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Research Article

SCIENTIFIC VALIDATION OF SOME NEPALESE MEDICINAL PLANTS FROM NANGLE BHARE OF KATHMANDU, NEPAL

BIMALA SUBBA*, MAHESH PRASAD TIMILSINA

Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal. Email: bimalasubba@gmail.com

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ABSTRACT

Objective: The present study was designed to provide scientific support for the medicinal properties of traditionally used medicinal plants of Nangle Bhare, Kathmandu, Nepal.

Methods: Plant extracts were prepared by cold percolation method using methanol solvent. Analysis of phytochemical constituents was carried out using standard methods. The brine shrimp toxicity assay for each extract was carried out according to Mayer *et al.* The 1, 1-diphenyl-2-picrylhyrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay were used to evaluate *in vitro* antioxidants activities. The antimicrobial activity of the plant extracts was carried by the disc diffusion method.

Results: Phytochemical analysis revealed the availability of secondary metabolites such as reducing compounds, glycosides, and flavonoids in the methanolic extract of eight plants, namely, frond of *Cheilanthes farinosa*, leaves of *Lyonia ovalifolia*, leaves and roots of *Rumex nepalensis*, aerial stem of *Equisetum debile*, roots of *Coix lacrema-jobi*, leaves of *Justica adhatoda*, leaves of *Osyris wightiana*, and roots of *Rubus ellipticus* collected from Nangle Bhare village of Kathmandu district. The brine shrimp bioassay revealed that roots and leaves of *R. nepalensis* only exhibited lethality against brine shrimp. Frond of *C. farinosa* and roots of *R. nepalensis* exhibited good antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Both DPPH and FRAP assays showed that among the plant under study, *R. ellipticus* has lower inhibitory concentration IC_{50} values than other plants such as *L. ovalifolia, R. nepalensis,* and *C. farinose.*

Conclusions: On the basis of this study, it was found that scientifically only four, namely *C. farinosa*, *L. ovalifolia*, *R. nepalensis,* and *R. ellipticus* have medicinal potentials.

Keywords: Medicinal plants, Phytochemicals, Biological screening, Antioxidant activity, 1, 1-Diphenyl-2-picrylhyrazyl assay, Ferric reducing antioxidant power assay.

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INTRODUCTION

A natural product is a term used commonly in reference to a compound found in nature with unique pharmacological effects. Nature has bestowed Nepal with natural biodiversity having a large number of medicinal plants [1].

Traditional use of plant and plant resources for the medicinal purpose has a long history in Nepal, and its use is rapidly spreading due to having no side effects, easily available at affordable prices and sometimes the only source of health care available to the poor [2]. Nepal is a multiethnic nation with more than 60 different ethnic groups speaking about 75 languages. These ethnic communities have a trivial customary acquaintance on the utilization of plant and plant parts, and there is a long tradition of transferring this indigenous knowledge orally from generation to generation [2]. The modern tools of chemistry and biology now allow scientists to point the exact nature of the biological effects of natural compounds on the human body as well as to expose potential synergies, which hold much assurance for the development of new therapies against many overwhelming diseases, including dementia and cancer [3]. The citations of traditional knowledge, especially on the medicinal uses of plants have delivered many important drugs of the modern day [4].

In this scenario, only a few studies have been attempted to evaluate the bio-efficacy of traditionally used medicinal plants scientifically in Nepal [5-8]. Thus, the present study was carried out to evaluate the phytochemicals and biological assay of following eight locally available medicinal plants, that is, *Cheilanthes farinosa* (Forsk.) Kaulf, *Lyonia ovalifolia* (Wall.) Drude, *Rumex nepalensis* (Spreng), *Equisetum debile* (Roxb. ex Vaucher), *Coix lacryma-jobi, Justica adhatoda, Osyris wightiana, and Rubus ellipticus* from Nangle Bhare of Kathmandu District of Nepal. The plants were selected on the basis of their traditional use by villagers. The outcomes from this research work may add to the overall value of the medicinal potential of the plants.

MATERIALS AND METHODS

Selection and collection of plants

In this study, medicinal plants are selected on the basis of their medicinal importance in literature and native people from Nangle Bhare of Kathmandu district at the altitude of 1550–1600 m. The collected plants were identified from the Central Department of Botany, TU Kathmandu, Nepal.

Chemicals

Methanol from Merck, Germany and 1, 1-diphenyl-2-picrylhyrazyl (DPPH), TPTZ and Ascorbic acid from Sigma-Aldrich, USA, were used. All other chemicals used were the highest grade.

