

Research article

Phytochemical screening and antibacterial activity of selected mistletoes of Kathmandu Valley

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Abstract

Mistletoes are a unique group of green, parasitic plants which require host plants for their survival. Among the 19 species of mistletoes so far reported from Nepal, the majority of them have traditionally been used as fodder and to cure various ailments, including nausea, vomiting, and bloody diarrhoea. In this study, seven common mistletoe species were collected from the Kathmandu Valley and surrounding region in Central Nepal. Methanolic extracts of the plant samples were selected for qualitative phytochemical screening, and antibacterial activity against four ATCC (American Type Culture Collection) bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. epidermidis*). Sonication method was used for the extraction and resulted with the highest percentage yield of extract for *Viscum album* (24.26 %) and lowest for *Scurrula parasitica* (11.86 %). Qualitative phytochemical screening showed the presence of saponin, flavonoid, steroid, terpenoid, glycosides, alkaloids and phenol. Among the methanolic extracts of seven species of mistletoe, only four have shown antibacterial properties against the bacterial strains used. Methanolic extracts of *Helixanthera ligustrina* and *Viscum articulatum* showed antibacterial activity against all tested organisms. *Macrosolen cochinchinensis* showed the significant inhibition in all bacteria except *S. aureus*. Similarly, the extract of *Scurrula parasitica* was found ineffective against gram-negative bacteria (*E. coli* and *P. aeruginosa*) but found effective against gram-positive bacteria (*S. aureus* and *S. epidermidis*).

Key-words: Antibacterial activity, Methanolic extract, Mistletoes, Phytochemical screening.

Introduction

Mistletoes are being used in the treatment of some diseases, both in traditional and complementary medicines, in several cultures (Pterus 2011). For example, direct ingestion of berries of mistletoes has been found to be effective against nausea, vomiting, bloody diarrhoea and shock-induced hypertension (Bussing 2000). Similarly, traditional uses of *Loranthus parasiticus* as a neuroprotective, tranquilizing, anticancer, immunomodulatory, antiviral, diuretic, and hypotensive agent have been

proved scientifically (Moghadamtousi *et al.* 2014). Additionally, *L. micranthus* in Nigeria and South Africa has been widely used as ethnomedicine for the treatment of hypertension, diabetes, and schizophrenia and as an immune system booster (Moghadamtousi *et al.* 2013).

A wide variety of pharmacological activities has been the basis for herbal medicines, which include plant-derived drugs for the treatment of different ailments and diseases, to gain importance in modern medicine (Wang *et al.* 2012). Plants are known for their antimicrobial properties due to compounds synthesized during secondary

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metabolism. The presence of metabolites, such as phenolics, alkaloids, steroids, glycosides, saponins, tannins, flavonoids, essential oils, and resins enable the medicinal plants effective against multiple pathogens (Janssen *et al.* 1987; Saxena *et al.* 1994).

Plant extracts not only inhibit the growth of microorganisms but also interfere with some metabolic processes or may modulate gene expression and signal transduction pathways (Manson 2003; Surh 2003). Antibacterial activity has been reported for an organic solvent fraction of mistletoe from its twigs and leaves against both gram-positive and gram-negative bacteria (Hussain *et al.* 2011). Furthermore, *Scurrula ferruginea* extract was found to be effective against skin pathogen *Staphylococcus aureus* (Marvibaigi *et al.* 2014), *Loranthus micranthus* extract against *Bacillus subtilis* (Artanti *et al.* 2012), and *Viscum album* extract against *Pseudomonas aeruginosa* (Kusi *et al.* 2015). The present work aimed to screen common Nepalese mistletoes for the similar activities.

Materials and Methods

PLANT MATERIALS

Seven species of mistletoe belonging to two families, Loranthaceae (*Helixanthera ligustrina*, *Macrosolen cochinchinensis*, *Scurrula elata* and *S. parasitica*) and Viscaceae (*Viscum album*, *V. articulatum* and *V. liquidambaricola*), were collected from the Kathmandu Valley and surrounding region in Central Nepal (Table 1). The specimens were identified with the help of a book "Flora of

Kathmandu Valley" (Malla *et al.* 1986) and then cross-checked with herbarium specimens deposited at National Herbarium and Plant Laboratories (KATH). Collected specimens were dried and fixed in herbarium sheet and deposited at Tribhuvan University Central Herbarium (TUCH).

