

PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF TRADITIONALLY USED MISTLETOES IN NEPAL

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Abstract: Mistletoes are being used for food or beverage and for variety of medicinal purposes. In Nepal, mistletoes are traditionally used as fodder and as remedy to cure various ailments. In this study, methanolic extracts of seven common mistletoe species (*Helixanthera ligustrina*, *Macrosolen cochinchinensis*, *Scurrula elata*, *S. parasitica*, *Viscum album*, *V. articulatum* and *V. liquidambaricola*) were analyzed for their phytochemical constituents and antioxidant potential. The total flavonoid content (TFC) and total phenol content (TPC) were quantitatively determined by aluminum chloride colorimetric and folin-cioalteau reagent methods, respectively. The TFC was found highest for *V. album* (31.5 ± 2.3 mg QE/g) and lowest for *M. cochinchinensis* (24.9 ± 2.3 mg QE/g). Similarly, highest total phenolic content was found in *S. parasitica* (32.9 ± 2.5 mg GAE/g) and lowest in *V. album* (20.6 ± 2.1 mg GAE/g). The antioxidant activity measured by DPPH radical scavenging assay was found best for *S. parasitica* (with lowest IC₅₀ value; 26.0 ± 0.7 µg/ml) among the studied mistletoes. Overall analysis provides scientific evidence in favor of indigenous use of these mistletoes as a source of fodder, food and for medicinal purposes.

Keywords: Mistletoes; Phenolic content; Flavonoid content; Antioxidant activity.

INTRODUCTION

Mistletoes are highly specialized flowering plant of sandalwood family (Order; Santalales), that exploit and (or) parasitize a wide range of host plants^{1,2,3}. Altogether, 1500 species of mistletoes are known worldwide⁴. They are occasionally used as food or beverage and also for variety of medicinal purposes for humans and animals to cure muscular swelling, sprains, fractures and dislocations^{1,5}. In Africa, mistletoes are used in treatment of various stomach troubles of children including diarrhea, hypertension, diabetes, and schizophrenia and also used as an immune system booster⁶. Similarly, the people of ancient Greek and Argentina uses these plants in spleen diseases and for problems related to menstruation, respectively. However,

Indian people used mistletoes as a tea against diabetes⁷.

Mistletoes like *V. album*^{8,9}, *Taxillus yadoriki*, *T. kaempferi* and *Korthalsella japonica*¹⁰, *Loranthus parasiticus*¹¹, species of *Scurrula* and *Viscum*¹² and *L. micranthus*¹³ were phytochemically explored and found to contain different chemicals of medicinal importance. Similarly, few mistletoe species such as *S. ferruginea*¹⁴, *L. micranthus*¹⁵, *L. parasiticus*¹¹, *L. europaeus*¹⁶, *L. regularis*¹⁷, *V. album*¹⁸, and *Dendrophthoe pentandra*¹⁹ were assayed for their antioxidant properties.

Among the 19 species of mistletoes found in Nepal some are being used traditionally by indigenous people since long but remained unused by the modern pharmacological practice. Mistletoes like *Dendrophthoe falacata*, *V. album*

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Received: 28 Aug 2020; Review: 27 Sep 2020; Accepted: 30 Sep 2020.
Doi: <https://doi.org/10.3126/sw.v14i14.34999>

and *V. articulatum* were reported to be used by indigenous people of Nepal²⁰. Similarly, 11 species of mistletoes and their uses by peoples of Bardia (tropical region) and Godawari-Phulochoki area (temperate region) have been documented⁵. The recent study also included the information of indigenous use of 15 mistletoes from different parts of the country²¹. Present study aimed to analyze phytochemical constituents and antioxidant potential of common mistletoes of Kathmandu valley which were already been documented for their indigenous use by previous researchers.

