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Journal of Institute of Science and Technology

Volume 22, Issue 2, January 2018

ISSN: 2469-9062 (print), 2467-9240 (e)

JIST, **22** (2): 140-147 (2018)

Published by:

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

JIST 2018, 22 (2): 140-147
ISSN: 2469-9062 (p), 2467-9240 (e)

Research Article

ANALYSIS OF PHYTOCONSTITUENTS AND BIOLOGICAL ACTIVITIES ON THE SELECTED MEDICINAL PLANTS OF DOLAKHA AND SINDHUALCHOWK DISTRICT OF NEPAL

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***Received:** 25 October, 2017; **Revised**: 10 November, 2017; **Accepted:** 14 November, 2017*

ABSTRACT

Phytochemical and biological activities of methanolic extract of seven plants viz. *Scindapsus officinalis* Schott. , *Lepisorus loriformis, Nicotiana tabacum* L., *Clematis buchananiana* DC., *Astilbe rivularis* D. Don, *Potentilla fulgens* Wall. ex Hook and *Taxus wallichiana* Zucc. were carried out. The brine shrimp bioassay showed that *N. tabacum, A. rivularis*, and *P. fulgens* were pharmacologically active. The antibacterial potential was studied against *Staphylococcus aureus* (gram positive bacteria) and *Escherichia coli* (gram negative bacteria) using Agar Well Diffusion Method. Roots of *P. fulgens* showed inhibition against gram positive bacteria while the rhizome of *A. rivularis* showed inhibition against both gram positive and gram negative bacteria. Antioxidant activity was evaluated by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant power FRAP assay. Both assays showed that *P. fulgens* has high antioxidant activities with (IC_{50} =15.57 ± 3.6) lower than standard ascorbic acid.

Keywords: Antioxidant activity, Agar well diffusion method, Brine shrimp bioassay, DPPH assay, FRAP assay.

INTRODUCTION

Natural product is a term used commonly in reference to chemical substances found in the nature that has distinctive pharmacological effects. Natural products and their derivatives have been and continue to be rich sources for drug discovery. They are known to be chemically balanced, effective and least injurious with none or much reduced side effects as compared to synthetic medicines. Plants for years have been a valuable source of natural product for maintaining human health, especially in the last decade with more intensive studies devoted to natural therapies Ellof (1998). It is reported that more than 400,000 species of tropical flowering plants have medicinal properties. However, natural products are not drugs; they are produce in the nature and through efforts made by researchers, which become candidates for drug development (N' guessan *et al*., 2007; Thomass, 1997). In this context, Nepal has many plants with medicinal and aromatic values as it is rich in biodiversity due to its geographical features. About 1600-1900 species of plants are commonly used in traditional medicinal practices in Nepal (Ghimire, 2008; Baral & Kurmi, 2006). Many of them are still not explored scientifically for their medicinal potential (Baral & Kurmi, 2006; Rajbhandari, 2001). The present study was carried out to evaluate the phytochemicals and biological assay of following six locally available medicinal plants from Dolakha and Sindhualchowk District of Nepal. The findings from this work may add to the overall value of the medicinal potential of the plants.

Scindapsus officinalis (Roxb.) Schott

Scindapsus officinalis (Roxb.) Schott commonly known as 'Kanchirno' (in Nepali community) belongs to the family Aracaceae. The plant is large, epiphytic, perennial climber with adventitious aerial roots growing on trees and rocks. Leaves are ovate. It is distributed in Tropical Himalayan, India, Burma, Indo-China etc. The plant parts used in medicine are dried mature inflorescence, shoots, roots and leaves. Fruits are most important part of *S. officinalis* and accepted in both unani and ayurveda for its beneficial properties. Traditionally, paste of this whole plant is used in fractures. In Ayurveda, its fruits are used as antihelmintic, appetizer, for curing asthma, dysentery and throat problems (Shivhare *et al.*, 2011).