PREPARATION OF PLANT EXTRACTS

The plant materials were air/shade dried at room temperature for 6 days, then powdered and passed through a wire sieve (porosity 220 microns). Fifteen gram of fine powder of each plant sample was dissolved in 150 ml of 100 % methanol. The mixtures were placed in a sonicator (E-Chrom Tech, Taiwan, UC-7240BDT) for 2 hours and filtered with the help of Whatman No. 1 filter paper. The filtrate was allowed to evaporate until completely dry and form a solid mass. For each sample, the extract was prepared individually and kept at 4°C for further use. Finally, 100 mg of crude plant extract was dissolved in 1 mL methanol and used for qualitative phytochemical analysis and antibacterial activity. The percentage yield of plant extract was calculated by using the following formula:

Percentage yield = $100 \times \text{dry weight of extract} / \text{dry weight of plant material}$

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Crude methanolic extracts of the plant samples were subjected to screening for secondary metabolites, like saponins, terpenoids, flavonoid, steroid, alkaloids, glycoside and phenol (Harborne 1973; Trease and Evans 1989).

Table 1. List of the mistletoe species with location, elevation and parts used.

Mistletoe Species	Location*	Elevation (m.a.s.l.)	Parts used
<i>Helixanthera ligustrina</i> (Wall.) Danser	A	1690	Leaf
<i>Macrosolen cochinchinensis</i> (Lour.) Tiegh.	B	1667	"
<i>Scurrula elata</i> (Edgew.) Danser	C	2000	"
<i>Scurrula parasitica</i> L.	C	1933	"
<i>Viscum album</i> L.	C	2102	Whole plant
<i>Viscum articulatum</i> Burm. f.	C	2484	"
<i>Viscum liquidambaricola</i> Hayata	C	2300	"

*A: Champadevi Hill, Kathmandu; B: Suryabinayak, Bhaktapur; C: Chitlang, Makawanpur.

ANTIBACTERIAL ACTIVITY

Microorganisms

The microorganisms used for the antimicrobial screening was American Type Culture Collection (ATCC) gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *S. epidermidis* (ATCC 12228). These organisms were obtained from the National Public Health Laboratory, Teku, Kathmandu.

Preparation of culture media

Nutrient Broth (NB)

About 6.5 gram of NB powder was weighed and dissolved in 500 mL distilled water. This media was sterilized in an autoclave at 15 lbs pressure and 121°C for 15 minutes. The sterilized media was cooled in laminar airflow and used for the suspension type of bacterial culture.

Muller Hinton Agar (MHA) medium

Nineteen gram of MHA powder was weighed and dissolved in distilled water. The final volume was maintained at 500 mL. About 20 mL of sterilized media was poured on each sterilized Petri plates of 9 cm diameter.

Preparation of the standard culture inoculums

The individual pure ATCC cultures of bacteria *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* were streaked on the different MHA plates. The plates were incubated at 37°C for about 24 hours to obtain pure and isolated colonies. Each distinct colony was aseptically transferred to the nutrient broth for the suspension culture with the help of the sterilized inoculating loop. The inoculated bottles were kept on the shaking incubator at 37°C and 127 rpm for overnight. The turbidity of the bacterial suspension was adjusted at the 0.5 McFarland standards for the antibacterial test. These inoculums were used for the swapping of the plates to test the antibacterial effects to the plant extracts.

Transfer of bacteria on the Petri plates

The test plates for the antimicrobial activity were first labelled with the date, name of bacteria and name of the plant sample and the concentration of the plant extract to be added. The MHA plates were inoculated with the appropriate bacterial culture by a sterile cotton swab. One cotton swab was used for one bacterium. The culture plates were allowed to dry for minutes under aseptic condition.