MATERIALS AND METHODS

Collection and identification of plant samples

Different species of mistletoes were collected from surroundings of Kathmandu valley (Table 1). The specimens were identified with the help of “Flora of Kathmandu Valley” and then cross checked with specimen deposited at National Herbarium and Plant Laboratories (KATH). Collected specimen were dried and fixed in herbarium sheet and deposited at Tribhuvan University Central Herbarium (TUCH).

Preparation and extraction of plant material

The collected plant materials were air/shade dried at 32-35°C for 6 days to remove moisture and powdered. Fifteen grams of fine powder of each plant sample was weighed separately and dissolve in 150 ml of 100 % methanol (Thermo Fisher Scientific, India). The mixtures were placed in Sonicator (UC-7240BDT E-Chrome Tech, Taiwan) using ultrasonic wave at 40 Hz for 2 hours and then filtered using Whatman No. 1 filter paper. The step was repeated with the residue for complete extraction. Then the filtrate recovered from both steps was allowed to evaporate under reduced pressure until completely dry and form solid mass (waxy). Obtained solid mass was weighed to express the gram of extract extracted per 15 grams of the plant powder. For each sample, extract was prepared individually and kept at 4°C for further use. Finally, 100mg of crude plant extract dissolved in 1 ml methanol was used for quantification of total phenol, total flavonoids, and antioxidant activity.

Table 1. List of plants under study with elevation and parts used Fam.^a: Family; Lor: Loranthaceae, Vis: Viscaceae. Ele.^b: Elevation. Loc.^c: Location; A: Below Champadevi, B: Suryabinayak, C, D, F: Between Godam to Chitlang Deurali, E: Chitlang Deurali, G: Chitlang. Parts used ^d: L: Leaf, YL: Young Leaf, WP: Whole Plant

S.N	Mistletoe Species	Acronyms	Fam. ^a	Ele. ^b (m)	Host species	Loc. ^c	Parts used ^d
1	<i>Helixanthera ligustrina</i> (Wall.) Danser	HLI	Lor	1690	<i>Pyrus pashia</i> Buch.-Ham. ex D. Don	A	L
2	<i>Macrosolen cochinchinensis</i> (Lour.) Tiegh.	MCO	Lor	1667	<i>Schima wallichii</i> Choisy	B	YL
3	<i>Scurrula elata</i> (Edgew.) Danser	SEL	Lor	2000	<i>Myrica esculenta</i> Buch.-Ham. ex D. Don	C	L
4	<i>Scurrula parasitica</i> L.	SPA	Lor	1933	<i>Pyrus pashia</i> Buch.-Ham. ex D. Don	D	L
5	<i>Viscum album</i> L.	VAL	Vis	2102	<i>Prunus cerasoides</i> Buch.-Ham. ex D. Don	E	WP
6	<i>Viscum articulatum</i> Burm. f.	VAR	Vis	2484	<i>Quercus semecarpifolia</i> Sm.	F	WP
7	<i>V. liquidambaricola</i> Hayata	VLI	Vis	2300	<i>Betula alnoides</i> Buch.-Ham. ex D. Don	G	WP

Total flavonoid content (TFC)

The TFC was determined by using the aluminum chloride colorimetric method with slight modification²². 0.25 ml of extract (10 mg/ml) was separately mixed with the 0.75 ml of ethanol, 0.05 ml of the 10% aluminum chloride, 0.05 ml of the 1 M potassium acetate and 1.4 ml of the distilled water. The solution mixture was shaken and allowed to stand at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm using UV-visible spectrophotometer (CT8600, E-Chrome Tech, Taiwan). Quercetin was used as standard solution in methanol with the concentration ranging from 10-100 µg/ml. and blank was prepared by adding all the reagents except the plant sample. The TFC was expressed in terms of milligram Quercetin per gram of dry mass (mg QE/g).