Astilbe rivularis Buch.-Ham. ex D. Don

Astilbe rivularis Buch.-Ham.exD. Don commonly known as 'Thuloausadhi' (in Nepalese language)

belongs to the family Saxifragaceae. It is distributed throughout Nepal at an altitude between 2000-3600 m asl on moist rocky hills. It is a shrubby plant propagated by root-stocks. The juice of this plant is applied traditionally on the sprain and muscular swelling. The rhizome is valuable in curing diarrhea, dysentery, peptic ulcer, headaches, hemorrhages, prolapsed of uterus and to improve fertility (Manandhar, 2002).

***Potentilla fulgens* Wall. ex Hook.**

Potentilla fulgens Wall. ex Hook., commonly known as 'Bajradanti', is called 'Himalayan cinquefoil' in English and belongs to the family Rosaceae. It is found in temperate and higher Himalayan up to an altitude of 1800-4350 m asl in Nepal, Bhutan and Tibet. More than three hundred species of genus *Potentilla* Linn. are used in Ayurvedic due to high content of polyphenols in their aerial and underground parts. Whole plant of *P. fulgens* is utilized as astringent and tonic for curing gum and tooth ailments (Pyorrhea, toothache and caries), diarrhea, stomach problems, cough, cold and diabetes (Farooqui *et al.*, 1998; Syiem *et al.*, 2002). Pharmacological studies reported that *P. fulgens* possesses antihyperglycemic, antihyperlipidemic, antitumor, antioxidant, anti-inflammatory and antiulcerogenic properties (Syiem *et al.*, 2002).

***Nicotiana tabacum* Linn.**

Nicotiana tabacum Linn., commonly known as 'Kaccho-paat' belongs to the family Solanaceae. It is native of tropical and subtropical area. It is now commonly cultivated worldwide. Leaves are applied externally for headache, wounds and ulcers. Hot water extract of the dried leaf is applied externally for ring worms. Leaf is orally taken for kidney diseases (Charlton, 2004).

Clematis buchananiana

Clematis buchananiana is commonly known as 'Pinaselahara'. It belongs to the family Ranunculaceae. It is distributed from temperate Himalayan from Kashmir east ward to Assam, Burma, India, China found from 1200 to 3000 m asl. Dense wooly hair is present outside the climber. Traditionally, it is used against sinusitis. A paste of the roots is used as a poultice to treat inflammation. The juice of the root is used for the treatment of peptic ulcers. A paste of the root bark is kept pressed against the teeth for about 15 minutes to relieve toothache. It can be administered orally to treat sexually transmitted infection, rheumatoid arthritis, bone disorder, chronic skin diseases and as a diuretic (Saha *et al.*, 2011).

Lepisorus loriformis

Lepisorus loriformis is commonly known as 'Surtipaat'. It belongs to the family Polypodiaceae. This plant is found on tree trunks or in rock crevices in forest. It is distributed in Nepal, Bhutan, Myanmar at the altitude of 2000-3000 m asl. This plant is 10-20 cm long with long creeping, densly scaly rhizomes. Traditionally the aqueous extract of the root of this plant is used against urinary disorders.

Table 1: Important medicinal plants and their uses

S. N.	Scientific name	Family	Local name	Parts taken	Therapeutic application	Collected area/ Altitude
1.	*S. officinalis*	Aracaceae	Kanchirno	Stem	Paste of the whole plant is used in fracture of bones.	Sindhupalchowk (1582 m)
2.	*L. loriformis*	Polypodiaceae	Surtipaat	Rhizome	Used in dysentery.	Jiri,Dolakha (1905m)
3.	*N. tabacum*	Solanaceae	Kachho-paat	Leaves	Used to make fertilizer. (Insecticidal).	Sindhupalchowk
4.	*C.buchananiana*	Ranunculaceae	Pinase lahara	Root	Sinusitis (burnt and it's smoke is inhaled 4 times daily a week).	Jiri, Dolakha
5.	*A. rivularis*	Saxifragraceae	Thulo Okhati	Rhizome	Curing diarrhea, dysentery, headache, to improve fertility, bleeding after child birth.	Jiri, Dolakha
6.	*P. fulgens*	Rosaceae	Banmula	Root	Root powder for tooth ache.	Jiri, Dolakha

MATERIALS AND METHODS

Selection and collection of plants

In this study medicinal plants were selected on the basis of their medicinal importance to native people, especially in Dolakha and Sindhupalchowk district of Nepal. The collected plants were identified at Central Department of Botany, Tribhuvan University, Kathmandu, Nepal.

