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RESEARCH ARTICLE

Phytochemical Screening and *In Vitro* Antioxidant Activity of Three Nepalese Plants

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Abstract

Nepal is rich in plant resources and holds the 49th spot in the world's biodiversity. The variation in altitude, climatic conditions, and geographical features across Nepal make it a rich source of Medicinal and Aromatic Plants (MAPs). The study was carried out to perform the preliminary phytochemical screening and assess antioxidant properties in *Ficus neriifolia, Rubus treutleri,* and *Periploca calophylla.* Plants were selected based on their ethnomedical use and scant scientific research. Ethanol (70%) and aqueous extracts were prepared by using the cold maceration method and preliminary phytochemical screening was conducted using the previously established method to test the presence of biologically active phytoconstituent. *In vitro* antioxidant activity was examined by DPPH free radical scavenging assay. The results revealed the variation in phytoconstituents among the 70% ethanol and aqueous extracts of all three plant samples. Among the samples, 70% ethanol extract of *P. calophylla* exhibited the highest antioxidant activity with an IC_{50} value of 94.36 μg/mL, while the aqueous extract of *F. neriifolia* showed the minimum antioxidant activity with an IC_{50} value of 902.23 μg/mL. This study indicates that these plant samples possess potent natural antioxidants capable of scavenging free radicals that cause cellular damage and contribute to various diseases in our bodies.

Keywords: DPPH, phytochemicals, *Periploca calophylla, Ficus neriifolia, Rubus treutleri*

1. Introduction

The diverse geographical, altitudinal, climatic, and ecosystem variation of Nepal has led to the availability of a wide range of flora and fauna. In Nepal, about 1515 to 2331 Medicinal and Aromatic Plants (MAPs) have been used for the treatment of several ailments [1]. Medicinal plants have been used as the source of medicine in allopathic systems and alternative systems of medicine like Ayurveda, Homeopathy, Unani, Siddha, and Shamanistic systems [2]. The knowledge about the use of medicinal plants has been transferred from one generation to another generation mainly through oral means of communication [3]. However, the lack of proper documentation, hesitation to share the knowledge with common people, and difficulties in the collection of medicinal plants have led to many medicinal plants being scientifically unexplored.

In the present study, three Nepalese plants were selected based on their traditional medicinal uses and limited scientific exposure. This study was conducted to explore the phytochemical constituents and antioxidant activity, which can be the basis for future investigations on their pharmacological properties. The selected plants for the study are briefly described here.

Ficus neriifolia **Sm.** is one of the common fodder species found in Nepal, Bhutan, India, China, Indochina, and Myanmar belonging to the Moraceae family. The twigs and foliage of the tree can be collected throughout the year for livestock purposes [4-5]. It is found at the altitude of 1400-2200 m in Nepal. Traditionally, its bark and stem juice were used in boils and conjunctivitis [6].

Rubus treutleri **Hook. F.** is a shrub belonging to the family Rosaceae, distributed throughout Nepal, India, Bhutan, and China. It is about 2 m tall with stalked, alternate leaves, slightly hairy on both surfaces, and the base is cordate. Flowers are white and fruits are red. The flowering season is from June to August and the fruiting season is in between September and November. It is propagated by roots, offshoots, or seeds [7]. In the Tanahun district of Nepal, the roots are traditionally used in combination with other plant species including *Ficus racemosa, Castanopsis indica, Poranopsis paniculata, Cheilocostus speciosus, Pogostemon benghalensis* for the treatment of burning sensation of stomach, urine, feet, and hands.

Periploca calophylla **(Wight) Falc**. is a trailing shrub under the family Apocynaceae, found in Nepal, India, Bhutan, Tibet, and Central West China. Leaves are lanceolate, long acuminate, leathery, and shiny stalked. Flowers occur in lax cymes. The flowering season is from April to May and the fruiting season is from November to January. It is propagated by seeds. In Nepal, it is used for treating multiple ailments such as bone fractures and muscle pain [8]. Stem of the plant is used for the treatment of traumatic injury and snakebite [9].

2. Materials and Methods

2.1 Plant Materials

The plant species (Table 1) were collected from the Kaski and Baglung districts of Nepal during April. Identification of plants was done with the help of a botanist and by comparing the herbarium plant databases. The herbarium of plant samples was prepared, registered, and stored at the Pharmacognosy Laboratory of Novel Academy, Purbanchal University, Pokhara, Nepal.