Antibacterial screening

The antimicrobial test was performed by modified agar diffusion MHA media with the help of sterile cork borer of 5 mm diameter and labelled properly with the sterile marker pen. Five different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) of the plant samples were prepared in the DMSO. In each well, sterilized Whatman filter paper disc was placed with the help of sterile forceps for fast diffusion. With the help of a sterile micropipette 30 µl of each plant extract was poured in the above prepared well. The DMSO was taken as the negative control while the gentamicin disc at a concentration of 10 µg was taken as a positive control. The plates were incubated on the microbial incubator overnight at 37°C and the zone of inhibition was observed for individual plant extract for individual bacteria at the different concentration for further analysis.

Results

THE PERCENTAGE YIELD OF PLANT EXTRACTS

The methanolic extracts of seven mistletoe species were found greasy and sticky. The crude methanolic extracts, obtained from 15 grams of plant powder, were expressed in percentage yield, which varied from 11.86 to 24.26 % (Figure 1). The highest yield percentage was obtained for *Viscum articulatum* (24.3) while the lowest was for *Scurrula parasitica* (11.9). The result was inconsistent even among different mistletoe species of the same genus as well as among genera.

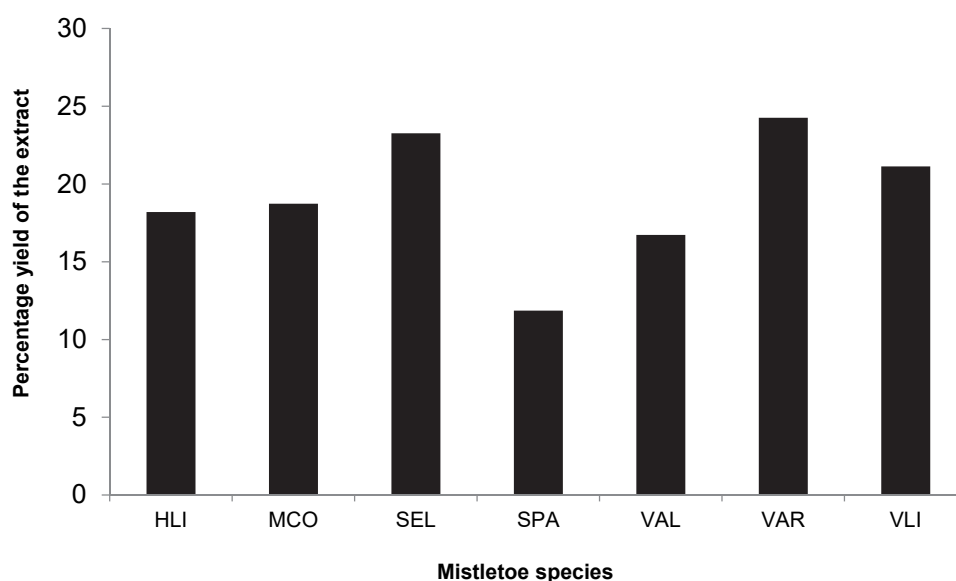


Figure 1: Percentage yield of the extract of different plant samples. Mistletoe species are HLI: *Helixanthera ligustrina*, MCO: *Macrosolen cochinchinensis*, SEL: *Scurrula elata*, SPA: *Scurrula parasitica*, VAL: *Viscum album*, VAR: *Viscum articulatum*, and VLI: *Viscum liquidambaricola*.

Table 2. Qualitative test for the presence of phytochemicals in mistletoes.

Mistletoe species ¹	Saponin	Terpenoids	Flavonoid	Steroid	Alkaloid	Glycoside	Phenol
HLI	+	+	+	+	-	+	+
MCO	-	+	+	+	+	+	+
SEL	+	+	+	+	-	+	+
SPA	-	+	+	+	-	+	+
VAL	+	+	+	+	-	+	+
VAR	+	+	+	+	-	+	+
VLI	+	+	+	+	+	+	+

¹ '+' presence, '-' absence.

¹Mistletoe species: HLI = *Helixanthera ligustrina*, MCO = *Macrosolen cochinchinensis*, SEL = *Scurrula elata*, SPA = *Scurrula parasitica*, VAL = *Viscum album*, VAR = *Viscum articulatum*, VLI = *Viscum liquidambaricola*.