Preparation of extracts

The collected plants were cleaned, air dried in shade. Exposure to the sunlight is avoided to prevent the loss and transformation of the active components. The completely dried samples were grinded into fine powder. The extraction of chemical constituents of plant material was carried out with methanol by the process of cold percolation. The powdered material was kept in clean and dry conical flask and dipped in methanol. It was left for 2-3 days at room temperature with shaking at intervals. Then it was filtered and filtrate was concentrated using rotator evaporator. This process was repeated for 6-7 times. The concentrated filtrate was air dried to obtain solid or semisolid residue. The same process was carried out for all the plants. After completely drying they were kept in beaker. The dried extracts were used for different tests.

Phytochemical screening

Analysis of crude methanolic extracts of above seven medicinal plants for various phytochemical constituents were carried out using standard methods (Culie, 1982).

DPPH radical scavenging activity (rsa) assay

The free radical scavenging activity of samples and standard ascorbic acid solution in methanol was determined based on their ability to react with stable 1, 1-diphenyl-2-picrylhyrazyl (DPPH) free radical (Blois, 1958). The plant samples at various concentrations (15-250 μg/ml) were added to a 100 μM solution of DPPH in methanol. After incubation at 37 °C for 30 min, the absorbance of each solution was determined at 517 nm. The measurement was performed in triplicates. The antioxidant activity of the samples was expressed as IC_{50} (inhibitory concentration), which was defined as the concentration (in μg/ ml) of sample required to inhibit the formation of DPPH radicals by 50%. Ascorbic acid was used as positive control. Free radical scavenging activity was calculated by using following equation:

$$\% \text{ of free radical scavenging activity} = \frac{(A_0 - A_T) \times 100}{A_0}$$

Where, A_O = Absorbance of DPPH solution and A_T = Absorbance of test or reference sample. The % scavenging was then plotted against concentrations used and from the graph IC_{50} was calculated.

FRAP assay

The antioxidant activity by FRAP assay was conducted according to the procedure given by Benzie and Strain with slight modification (Benzie & Strain, 1996). The FRAP reagent was prepared by mixing acetate buffer of pH 3.6 (300 mM), TPTZ (tripyridyltriazine) solution of 10 mM and ferric chloride solution of 20 mM in the ratio of 10:1:1. Antioxidant activity was calculated with the standard calibration of ferrous sulfate. The leaf extracts (5 mg/ml) was prepared by adding methanol and was used as sample. Finally, absorbance was taken at 593 nm keeping the temperature 37 °C.

Antibacterial assay

Staphylococcus aureus (gram positive) and *Escherichia coli* (gram negative) used for this assay were obtained from Central Department of Microbiology, TU, Nepal. The antimicrobial activity of the plant extracts was determined by disc diffusion method (Bauer *et al.*, 1996). A suspension of test micro organisms was spread on Muller-Hinton Agar (MHA) medium. The sterile filter paper discs (6 mm in diameter) were individually impregnated with different concentration of plant extract prepared in dimethyl sulphoxide (DMSO) and then placed into the agar plates which have been previously inoculated with the test micro organisms. The plates were subsequently incubated 24 hours at 37 ^{o}C. After incubation the growth inhibition zones were quantified by measuring the diameter of the zone of inhibition in mm. Dimethyl sulphoxide (DMSO) discs were used as control. All the tests were performed in triplicate.