Table 1. List of plants used in the study

S. N.	Plants	Local name	Parts collected	Place of collection	Herbarium registration number
1	*Ficus neriifolia*	Dudhilo	Leaves	Baglung	NAH-2022-01
2	*Rubus treutleri*	Thulo aiselu	Stem	Kaski	NAH-2022-02
3	*Periploca calophylla*	Sikari laharo	Aerial parts	Kaski	NAH-2022-03

2.2 Extraction and Yield Value Calculation

The collected plant samples were dedusted, washed thoroughly with clean water, and chopped into pieces. The chopped plant materials were shade-dried and crushed into coarse powder by using an electric grinder. The dried plant samples (15 g) were macerated with 70% ethanol (150 mL) and distilled water (150 mL) at the sample: solvent ratio of 1:10 (w/v) for 48 hours at room temperature. The extracts obtained were concentrated using a rotary evaporator under reduced pressure (40ºC, 90 rpm). Then, the extracts were packed in closed vials and stored at 4ºC until further experiment was performed.

The extract yield percentage was calculated using the formula:

$$Extract\ Yield\ Percentage = \frac{Weight\ of\ extract\ after\ solvent\ evaporation}{Weight\ of\ dried\ plant\ sample\ taken} \times 100$$

2.3 Phytochemical Screening

Freshly prepared plant extracts were subjected to

Table 2. *List of phytochemical screening methods used in the study*

Phytoconstituents	Tests	Methods	Observations
Carbohydrates	Molisch's test	Extract + Molisch's reagent + sulphuric acid	Purple/violet ring at the junction
	Benedict's Test	(Extract + Benedict's reagent) heated	Green/orange/red precipitate indicates the presence of reducing sugars
	Fehling's test	(Extract + Fehling's A + Fehling's B) heated	Red precipitate indicates the presence of reducing sugars
Flavonoids	Shinoda test	Extract + ethanol + a few pieces of magnesium metal + conc. HCl	The orange color indicates the presence of flavones. Red or magneta color indicates the presence of flavonols.
	Alkaline test	Extract + 2% NaOH	Yellow color that becomes colorless with the addition of HCl
Glycosides	Killer-killani's test	Extract + glacial acetic acid + 2% $FeCl_3$+ sulphuric acid	The formation of a brown ring between the layers indicates the presence of cardiac steroidal glycosides
Saponins	Froth test	The filtrate is shaken vigorously with distilled water	Persistent foam for 2-5 minutes
Tannins	Ferric chloride test	Extract + 5% ferric chloride	A dark blue precipitate indicates hydrolyzable tannin. Green precipitate indicates condensed tannin.
Quinones	Sulphuric acid test	Extract + isopropyl alcohol + conc. sulphuric acid	Red color
Terpenoids	Salkowski test	Extract + choloroform + conc. sulphuric acid	Reddish-brown precipitate at the interface
Proteins	Xanthoproteic Test	Filtrate + ethanol + conc. Nitric acid (few drops)	Yellow color

phytochemical screening to detect the presence of phytoconstituents such as carbohydrates, flavonoids, glycosides, saponins, tannins, quinones, terpenoids, and proteins by following the standard methods [10-15] as described in Table 2.

2.4 Antioxidant Activity

DPPH free radical scavenging assay [16] was used to determine the antioxidant activity of the plant extracts. 50 μL of each extract of various concentrations (250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL, 15.62 μg/mL, and 7.81 μg/mL) was mixed with 150 μL of ethanolic solution of DPPH (150 μM) in 96 well plates. The reaction mixture was incubated for 30 minutes at room temperature in dark condition and the absorbance value of the resulting solution was measured at 517 nm using an ELISA plate analyzer. Upon reduction, the color of the solution faded from violet to pale yellow. Percent DPPH free radical scavenging activity was calculated as follows:

$$DPPH\ free\ radical\ scavenging\ activity = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Control was the test solution without a sample. Ascorbic acid was used as the standard. The antioxidant activity was expressed in terms of IC_{50} and was calculated by plotting a graph of percentage DPPH free radical scavenging activity against concentration.

2.5 Statistical Analysis

Antioxidant assay was performed in triplicate and the results were expressed as mean±standard deviation (SD). The IC_{50} was calculated using a linear regression equation. All statistical analysis was carried out through Microsoft Excel 2007.