QUALITATIVE ESTIMATION OF PHYTOCHEMICALS

A qualitative test revealed the presence of terpenoids, flavonoids, steroids, glycoside and phenols in all of the methanolic extracts (Table 2). Saponin was reported in all of the extracts except *Scurrula parasitica* and *Macrosolen cochinchinensis*. In contrast, alkaloids were absent in all extracts except *M. cochinchinensis* and *Viscum liquidambaricola*. Similarly, saponin was absent in *Macrosolen cochinchinensis* and *Scurrula parasitica*.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of methanolic plant extracts of seven mistletoe species were tested against four ATCC bacterial strains. Gentamicin and dimethyl sulphoxide (DMSO) were used as positive and negative controls, respectively. Five different concentrations of plant extract, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL were tested against four bacterial strains and compared with antibiotic gentamicin.

Among the seven mistletoe species selected for antibacterial activities, the plant extract of four species was found to show the antibacterial property (Table 3). Two mistletoe species, *Helixanthera ligustrina* and *Viscum articulatum* showed antibacterial activity against all tested organisms. The extract of *Scurrula parasitica* was found ineffective against *E. coli* and *P. aeruginosa* but found effective against gram-positive bacteria. Similarly, the extracts of *Macrosolen cochinchinensis* inhibited all tested organisms except *S. aureus*. It

showed moderate antibacterial activity against *P. aeruginosa* at all concentration as compared to other bacterial strains. In contrast, the extract from three plants species namely *Scurrula elata*, *Viscum album* and *V. liquidambaricola* were found ineffective against all bacterial strains. Even in cases, where the extracts showed antibacterial effect, there was only moderate activity at concentrations of plant extract comparable to that of standard antibiotics (i.e., between 6.25 to 12.5 mg/mL).

Table3. Antibacterial activity of plant extracts against four bacterial strains.

Plant species*	Bacteria**	Zone of inhibition in mm (with a diameter of well 5 mm)***					
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	Antibiotic
HLI	ECO	15.3±3.1	13.7±1.5	11.7± 0.6	10.0±1.0	10.7±0.6	22
	PAE	19.0±3.6	15.7±1.5	13.3±1.5	10.7±2.1	-	21
	SEP	16.0±1.0	13.0±1.0	9.7± 0.6	9.0±1.0	-	22
	SAU	19.0±1.0	16.0±1.0	13.7±0.6	10.7±0.6	-	19
MCO	ECO	10.0±1.0	9.0±1.0	8.7±2.1	6.0 ± 4.4	-	22
	PAE	21.3±1.5	18.0±1.0	13.7±4.2	12.3±3.1	11.7±3.1	21
	SEP	16.7±1.5	11.7±0.6	-	-	-	22
	SAU	-	-	-	-	-	19
SEL	ECO	-	-	-	-	-	22
	PAE	-	-	-	-	-	21
	SEP	-	-	-	-	-	22
	SAU	-	-	-	-	-	19
SPA	ECO	-	-	-	-	-	22
	PAE	-	-	-	-	-	21
	SEP	10.7±0.6	8.7±0.6	-	-	-	22
	SAU	12.7±0.6	11.3±0.6	10.3±0.6	-	-	19
VAL	ECO	-	-	-	-	-	22
	PAE	-	-	-	-	-	21
	SEP	-	-	-	-	-	22
	SAU	-	-	-	-	-	19
VAR	ECO	14.0±1.0	13.0±1.0	12.7±1.5	8.7±0.6	-	22
	PAE	14.7±0.6	12.7±1.5	11.3±1.5	10.3±0.6	9.3± 0.6	21
	SEP	16.7±2.1	13.0±1.0	11.7±0.6	10.7±0.6	-	22
	SAU	11.7±0.6	9.7±0.6	9.7± 0.6	-	-	19
VLI	ECO	-	-	-	-	-	22
	PAE	-	-	-	-	-	21
	SEP	-	-	-	-	-	22
	SAU	-	-	-	-	-	19

*Plant species: HLI = *Helixanthera ligustrina*, MCO = *Macrosolen cochinchinensis*, SEL = *Scurrula elata*, SPA = *Scurrula parasitica*, VAL = *Viscum album*, VAR = *Viscum articulatum*, VLI = *Viscum liquidambaricola*. **Bacteria: ECO = *Escherichia coli*, PAE = *Pseudomonas aeruginosa*, SAU = *Staphylococcus aureus*, SEP = *Staphylococcus epidermidis*. ***The values represent mean ± standard deviation of three replicates.