Total phenolic content (TPC)

The TPC was determined by folin-ciocalteau method²³ with slight modification by mixing 0.1 ml of the sample extracts (2.5 mg/ml) with 1 ml folin-ciocalteau reagent (1:10 dilution with distilled water) and 0.8 ml of aqueous 1M sodium carbonate. The reaction mixture was left for 15 minutes and absorbance was measured at 765 nm. A calibration curve was obtained using Gallic acid in methanol using the concentration ranging from 25-250 µg/ml as standard. Based on the standard curve, the concentration of each sample was calculated. The TPC content was expressed in terms of the milligram of the Gallic acid equivalent per gram of the dry mass (mg GAE/g).

Antioxidant activity assay

Antioxidant activity of the plant extract was determined through the DPPH (2,2-Diphenyl-1-picrylhydrazyl; Fisher Scientific India Limited) free radical scavenging activity using ascorbic acid standard²⁴. The different concentration of plant extract (25-200 µg/ml) and ascorbic acid (10-100 µg/ml) were prepared in methanol. 0.5 ml of samples of plant extract as well as ascorbic acid of each concentration was taken separately in clean test tubes. Then 0.5 ml of DPPH solution (0.2mM) was added, properly mixed and incubated in dark for 30 minutes. The controls were prepared as above but without plant extract or ascorbic acid. Absorbance of the solution was measured at 517 nm. The free radical scavenging activity (RSA) of plant samples was calculated as follows and expressed in percentage: % Radical Scavenging activity (RSA) = 100* Abs. control-Abs. sample/Abs. control.

Statistical analysis

All the experiments were performed in triplicates (mean ± SD) and the data obtained were analyzed in Microsoft excel 2010.

RESULTS

Total flavonoid content (TFC)

Quercetin was used as standard to determine TFC in plant extracts. The equation obtained from graph of Quercetin was used for the estimation of TFC expressed in terms of mg QE/g ± SD. The highest TFC was estimated as 31.5 ± 2.3 mg QE/g for *V. album* and lowest as 24.9 ± 2.3 mg QE/g for *M. cochinchinensis*. The amount of TFC in rest of the species lied between these two extremes (Figure 1).

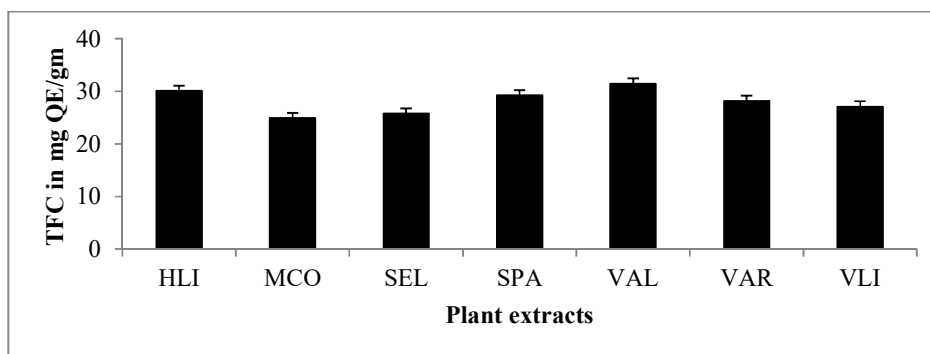


Figure 1: Total flavonoid content present in different mistletoes. Error bar indicate ±SD (N=3).

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

Total phenolic content (TPC)

Based on the equation of Gallic acid, total phenolic content present in methanolic extract of seven different samples was determined. The highest amount of TPC was

found in *S. parasitica* (32.9 ± 2.5 mg GAE/g) while lowest found in *V. album* (20.6 ± 2.1 mg GAE/g), rest of the species possessed the amount lies between two values (Figure 2).