3. Results and Discussion

3.1 Extract Yield Percentage

The extraction of the selected plant samples was carried out in 70% ethanol and distilled water. The extract yield percentage of the plant samples is given in Table 3. Among the different plant samples, the yield percentage was found the highest in the aqueous extract of *P. calophylla.*

Table 3. Extract yield percentage of plant samples

Plants names	Weight of extract after solvent evaporation (70% Ethanol extract)	Yield % (70% Ethanol extrac)	Weight of extract after solvent evaporation (Aqueous extract)	Yield % (Aqueous extract)
F. neriifolia	2.05 g	13.67	1.60 g	10.67
R. treutleri	1.31 g	8.73	1.67 g	11.13
P. calophylla	1.46 g	9.73	2.49 g	16.6

Wide varieties of solvents such as water, ethanol, methanol, acetone, etc., have been used for the extraction of phytoconstituents and their choice depends on the bioactive constituents to be extracted [17]. In the present study, 70% ethanol and distilled water were used as the extraction solvents and the effect of these extraction solvents on extraction yield, phytochemical constituents, and antioxidant activity were studied. More bioactive compounds are extracted in 70% ethanol than absolute ethanol as the addition

of water to ethanol improves the extraction rate. Water is known as a universal solvent and has been reported to be the better solvent for the extraction of phenolic compounds [18-21]. Traditionally, medicinal plants were taken with water, therefore, to explore the phytochemicals extracted with water; water was used as an extraction solvent in the present study.

In the present study, the yield value of *F. neriifolia* was found higher in 70% ethanol extract than in aqueous extract. This result was consistent with previous studies [21-23], where the ethanol extract of different plants showed a high yield value in comparison to the aqueous extract. In our study, the yield value of *R. treutleri* and *P. calophylla* was found higher in aqueous extract than in 70% ethanol extract. Several studies [17, 24-26] have also reported a similar finding, where yield value was found higher in aqueous extract than in ethanol extract. The differences in the extract yield values of these plant samples are due to the differences in the presence of bioactive constituents in them. The higher the solubility of phytoconstituents in the solvent, the higher is their extract yield value [27].

3.2 Phytochemical Screening

The results obtained from qualitative phytochemical screening of different plant extracts are presented in Table 4. The phytochemical evaluations were graded as (−) absence, (+) poor, (++) moderate, (+++) abundant based on the intensity of the colored reaction product of the test compared to the control in each case.

Table 4. Phytochemical screening of plant extract

S.N.	Phytochemicals	Tests	*F. nerrifolia*		*R. treutleri*		*P. calophylla*	
			E	***A***	***E***	***A***	***E***	***A***
1	Carbohydrates	Molisch's test	+++	-	+++	++	+++	++
		Benedict's test	+	+	+	+	+	+
		Fehling's test	+	+	+	+	+	+
2	Flavonoids	Shinoda test	++	+	+++	+	+	++
		Alkaline test	+++	+	++	++	+++	+
3	Glycosides	Killer-killani's test	-	-	+++	-	+++	-
4	Saponins	Froth test	+	+	-	-	++	+++
5	Tannins	Ferric chloride test	++	+	++	+++	+++	+
6	Quinones	Sulphuric acid test	+++	-	-	++	-	-
7	Terpenoids	Salkowski test	++	+	+++	++	++	++
8	Proteins	Xanthoproteic test	+	-	-	+	++	-

Note: E= 70% Ethanol extract; A= Aqueous extract

The 70% ethanol extract of *F. neriifolia* contains phytochemicals like carbohydrates, flavonoids, tannins, saponins, quinones, terpenoids, and proteins in different amounts, while the aqueous extract was devoid of quinones and protein. Both 70% ethanol and aqueous extracts were devoid of cardiac glycosides. In Shinoda test for flavonoids, *F. neriifolia* extract showed an orange color appearance, which indicates the presence of flavones. Phytochemical screening of different Ficus species has previously revealed the presence of flavonols, pentacyclic triterpenes, triterpenoids, alkaloids, balsams, carbohydrates, free anthraquinones, tannins, glycosides, resins, sterols and saponins [28].