Brine Shrimp Bioassay

The brine-shrimp toxicity assay for each extract was carried out according to Mayer *et al.* (1982). Briefly, sample solutions were prepared by dissolving 200 mg of each plant extract in DMSO up to the mark in 10 ml volumetric flasks. To calculated volume of the sample solution for 10, 100 and 1,000 μg/ml dose levels in three replicates was introduced into freshly hatched ten brine-shrimp nauplii in artificial sea water (total volume 10 ml). A control tube for each dose level was also prepared. After 24 h of illumination under a table

lamp (60 Watt), the number of survivors was counted. No death was observed in the control tubes. The LC_{50} (Lethal concentration for 50% mortality) values was determined using the probit method (Finney, 1971), as the measure of toxicity of the extracts.

RESULTS AND DISCUSSION

Phytochemical screening

The different phytochemicals in the crude methanol extracts were identified by the color reaction with different reagents. The results of phytochemical screening are shown in Table 2.

Table 2: Results of Phytochemical Screening.

Phytochemicals / Plants	Polyphenols	Reducing compounds	Glycosides	Quinones	Saponins	Basic alkaloids	Terpenoids	Flavonoids
S. officinalis	+	+	+	+	+	+	+	+
L. loriformis	-	-	-	+	-	+	+	+
N. tabacum	+	+	-	-	+	+	+	+
C. buchananiana	-	+	-	-	-	-	+	+
A.rivularis	+	+	+	-	-	+	+	+
P. fulgens	+	+	-	-	+	+	+	+

+sign indicates the presence of phytochemicals while - sign indicates the absence of phytochemicals.

From the above result it was observed that terpenoids and flavonoids were present in all plants extracts, while polyphenols and reducing compounds were present in most of the plants extracts. The alkaloids was present in five plant extracts. Similarly, glycosides was present only in *S. officinalis* and *A. rivularis.*

Various studies have revealed that phenolic compounds possess biological properties such as anticancer, antioxidant, anti-inflammatory, anti-aging etc. Similarly, flavonoids are known to inhibit many bacterial strains. The glycosides are found to possess antibacterial, antitumor and antioxidant properties. Likewise, the alkaloids are also found to be anticancer, antibacterial, analgesic and antimalarial. Further, saponins of plant are known to enhance antibody production. The present study gives insight to the presence of various phytochemicals which can be attributed to the potential antioxidant and antibacterial properties in the tested sample.

Biological screening

Brine-shrimp bioassay

The newly hatched brine-shrimp nauplii were exposed to the plant extracts and their biological activities were evaluated on the basis of their toxicity towards the nauplii. The LC_{50} values (µg/mL) for different fractions were determined and those having values less than 1000 are supposed to be pharmacologically active. Results obtained during brine shrimp bioassay is given in Table 3.

The results of brine shrimp bioassay showed that methanolic extract of leaf of *N. tabacum*, rhizomes of *A. rivularis*, and roots of *P. fulgens* were pharmacologically active while other plants are inactive. The literature also reported highly toxicity for *N. tabacum* and *A. rivularis* while no literatures were found on other plants toxicity against brine shrimps (Adhikari *et al.*, 2012; Bussmann *et al.*, 2011).

Table 3: LC_{50} value of different plant extracts on Brine Shrimp assay

S. N.	Plant name	LC_{50} (µg/ml)
1	*S. officinalis*	2.75×10^{22}
2	*L. loriformis*	7.06×10^{4}
3	*N. tabacum*	77.09
4	*C. buchananiana*	-
5	*A. rivularis*	79.25
6	*P. fulgens*	190.1

Antibacterial assay

Agar well diffusion method was used to evaluate antibacterial activity which was measured in the form of zone of inhibition (ZOI), given in Table 4. Two out of six plants studied possessed antibacterial activity on the dose dependent manner.

The activity was shown by the methanolic extract of rhizome of *A. rivularis* against both Gram positive and Gram negative bacteria and roots of *P. fulgens* against Gram positive bacteria. The negative control, 5% DMSO, did not produce any zone of inhibition whereas the positive control neomycin produced zone of inhibition. It can also be inferred that the plant extract have great potential as antimicrobial compounds, especially in the treatment of infectious diseases caused by resistant microorganisms.