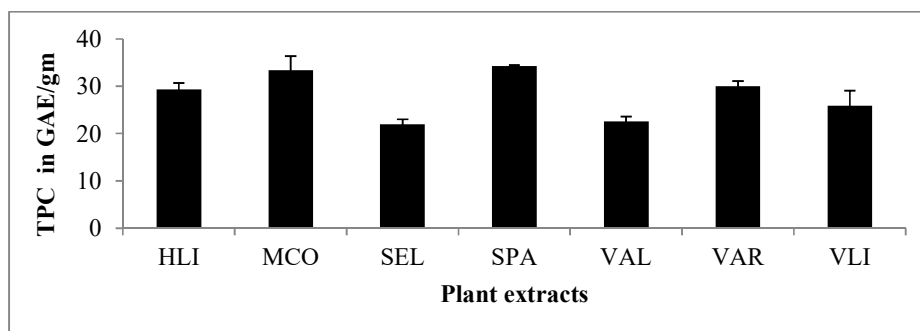


Figure 2: Total phenolic content present in different mistletoes. Error bar indicate \pm SD (N=3).

Legend: HLI: *Helixanthera ligustrina*, MOCO: *Macrosolen cochinchinensis*, SEL: *Scurrula elata*, SPA: *Scurrula parasitica*, VAL: *Viscum album*, VAR: *Viscum articulatum*, VLI: *Viscum liquidambaricola*.

Antioxidant activity of the plant extract

In this study, DPPH was used as the source of free radical and ascorbic acid as pure antioxidant reference compound. There was gradual increase in percentage radical scavenging activity as the concentration of extract increased (data not shown). The IC_{50} value for ascorbic acid was measured as $19.61 \mu\text{g/ml}$ while for plant extracts, highest and lowest IC_{50} values were obtained for *V. album* ($199.0 \pm 1.3 \mu\text{g/ml}$) and *S. parasitica* ($26.0 \pm 0.7 \mu\text{g/ml}$), respectively. The IC_{50} value of *S. parasitica* showed best

antioxidant activity which is more or less comparable to the standard i.e. ascorbic acid. Three mistletoe species; *M. cochinchinensis* ($65.9 \pm 2.8 \mu\text{g/ml}$), *H. ligustrina* ($88.2 \pm 0.7 \mu\text{g/ml}$) and *V. liquidambaricola* ($102.6 \pm 3.8 \mu\text{g/ml}$) showed moderate antioxidant activity as compared to *S. parasitica*. Similarly, *S. elata* ($186.4 \pm 6.5 \mu\text{g/ml}$), *V. album* ($199.0 \pm 1.3 \mu\text{g/ml}$), and *V. articulatum* ($183.1 \pm 0.6 \mu\text{g/ml}$) were found to show least antioxidant activity (Figure 3).

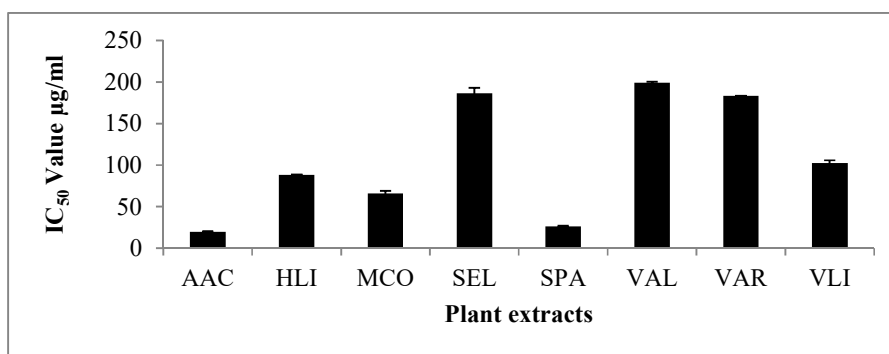


Figure 3: IC_{50} value of different mistletoe species (AAC: Ascorbic acid). Error bar indicate \pm SD (N=3).