Both the 70% ethanol and aqueous extract of *R. treutleri* contain phytochemicals such as carbohydrates, flavonoids, tannins, and terpenoids. Saponins were absent in both extracts, whereas quinones and protein were present only in the aqueous extract and cardiac glycoside was present only in the 70% ethanol extract. In the shinoda test for flavonoids, *R. treutleri* extract showed a magenta color appearance, which indicates the presence of flavonol. Basak et al. [2018] have previously reported the presence of phytochemical constituents such as proteins, tannins, phlobatannin, terpenoid, alkaloids, saponins, lipids, anthocyanins/betacyanin in a 70% methanol extract of ripe fruits of *R. treutleri* [29]. In the present study, the 70% ethanol extract of the *R. treutleri* stem was devoid of saponins. The differences in phytochemical constituents may be due to differences in plant parts used, solvent used, extraction method, geographical location of plants collected, etc.

Similarly, the 70% ethanol and aqueous extracts of *P. calophylla* revealed the presence of carbohydrates, flavonoids, saponins, tannins, and terpenoids. Both extracts were devoid of quinones, whereas cardiac glycosides and proteins were present only in the 70% ethanol extract. In Shinoda test for flavonoids, *P. calophylla* extract showed an orange color appearance, which indicates the presence of flavones. The relative amounts of phytochemicals were found to be more in 70% ethanol extract than in aqueous extract.

Many studies [30-33] have reported that different phytoconstituents such as tannins, flavonoids, terpenoids, saponins, phenols, and glycosides possess several biological activities such as anti-inflammatory, antioxidant, antidiabetic, antimicrobial, anticancer, antioxidant activities, etc. The presence of various phytoconstituents in these plant extracts might also contribute to the various biological actions, so further research should be conducted to explore their biological activity.

3.3 Antioxidant Activity

The antioxidant activity of the plant samples was carried out by DPPH free radical scavenging assay. The DPPH free radical scavenging activity of 70% ethanol and aqueous extract of plant samples are shown in Figure 1 and Figure 2, respectively. In the present study, the antioxidant activity increases with an increase in the concentration of the extract. The antioxidant activity was found higher in 70% ethanol extract in comparison to aqueous extract.

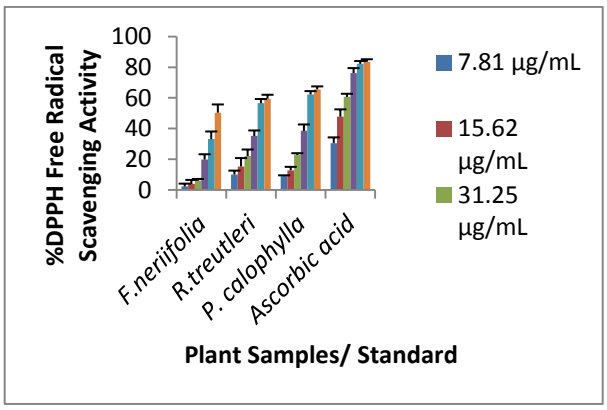

Figure 1. Graphical representation of percentage DPPH free radical scavenging activity of 70% ethanol extract of plant samples and standard ascorbic acid. The error bar represents the standard deviation of three independent determinations (n = 3).

In the 70 % ethanol extract, DPPH radical scavenging activity was found highest in *P. calophylla* (IC_{50} value = 94.36 μg/mL) and lowest in *F. neriifolia* (IC_{50} value = 233.36 μg/mL) in comparison to standard ascorbic acid (IC_{50} value = 24.28 μg/mL).