Preparation of extracts

We followed the methods of Subba *et al*. 2014 [9]. The collected plants were cleaned, air dried in the shade. Exposure to the sunlight is escaped to stop the loss and alteration of the active components. The entirely dried samples were pulverized 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 a clean and dry conical flask and immersed in methanol. It was left for 2–3 days at room temperature with shaking at intervals. Then, it was

filtered and the filtrate was concentrated using a rotary evaporator. This process was continued for 6–7 times until complete extraction. The concentrated filtrate was air-dried to obtain the solid or semisolid residue. The same process was repeated for all plants. After completely drying they were kept in a beaker. The dried extracts were used for different tests.

Phytochemical screening

Analysis of crude methanolic extracts of the above eight medicinal plants for various phytochemical constituents was carried out using standard methods [10].

DPPH radical scavenging 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 DPPH free radical [11]. Various concentrations (15–250 µg/ml) of plant extracts were added to a 100 µM solution of DPPH in methanol. After incubation at 37°C for 30 min, the absorbance was measured at 517 nm for each solution. The measurement was performed in triplicates. The antioxidant activity of the extract was conveyed as inhibitory concentration (IC_{50}), 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 using following the universal equation:

$$\% \text{ Of free radical scavengingactivity} = \frac{(A_0 - A_T)}{A_0} \times 100$$

Where, A_0 = Absorbance of DPPH solution and A_T = Absorbance of test or reference sample. The percentage scavenging was then plotted against concentrations used and from the graph IC_{50} was calculated.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity by FRAP assay was conducted according to the procedure given by Benzie and Strain [12]. Standard calibration curve of ferrous sulfate was plotted to calculated antioxidant activity. The leaf extracts (5 mg/ml) were prepared by adding methanol and were used as a sample. Finally, the absorbance was taken at 593 nm keeping the temperature at 37°C.

Microorganism

The microorganisms used in this study were identified strains obtained from Central Department of Microbiology, TU, Kathmandu, Nepal. In this study, one Gram-positive (*Staphylococcus aureus*) and one Gram-negative (*Escherichia coli*) were used.

Antibacterial assay

The antimicrobial activity of the plant extracts was carried by the disc diffusion method [13]. The sterile filter paper discs (6 mm in diameter) were individually soaked with different concentration of plant extract prepared in dimethyl sulfoxide (DMSO) and then placed into the agar plates which had previously been inoculated with the tested microorganisms for 30 min. The plates were subsequently incubated for 24 h at 37°C. After incubation, the growth inhibition rings were calculated by measuring the diameter of the zone of inhibition (ZOI) in mm. For control DMSO discs were used. All tests were performed in triplicate.

Brine shrimp bioassay

The brine shrimp toxicity assay for each extract was carried out according to Mayer *et al*. [14]. Briefly, sample solutions were prepared by dissolving 200 mg of each plant extract in DMSO up to the mark in 10 ml volumetric flasks and solutions of varying concentrations for 10, 100 and 1,000 µg/ml were obtained by serial dilution technique using artificial sea water. Ten, one-day nauplii were used in each test. Three replications were used for each concentration. A control tube (without plant extract) for each dose level was prepared in parallel. The number of survivors was counted after 24 h of illumination under a table lamp (60 Watt). No death was observed in the control tubes. The lethal concentration for 50% (LC_{50}) mortality values was determined using the probit method Finney, as the measure of toxicity of the extracts [15].

RESULTS AND DISCUSSION

Extraction and phytochemical screening

The different phytochemicals in the crude methanol extracts were identified by the color reaction with different reagents. The results of percentage yields during extraction and phytochemical screening are shown in Tables 1 and 2, respectively.

Table 1: % Yield of different plant extracts in methanol

Name of plant species	Dry weight of sample (g)	Dry weight of extract (g)	% Yield
C. farinose	50.260	10.286	20.466
L. ovalifolia	50.665	15.591	30.773
R. nepalensis (leaves)	60.495	6.202	10.252
R. nepalensis (roots)	50.200	11.249	22.408
E. debile	75.806	6.094	8.039
C. lacrema jobi	75.826	5.152	6.795
J. adhatoda	75.808	5.673	7.483
O. wightiana	50.597	12.279	24.268
R. ellipticus	50.505	5.596	11.080

C. farinosa: Cheilanthes farinose, C. lacrema jobi: Coix lacryma-jobi, L. ovalifolia: Lyonia ovalifolia, J. adhatoda: Justica adhatoda, R. nepalensis: Rumex nepalensis, O. wightiana: Osyris wightiana, R. ellipticus: Rubus ellipticus, E. debile: Equisetum debile

Table 2: Phytochemical screening of different plant extracts

Group of compounds	*C. farinosa*	*L. ovalifolia*	*R. nepalensis* (leaves)	*R. nepalensis* (roots)	*E. debile*	*C. lacryma-jobi*	*J. adhatoda*	*O. wightiana*	*R. ellipticus*
Glycosides	-	-	+	-	-	+	-	-	+
Quinones	-	-	-	-	-	-	-	+	-
Saponins	-	-	-	+	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	+	-	-	-
Flavonoids	+	+	+	-	-	-	-	+	+
Polyphenols	+	-	-	-	-	-	-	-	-
Reducing compounds	-	-	+	-	-	+	-	-	-

+: Detected, -: Not detected, *C. farinosa: Cheilanthes farinose, C. lacryma-jobi: Coix lacryma-jobi, L. ovalifolia: Lyonia ovalifolia, J. adhatoda: Justicia adhatoda, R. nepalensis: Rumex nepalensis, O. wightiana: Osyris wightiana, R. ellipticus: Rubus ellipticus, E. debile: Equisetum debile*

The phytochemical analysis of different plant extracts in methanol is given in Table 2.