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

DISCUSSION

Secondary metabolites are the chemicals developed in plants for self-defense from environmental stress, animals

and plants including microorganisms. Among the secondary metabolites, flavonoids are a class of secondary plant phenolics with significant antioxidant and chelating

property²⁵. In this study, the TFC varies from 24.9 mg QE/g to 31.5 mg QE/g. The TFC in *M. cochinchinensis* (24.9 mg QE/g) of present study showed similarity with the value reported in the earlier work²⁶ on *M. parasiticus* (22.5 mg QE/g). Similarly, in another work²⁷, TFC was reported for *Dendrophthoe falcata* as 5.0, 9.0 and 15.0 mg QE/g for chloroform, methanol and hydroalcoholic extracts, respectively. These amounts were distinctly less than the amount of TFC of mistletoes in present study. These variations may be due to difference between the species and type of solvent used for extraction.

The amount of TPC obtained from methanolic extract of leaves of *S. parasitica* was higher than the amount reported by earlier researchers²⁸ (21.77 ± 1.41 mg GAE/g). However, highest amount of TPC was reported from ethanolic extracts of *S. parasitica* (210 mg GAE/g) and *M. cochinchinensis* (150 mg GAE/g) using high temperature batch extraction method²⁹. Similarly, variable amount of TPC has been reported for acetone extracts of *Scurrula ferruginea*¹⁴. The highest amount was obtained in stem (309.1 ± 1.2 mg GAE/g) followed by leaf (144.2 ± 0.7 mg GAE/g) and flower (126.4 ± 0.3 mg GAE/g). Furthermore, methanolic extracts of *V. album* grown on two different host trees was examined for TPC using tannic acid as standard compounds. *V. album* grew on Cocoa tree possessed higher TPC (182 mg/100g) than Cashew tree (160 mg/100g)³⁰. The lower total phenolic content in seven mistletoe species of present study may be due to the use of different plant parts for extraction, type of solvent in which extraction was performed and even type of host trees on which mistletoes grew.

Antioxidants derived from natural resources mainly from plants have been intensively used to prevent oxidative damages³¹. So the antioxidant properties of plant extract are very important for investigation of their pharmaceutical uses. Antioxidant property can be concluded on the basis of % radical scavenging activity (RSA) and IC₅₀ value. Antioxidant activity of the plant extract is expressed as percent inhibition of stable free radical or inhibition concentration fifty (IC₅₀) in reference to a standard compound. The plant with higher percentage

RSA and corresponding lowest IC₅₀ value is considered having better antioxidant properties. The higher IC₅₀ value (110.91 µg/ml) for methanolic extracts of *S. parasitica* than the present study (26.0 ± 0.7 µg/ml) has been reported earlier³². Much higher IC₅₀ value (599.6 µg/ml) for methanolic extract of *M. cochinchinensis* was also reported³³. The difference between the values obtained in present and previous studies may be due plant collection from different geographical locations and extraction process. In both studies, plants were collected from tropical climatic condition and used simple extraction procedure compared to the present study (temperate climatic condition and sonication method). The present finding is again supported by the previous work³⁴ where antioxidant potential was found increased with the rising altitude.

CONCLUSION

Present study attempted to explore phytochemical constituents and antioxidant potentialities of 7 mistletoes commonly available in surrounding mountains of Kathmandu valley. Overall analysis of phytochemical constituents and antioxidant activity suggested that all mistletoes traditionally been used for different purposes were rich in terms of different phytochemicals. Among the selected species, five species (*H. ligustrina*, *M. cochinchinensis*, *V. album*, *V. articulatum*, *V. liquidambaricola*) were used for medicinal purposes and two (*S. elata*, *S. parasitica*) as fodder by indigenous people of Nepal. Quantitative estimation of TPC and TFC also deduced that the selected mistletoes are rich in both phytochemicals. However, antioxidant potential of *S. parasitica* was found best among all, although traditionally been used as fodder and ignored for its medicinal importance.

ACKNOWLEDGEMENT

The authors are grateful to the Cornell Nepal Study Program (CNSP) for providing partial financial support to carry out this research work.

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