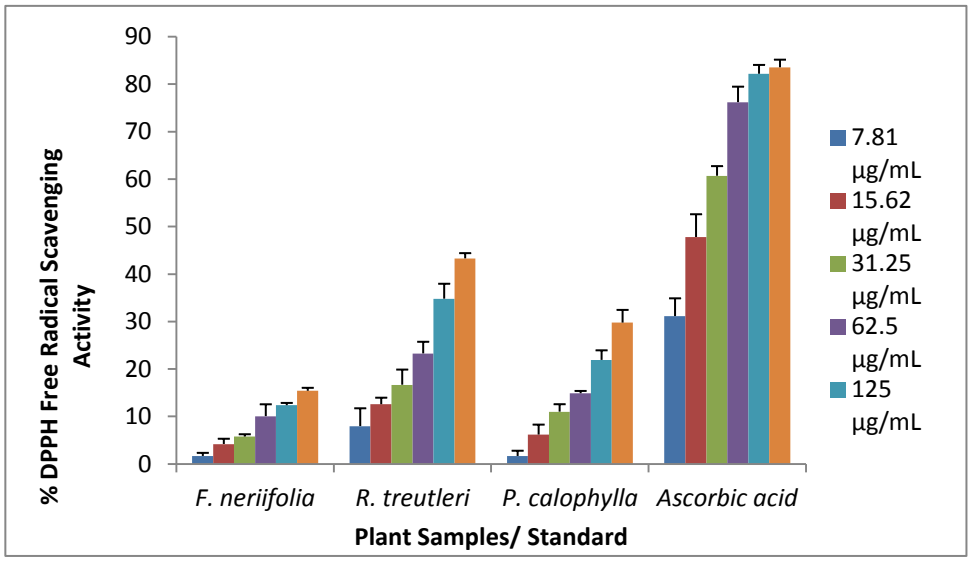

Figure 2. *Graphical representation of percentage DPPH free radical scavenging activity of aqueous extract of plant samples and standard ascorbic acid. The error bar represents the standard deviation of three independent determinations (n = 3).*

In aqueous extract, DPPH radical scavenging activity was found highest in *R. treutleri* (IC_{50} value = 274.64 μg/mL) and lowest in *F. neriifolia* (IC_{50} value = 902.23 μg/mL) in comparison to standard ascorbic acid (IC_{50} value = 24.28 μg/mL).

In the present study, the lowest antioxidant activity was found both in 70% ethanol and an aqueous extract of *F. neriifolia*. The relative number of secondary metabolites was also found less in *F. neriifolia* than in other extracts. This could be the possible reason behind the lowest antioxidant activity exhibited by *F. neriifolia*. Similarly, in this study, 70% ethanol and aqueous extracts of *R. treutleri* stem have shown antioxidant activity with an IC_{50} value of 105.4 μg/mL and 274.64 μg/mL, respectively. In a previous study, it was reported that a methanol extract from fruits of *R. treutleri* showed DPPH free radical scavenging activity with an IC_{50} value of 60.13 μg/mL [29]. Poudel et al. (2019) had previously reported that ethanol and aqueous extracts of *P. calophylla* stem exhibit antioxidant activity with an IC_{50} value of 11.69 μg/mL and 9.03 μg/mL, respectively [26]. The variation in antioxidant activity in different studies may be due to the difference in the amount and types of phytochemicals present in different parts of the plant and the disparity between the solvents and methods used for the extraction.

The antioxidant activity of plant samples may be attributed to the presence of different phytochemicals such as flavonoids, saponins, tannins,

terpenoids, proteins, etc. Flavonoids have been reported to be an antioxidant by inhibiting free radical-generating enzymes like xanthine oxidase, lipoxygenase, protein kinase C, cyclooxygenase, microsomal monooxygenase, and NADPH oxidase [34, 35]. Tannins function as antioxidants because of their capacity to interfere with oxidation reactions, chelate metal ions, and primarily can donate hydrogen atoms to oxidants [36]. Saponins show antioxidant activity by inhibiting the production of ROS and scavenging free radicals [37]. Terpenoids are an important class of phytoconstituents that show prominent antioxidant activity through their hydrogen-donating capacity and radical scavenging properties [38]. The protein shows unique antioxidant activity by inactivating ROS, reducing hydroperoxides, and chelating prooxidative transition metals [39].

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4. Conclusion

The result of this study revealed that both the 70% ethanol and aqueous extract of all three plants *F. neriifolia*, *R. treutleri*, and *P. calophylla* contain a diverse array of secondary metabolites. Among the studied plant samples, the 70% ethanol extract of *P. calophylla* displayed notable antioxidant activity, which might be due to the presence of phytoconstituents such as polyphenols, flavonoids, and tannins. This plant can be a potent source of natural antioxidants, which could aid in preventing various oxidative stress-related conditions potentially harmful to cellular physiology and cause several diseases. Further investigations can be performed to determine the particular mechanism and identify the specific chemical constituents responsible for exhibiting antioxidant activity.

Disclosure

The authors declare no conflicts of interest for this study.