Table 4: Mean Zone of inhibition (ZOI) shown by different medicinal plants against tested bacteria

Plant extract	**Mean Zone of inhibition (ZOI) (mm)**							
	Test organisms	*S. aureus* (Gram positive)				*E. coli* (Gram negative)			
	Concentration (mg/ml)	10	25	50	100	10	25	50	100
A. rivularis		10	12	15	16	9	13	15	20
P. fulgens		6	10	13	19	-	-	-	-
Neomycin		-	-	-	38	-	-	-	35

The result is well supported by reported literatures Bakht *et al.* (2012), Galvez (2016), Sharma (2016). Although the references are also available regarding antimicrobial activity of *S. officinalis* (Roxb.) Schott. Rakshit *et al.* (2011), such activity was not observed in this study for *S. officinalis*. These differences may be due to the altitude variation, time of collection of that plants and laboratory conditions. Phytochemical analysis of the two plant samples with potential antibacterial activity revealed that the two methanolic leaf extracts contain flavonoids, terpenoids, and phenolic compounds.

Antioxidant activity

The antioxidant activity of the methanolic solution of different samples were explored by using 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay and FRAP (Ferric reducing antioxidant power) assay.

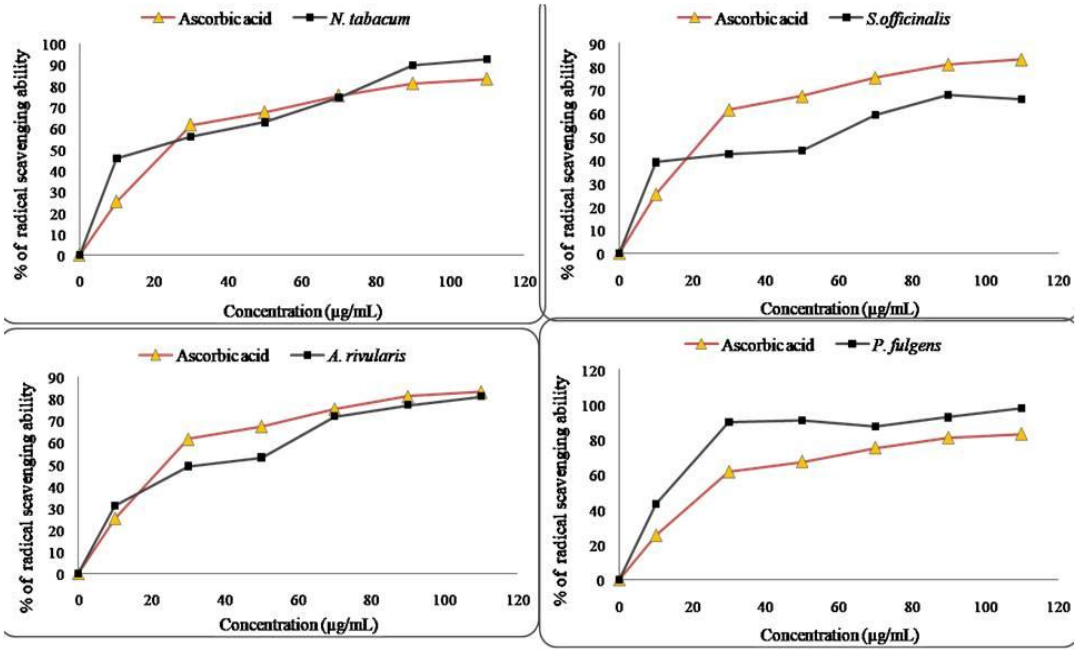

Fig.1. DPPH scavenging activities of the methanolic extracts of the plants.

DPPH assay

The methanol extracts of *S. officinalis, N. tabacum, A. rivularis* and *P. fulgens* were assessed for free radical scavenging activity. The graph of concentration against the corresponding % radical scavenging activity of different samples are plotted (Figure 1) and concentration providing 50% inhibition was determined, which is shown in the Table 3.

Table 5: Antioxidant activities of the plants extract.

