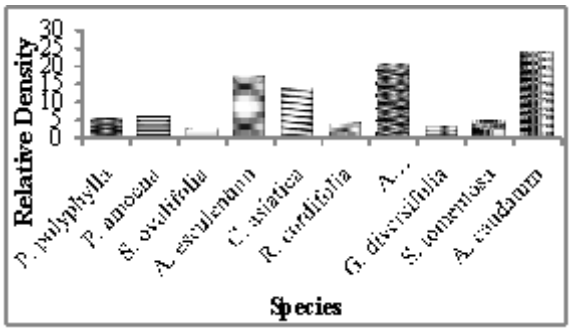

Fig. 4. Relative density of *P. polyphylla* and associated herbs and shrubs

The relative coverage pattern indicated that *A. caudatum* has highest (38.47%) and *S. ovalifolia* has lowest (1.02%) relative coverage, whereas, *P. polyphylla* shows relative coverage of 3.05% (Figure 5).

As illustrated in Figure 6, *S. ovalifolia* shows highest Important Value Index (IVI) of 81.30 and *A. intermedium* shows lowest value of 7.33 whereas *P. polyphylla* has IVI value of 14.6.

Antibacterial activity of P. polyphylla

The ZOI and MIC of different extracts obtained from *P. polyphylla* were determined. The ZOI of *P. polyphylla* extracts at 500 mg/ml on the tested organisms are shown in Table 2.

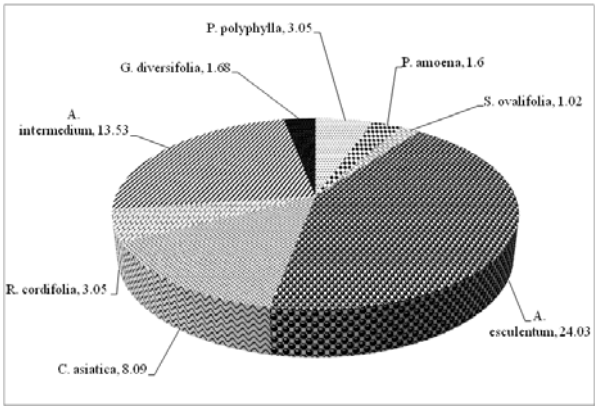

Fig. 5. Relative coverage (%) of *P. polyphylla* and associated herbs and shrubs

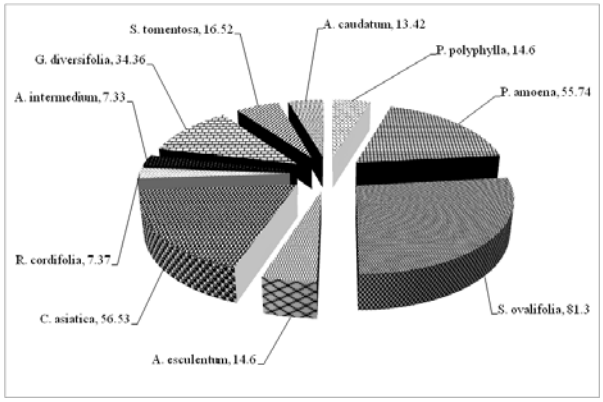

Fig. 6. Importance Value Index (IVI) of *P. polyphylla* and associated herbs and shrubs

The study indicated that *S. ovalifolia* is most important species, *P. polyphylla* the medium one and *A. intermedium* lowest important species.

Table 2. ZOI of different extracts of *P. polyphylla* on tested organisms

S.N.	Extracts and standards	ZOI (mm)				
		S. aureus	*P. aeruginosa*	*E. coli*	*S. flexinerae*	*B. subtilis*
1	P	15	7	8	8	NA
2	C	14	NA	NA	NA	NA
3	M	7	NA	NA	NA	NA
4	W	NA	7	NA	NA	NA
5	P1	18	NA	14	8	15
6	C1	10	10	11	10	10
7	M1	9	7	NA	9	7
8	W1	7	NA	NA	11	9
9	Cp	8	24	15	14	10
10	Nx	11	26	NA	32	24

Note: P, C, M and W are petrol ether, chloroform, methanol and water extracts of rhizomes of *P. polyphylla*. P1, C1, M1 and W1 are petrol ether, chloroform, methanol and water extracts of aerial parts of *P. polyphylla*. Cp and Nf are Ciprofloxacin and Norfloxacin standard antibiotics respectively, used in the assay. NA = Not active. Diameter of well = 4mm. ZOI e— 12mm was considered most active; 9-11 mm average; and from 7 to 8 mm less active (Cheesbrough, 1993).

Table 2 showed that different extracts of aerial part of *P. polyphylla* were active against *B. subtilis*, *S. aureus*, *P. aureginosa*, *E. coli* and *S. flexinerai* whereas, only petrolium ether and chloroform extracts of rhizome part is active against *S. aureus* but other extracts were found to be inactive.

The ZOI of both aerial and underground parts of *P. polyphylla* in petrol ether extract was high against *S. aureus*. Therefore dilutions of 250, 125, 62.5, 31.25 and 15.62 mg/ml were prepared following the serial dilution techniques to find out its MIC which was found to be 62.5 mg/ml, being the least concentration to inhibit the growth of *S. aureus*.

The study indicated that *P. polyphylla* was an important medicinal plant species having a number of ethnomedicinal, social and economical importance. This species can also be used to treat different bacterial infections for which detail investigation is needed. The distribution pattern, accessibility and perception of people towards this species and its antibacterial properties indicated that this species should be further studied to enhance the livelihood of rural communities of Nepal. Panchase forest can be a model forest for the study of *P. polyphylla* together with other valuable medicinal plant species from government sector and other research institutions.

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