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References

1. G. Lamichhane, G. Sharma, B. Sapkota, M. Adhikari, S. Ghimire, P. Poudel, H.J. Jung, Screening of antioxidant, antibacterial, anti-adipogenic, and anti-inflammatory activities of five selected medicinal plants of Nepal, *Journal of Experimental Pharmacology*, 2023, 93-106.
2. D. Khadka, M.K. Dhamala, F. Li, P.C. Aryal, P.R. Magar, S. Bhatta, M. S. Thakur, A. Basnet, D. Cui and S. Shi, The use of medicinal plants to prevent COVID-19 in Nepal, *Journal of Ethnobiology and Ethnomedicine*, 2021, **17**(1), 1-7.
3. A.G. Singh, A. Kumar and D. D. Tewari, An ethnobotanical survey of medicinal plants used in Terai forest of western Nepal, *Journal of Ethnobiology and Ethnomedicine*, 2012, **8**, 1-5.
4. Y.R. Subedi, R. Mulia, E. Cedamon, B. Lusiana, K. Shrestha and I. Nuberg, Local knowledge on factors

leading to agroforestry diversification in mid-hills of Nepal, *Journal of Forest and Livelihood*, 2018, **15**(2), 32-51.

5. L. B. Chaudhary, J. V. Sudhakar, A. Kumar, O. Bajpai, R. Tiwari and G.V. Murthy, Synopsis of the genus Ficus L.(Moraceae) in India, *Taiwania*, 2012, **57**(2), 193-216.

6. R. M. Kunwar and R.W. Bussmann, Ficus (Fig) species in Nepal: a review of diversity and indigenous uses, *Lyonia*, 2006, **11**(1), 85-97.

7. A. B. Chaudhuri, Forest Plants of Eastern India, Ashish Publishing House, 1993, 235.

8. J. Adhikari, S. Thapaliya, S. Singh, M. K. Sah and N. Paudyal, Anti-inflammatory properties of methanolic extract of “sikari laharo" (*Periploca calophylla*), *Journal of Agriculture and Forestry University*, 2020, 231-242.

9. U. Quattrocchi, CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology (5 Volume Set), CRC press, 2012, 2852.

10. R. S. Farag, M. S. Abdel-Latif, H. H. Abd El Baky and L. S. Tawfeek, Phytochemical screening and antioxidant activity of some medicinal plants’ crude juices, *Biotechnology Reports*, 2020, 28:e00536.

11. V. Sharma, A. Agarwal, U. Chaudhary and M. Singh, Phytochemical investigation of various extracts of leaves and stems of *Achyranthes aspera* Linn, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2013, **5**(1), 317-320.

12. S. Ali, M. R. Khan, M. Sajid and Z. Zahra, Phytochemical investigation and antimicrobial appraisal of *Parrotiopsis jacquemontiana* (Decne) Rehder, *BMC Complementary and Alternative Medicine*, 2018, **18**, 1-5.

13. M. Joseph, A. Charles and V. A. Ramani, Phytochemical and antimicrobial studies of *Phyllanthus wightianus* leaf extract, *International Journal of Phytomedicine*, 2012, **4**(4), 456.

14. R. Gul, S. U. Jan, S. Faridullah, S. Sherani and N. Jahan, Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan, *The Scientific World Journal*, 2017, 2017.

15. R. Maria, M. Shirley, C. Xavier, S. Jaime, V. David, S. Rosa and D. Jodie, Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador, *Journal of King Saud University-Science*, 2018, **30**(4), 500-505.

16. K. J. Lee, Y. C. Oh, W. K. Cho and J. Y. Ma, Antioxidant and anti-inflammatory activity determination of one hundred kinds of pure chemical compounds using offline and online screening HPLC assay, *Evidence-Based Complementary and Alternative Medicine*, 2015, 2015.

17. D. H. Truong, D. H. Nguyen, N. T. Ta, A. V. Bui, T. H. Do and H. C. Nguyen, Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*, *Journal of Food Quality*, 2019, 2019.

18. B. Vongsak, P. Sithisarn, S. Mangmool, S. Thongpraditchote, Y. Wongkrajang and W. Gritsanapan, Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method, *Industrial Crops and Products*, 2013, **44**, 566-571.

19. P. Siddhuraju and K. Becker, Antioxidant properties of various solvent extracts of total phenolic

constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves, *Journal of Agricultural and Food Chemistry*, 2003, **51**(8), 2144-2155.