The preliminary screening of phytochemical constituent of different plant extracts revealed the presence of reducing compounds, glycosides, and flavonoids. The studies have shown that the plant extracts: *C. farinosa* (Forsk.) Kaulf, *L. ovalifolia* (Wall.) Drude*, Rumex nepalensis* (Spreng), *C. lacryma-jobi, and O. wightiana* contain the major pharmaceutical constituents such as glycosides, flavonoids, terpenoids, and saponins. The obtained resulted is well supported by the literature [16,17]. These are the bioactive components which are known to be as bactericidal, pesticidal in nature [18-20]. The existence of these metabolites suggests a great potential of these plant sample as a major source of valuable phytomedicines. However, many phytochemicals are reported from *E. debile* (Roxb. ex Vaucher) and *J. adhatoda,* in this study any phytochemicals were not observed in these plants [21,22]. The results presented in Table 2 are slightly different than the data present in the literature of these different plants. This may be due to the variation in altitude of plants, extraction procedure, etc. [17,23].

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 toward the nauplii. The LC_{50} values (µg/mL) for different fractions were determined and those having values <1000 are supposed to be pharmacologically active. Results obtained during brine shrimp bioassay are given in Table 3. The results of brine shrimp bioassay showed that methanolic extract of *R. nepalensis* (roots) was pharmacologically active while other plants are inactive. The literature also reported moderate toxicity for *R. nepalensis* while no literature was found on other tested plants toxicity against brine shrimps [23].

Antibacterial activity

The results of Antibacterial activity of plant extracts are given in the Table 4. There were two out of nine plants studied possessed antibacterial activity in a dose-dependent manner.

The antibacterial activity was studied by measuring the inhibition zone around the disc (6 mm). The antibacterial activities of nine different plant extracts were determined in comparison with Streptomycin and DMSO. The blind control 5% DMSO did not produce any ZOI whereas the positive control streptomycin produced a ZOI. The organisms taken were *S. aureus* as Gram-positive bacteria whereas *E. coli* as Gram-negative bacteria.

Table 3: Calculation of LC_{50} value of plant extracts

Plant extracts	LC_{50}
R. nepalensis (Leaves)	31622.7±0.68
R. nepalensis (roots)	251.18±0.21

Values are expressed as mean+ SD; values are from triplicate readings,
R. nepalensis: Rumex nepalensis

The activity was shown by the methanolic root extract of the rhizome of *R. nepalensis* and frond of *C. farinosa* against both Gram-positive and Gram-negative bacteria. The result is well supported by reported kinds of literature [24,25]. Phytochemical analysis of the two plant samples with potential antibacterial activity revealed that the two methanolic leaf extracts contain flavonoids, polyphenol, and saponin (Table 2). It also revealed that the plant extract has great potential as antimicrobial compounds, especially in the treatment of infectious diseases caused by resistant microorganisms. In contrast, the references are also available regarding the antimicrobial activity of *E. debile* [26], *L. ovalifolia* and *O. wightiana* [27], *J. adhatoda,* [28], *R. ellipticus* [29], and *C. lacrema-jobi* [16], but for all these plant extracts antibacterial activity has not been observed.

These differences may be due to variation in genetic, geographical, and seasonal factors as well as the developmental stages of the concerned plant, its parts/tissues [30].

Antioxidant activity

The antioxidant activity of the methanolic solution of different samples was explored by using DPPH radical scavenging assay and FRAP assay.

DPPH assay

The methanol extractives of *C. farinosa, L. ovalifolia, R. nepalensis,* and *R. ellipticus* were assessed for free radical scavenging activity. The graph of concentration against the corresponding percentage radical scavenging activity of different samples was plotted (figures not shown) and concentration providing 50% inhibition was determined.

An IC_{50} value of four plant species and ascorbic acid is presented in Table 5.