S.N.	Plants name	IC_{50} value (µg/ml)
1	*S. officinalis*	50.98 ± 7.12
2	*N. tabacum*	29.21 ± 2.05
3	*A. rivularis*	32.05 ± 2.59
4	*P. fulgens*	15.57 ± 3.6
5	Ascorbic Acid	21.20 ± 2.52

IC_{50} value of the standard i.e., Ascorbic acid was found to be 21.20 µg/ml. Among the plant under study *P. fulgens* have lower IC_{50} values and other plants like *N. tabacum, A. rivularis,* and *S. officinalis* have higher IC_{50} value than that of Ascorbic acid. The high antioxidant activity of the plant *P. fulgens* must be due to the phytochemicals like polyphenols, flavonoids, basic alkaloids etc. The summarized results are shown Table 5.

FRAP assay

The FRAP assay measures the total antioxidant activity on the basis of the ability to reduce a ferric salt Fe(III)(TPTZ)2Cl3 to Fe(II) ions. The FRAP assay was carried out under acidic conditions (pH 3.6) in order to maintain the iron solubility. With reference to the calibration curve obtained at 593 nm for ferrous sulphate solution (R2 = 0.992) (Figure 2).

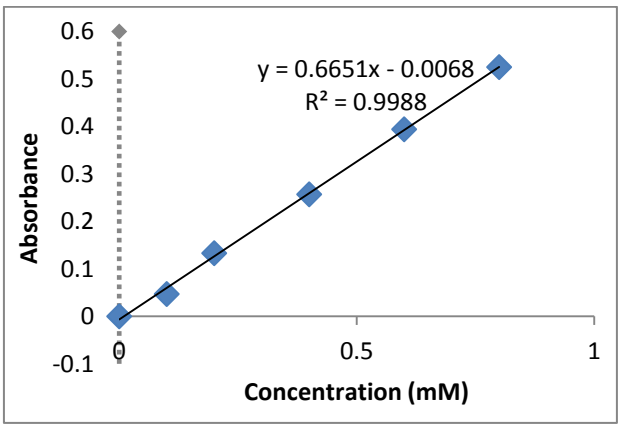

Fig. 2. Absorbance versus concentration of ferrous sulphate.

The FRAP values of extracts of leaves of *N. tabacum, A. rivularis* and *P. fugens* were found 1.74, 2.069, and 1.91 mM Fe+2/liter respectively. The results obtained from the assay are given in Table 6.

Table 6: Antioxidant power of methanol extract of Different plants.

Botanical name	Concentration (mg/ml)	Antioxidant power [mM Fe(II)/L]
N. tabacum	1	1.74 ± 0.023
A. rivularis	1	1.91 ± 0.13
P. fulgens	1	2.096 ± 0.21

The highest antioxidant activity was found in *P. fulgens* extract in both methods. The other two plants showed almost similar activities in both assay. It becomes evident that the antioxidant activities of all the extracts are due to the presence of flavonoids and polyphenols in all the plants. Many reports have been published on the phytochemical screening and antioxidant activities of these plants (Sharma, 2016; Jaitak *et al.,* 2010; Hori *et al.,* 2017). However, the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants. The present study also correlates with the results of previously reported with little variations (Sharma, 2016; Jaitak *et al.,* 2010; Hori *et al.,* 2017).

CONCLUSION

Phytoconstituents and biological activities on the selected six medicinal plants of Dolakha and Sindhualchowk districts of Nepal have been successfully carried out. Based on the results of this study, *P. fulgens*. *N. tabacum* and *A. rivularis* were found to have medicinal potentials. A wide range of phytochemicals is present in all the plants. From Brine shrimp bioassay, leaves of *N. tabacum*, rhizome of *A. rivularis* and roots of *P. fulgens* were found to be highly toxic against brine shrimp nauplii. Roots of *P. fulgens*, and rhizomes of *A. rivularis* showed antibacterial activity against gram positive bacteria. The rhizome of *A. rivularis* also showed antibacterial activity against gram negative bacteria. *P. fulgens* showed highest antioxidant activity followed by *A. rivularis* and

N. tabacum showing almost similar activities. Hence, the three plants can be developed further as plant-based drugs. This report complements the previously reported curative values and it also highlights urgency for the further investigations of these pharmaceutically relevant plants.

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