Discussion

THE PERCENTAGE YIELD OF PLANT EXTRACTS

The percentage yield of the plant extract has been suggested to vary and depends on parts of the plant used (root, stem, leaf, flower and fruits), type of solvent and process of extraction (Cemaluk and Nwankwo 2012; Gurer-Orhan et al. 2004; Handa et al. 2008; Vicas et al. 2009). The variation in yield percentage among the species may be due to species specific factors, including plant parts used, degree of maturity, and environmental conditions under which plants are grown. However, the lowest yield for *Scurrula parasitica* is probably related to the low proportion of leaf tissues (due to thin, papery nature) in the plant samples used for extraction.

QUALITATIVE SCREENING OF PHYTOCHEMICALS

Secondary metabolites are the chemicals developed in plants for self-defense against environmental stresses, animals and plants, including micro-organisms. Among the mistletoes, *Viscum album* is previously reported to contain terpenoid (Wahab et al. 2010) which is also verified by the present result. However, the presence of alkaloids reported by Kusi et al. (2015) was not observed in this study which may be due to the differences in plant parts used for extraction. The secondary metabolites like alkaloids and saponins were reported from African mistletoes, i.e. *Tapinanthus dodoneifolius* (Deeni and Sadiq 2002), and flavonoids and glycosides from Japanese mistletoes, i.e. *Taxillus yadoriki*, *T. kaempferi* and *Korthalsella japonica* (Fukunaga et al. 1989). In this study, saponin and alkaloid were absent in two and five species, respectively. Besides, the presence of major secondary metabolites in all mistletoes may suggest their future use as potential medicinal plants.

ANTIBACTERIAL ACTIVITY

Plant extracts usually contain antibacterial substances and able to suppress the growth of tested bacteria in different concentrations. Plant extracts were screened for antibacterial test against four pathogenic strains. The ATCC strains of gram-

negative and gram-positive bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. epidermidis* were used.

Among the seven mistletoe species studied, methanolic extracts of only four species showed antibacterial activities. Of them, *Helixanthera ligustrina* and *Viscum articulatum* showed growth inhibition of all bacteria tested. *Macrosolen cochinchinensis* also showed significant growth inhibition of all bacteria except *Staphylococcus aureus*. However, the inhibitory effect of *Macrosolen cochinchinensis* was strongest against *Pseudomonas aeruginosa* as compared to *Staphylococcus epidermidis* and *Escherichia coli*. According to Tripathi et al. (2013) *M. cochinchinensis* shows no inhibition against *E. coli*. The extracts of *Viscum album* did not show any inhibition zone against all tested organisms. These results are consistent with those of Tripathi et al. (2013). Kusi et al. (2015), on the other hand, reported antibacterial activity in the methanolic leaf extract of *Viscum album*. Deeni and Sadiq (2002) reported dependence of antimicrobial activity of African mistletoes on the host physiological states of development, diurnal and seasonal variations. The antibacterial activity shown by the members of the genus *Scurrula* against *S. aureus* is supported by previous study (Marvibaigi et al. 2014). In the present study, *Scurrula parasitica*, *Viscum album* and *V. liquidambaricola* extracts did not show antibacterial activity against the tested organisms. These may be moderate antibacterial against bacteria because the plant extracts have secondary metabolites, such as alkaloids, terpenes, phenolics, and glycoside.

Conclusion

Qualitative phytochemical screening showed the presence of major secondary metabolites in almost all of the tested mistletoes, which suggest their importance as potential medicinal plants. Similarly, among the seven mistletoe species studied, four have potentials to be used as antibacterial agents as they exhibited inhibitory effects against tested

bacteria. *Helixanthera ligustrina* and *Viscum articulatum* are even more effective as they showed antibacterial activity against all gram-positive and gram-negative bacteria tested. *Macrosolen cochinchinensis* exhibits the best antibacterial activity against *Pseudomonas aeruginosa*.

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