IC_{50} value of the standard, that is, ascorbic acid was found to be 21.17±0.5 µg/ml. Among the plant under study, *R. ellipticus* has lower IC_{50} values than other plants such as *L. ovalifolia, R. nepalensis,* and *C. farinose.* The high antioxidant activity of the plant *R. ellipticus* is may be due to the phytochemicals such as flavonoids and glycosides. Rest of five plants were not considered for the determination of antioxidant activity after the preliminary test was performed. However, *E. debile* [26], *J. adhatoda* [31], and *O. wightiana* [32] also showed the significant antioxidant activity. This variation may be due to the collected plant from various altitudes, genetic variation, etc.

FRAP assay

This method measures the total antioxidant activities of samples on the basis of their ability to reduce a ferric salt Fe (III)$(TPTZ)_2Cl_3$ to Fe(II) ions. Acidic condition (pH 3.6) was maintained during the FRAP assay to maintain the iron solubility. With reference to the calibration curve (figure not shown) obtained at 593 nm for ferrous sulfate solution (R^2=0.992), the FRAP values of extracts of leaves of *R. ellipticus*, *L. ovalifolia*, *R. nepalensis,* and *C. farinosa* were found 2.266±0.04, 2.176±0.05, 1.983±0.01, and 1.228±0.011 mM Fe^{++}/L, respectively, Table 6.

The above data show that high antioxidant power for these four plants. This result is comparable to the data obtained from the DPPH

Table 4: Mean ZOI shown by different medicinal plants against tested bacteria

Plant extract	Mean ZOI (mm)							
	Test organisms	*S. aureus* (Gram-positive)				*E. coli* (Gram-negative)			
	Concentration (mg/ml)	10	25	50	100	10	25	50	100
C. farinosa		9±0.26	11±0.36	14±0.32	17±0.32	8±0.32	11±0.50	13±0.5	16±0.5
R. nepalensis (roots)		11±0.28	14±0.32	16±0.28	18±0.32	13±0.5	15±0.25	17±0.5	19±0.24
Streptomycin		-	-	-	20±0.5	-	-	-	21±0.32

S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, C. farinosa: Cheilanthes farinose and R. nepalensis: Rumex nepalensis, ZOI: Zone of inhibition, ZOI includes hole size 6 mm, ZOI: Zone of inhibition

Table 5: IC_{50} values of different plant extracts in comparison with ascorbic acid

Plants name	IC_{50} values (µg/ml)
Ascorbic acid	21.17±0.5
C. farinosa	40.72±0.21
L. ovalifolia	24.31±0.28
R. nepalensis	25.23±0.24
R. ellipticus	23.66±0.4

Values are expressed as mean+ SD; values are from triplicate readings, *C. farinosa: Cheilanthes, L. ovalifolia: Lyonia ovalifolia, R. nepalensis: Rumex nepalensis, R. ellipticus: Rubus ellipticus*

Table 6: The antioxidant power of the methanolic extract of different plants

Botanical name	Concentration (mg/ml)	Antioxidant power (mM Fe(II)/L)
C. farinosa	1	1.228±0.011
L. ovalifolia	1	2.176±0.05
R. nepalensis	1	1.983±0.01
R. ellipticus	1	2.266±0.04

Values are expressed as mean+ SD; values are from triplicate readings, *C. farinosa: Cheilanthes, L. ovalifolia: Lyonia ovalifolia, R. nepalensis: Rumex nepalensis, R. ellipticus: Rubus ellipticus*

assay also. Many reports have been published in the literature on the phytochemical screening and antioxidant activities of these plants [33,34]. However, the natural products profile and consequently the bioactivity are known to vary with the climate, geographic location of the plants, and genetic variation. The results of this study link well with the results of previously stated with slight variations.

CONCLUSIONS

Phytoconstituents and biological activities on the selected eight medicinal plants of Nangle Bhare of Kathmandu District of Nepal have been successfully carried out. Based on the results generated from the study, only four, namely, *C. farinosa, L. ovalifolia, R. nepalensis,* and *R. ellipticus* have medicinal potentials. A wide range of phytochemicals is present in all plants. From brine shrimp bioassay, the root of *R. nepalensis* was found to be toxic against brine shrimp nauplii. The two plants extract, namely frond of *C. farinosa* and roots of *R. nepalensis* only showed the antibacterial activity among nine different extracts. The result indicated that these plants could be the source of potent antibacterial medicine in the treatment of bacterial diseases. The front of *C. farinosa*, leaves of *L. ovalifolia*, roots of *R. nepalensis,* and roots of *R. ellipticus* exhibited the highest antioxidant activity. Hence, the four plants can be developed further as plant-based antioxidants. This report complements the previously reported curative values, and it also highlights the urgency for the further investigations of these pharmaceutically relevant plants.

Statistics

All the analysis was carried out in triplicate, and the results are expressed as mean ± SD.

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CONFLICTS OF INTEREST

The authors announce that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Subba *et al*. analyzed the data, and wrote the manuscript, whereas MP Timilsina carried out the laboratory work. Both authors read and approved the final manuscript.

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