20. O. R. Alara, N. H. Abdurahman and C. I. Ukaegbu, Extraction of phenolic compounds: A review, *Current Research in Food Science*, 2021, **4**, 200-214.

21. Q. D. Do, A. E. Angkawijaya, P. L. Tran-Nguyen, L. H. Huynh, F. E. Soetaredjo, S. Ismadji and Y. H. Ju, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*, *Journal of Food and Drug Analysis*, 2014, **22**(3), 296-302.

22. J. O. Arawande, A. Akinnusotu and J. O. Alademeyin, Extractive value and phytochemical screening of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) using different solvents, International Journal of Traditional and Natural Medicine, 2018, **8**(1), 13-22.

23. N. I. Pujirahayu, H. A. Ritonga and Z. A. Uslinawaty, Properties and flavonoids content in propolis of some extraction method of raw propolis, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2014, **6**(6), 338-340.

24. T. Dhanani, S. Shah, N. A. Gajbhiye and S. Kumar, Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*, *Arabian Journal of Chemistry*, 2017, **10**, S1193- S1199.

25. C. L. Chang and C. S. Lin, Phytochemical composition, antioxidant activity, and neuroprotective effect of *Terminalia chebula* Retzius extracts, *Evidence-Based Complementary and Alternative Medicine,* 2012, 2012.

26. S. Poudel, B. Sapkota, P. Timilsina, K. Parajuli and A. Kaundinnyayana, Phytochemical analysis, in vitro antioxidant activity and in vivo anti-inflammatory and analgesic activities of selected medicinal plants of Nepal, *Journal of Pharmacognosy and Phytochemistry,* 2019, **8**(6), 14-25.

27. D. Khatri and S. B. B. Chhetri, Reducing sugar, total phenolic content, and antioxidant potential of Nepalese plants, BioMed Research International, 2020, 2020.

28. M. Z. Salem, A. Z. Salem, L. M. Camacho and H. M. Ali, Antimicrobial activities and phytochemical composition of extracts of Ficus species: An over view, *African Journal of Microbiology Research*, 2013, **7**(33), 4207-4219.

29. M. Basak, S. Dutta and M. Chowdhury, Wild raspberry: antioxidant fruits from Eastern Himalaya, *Journal of Food Biochemistry*, 2018, **42**(5), e12560.

30. A. Ullah, S. Munir, S. L. Badshah, N. Khan, L. Ghani, B. G. Poulson, A. H. Emwas and M. Jaremko, Important flavonoids and their role as a therapeutic agent, *Molecules*, 2020, **25**(22), 5243.

31. M. Fraga-Corral, P. Otero, J. Echave, P. Garcia-Oliveira, M. Carpena, A. Jarboui, B. Nuñez-Estevez, J. Simal-Gandara and M. A. Prieto, By-products of agri-food industry as tannin-rich sources: A review of tannins' biological activities and their potential for valorization, *Foods*, 2021, **10**(1), 137.

32. J. C. Chen, M. H. Chiu, R. L. Nie, G. A. Cordell and S. X. Qiu, Cucurbitacins and cucurbitane glycosides: structures and biological activities, Natural Product Reports, 2005, **22**(3), 386-399.

33. Y. Zhang, P. Cai, G. Cheng and Y. Zhang, A brief review of phenolic compounds identified from plants: Their extraction, analysis, and biological activity, *Natural Product Communications*, 2022, **17**(1), 1934578X211069721.

34. P. G. Pietta, Flavonoids as antioxidants, *Journal of Natural Products*, 2000, **63**(7), 1035-1042.

35. S. D. Banjarnahor and N. Artanti, Antioxidant properties of flavonoids, *Medical Journal of Indonesia*, 2014, **23**(4), 239-244.

36. R. Amarowicz, Tannins: the new natural antioxidants?, *European Journal of Lipid Science and Technology,* 2007, **109**(6), 549-551.

37. P. Sur, T. Chaudhuri, J. R. Vedasiromoni, A. Gomes and D. K. Ganguly, Antiinflammatory and antioxidant property of saponins of tea [*Camellia sinensis* (L) O. Kuntze] root extract, Phytotherapy Research, 2001, **15**(2), 174-176.

38. J. Grabmann, Terpenoids as plant antioxidants, Vitamins & Hormones, 2005, **72**, 505-535.

39. R. J. Elias, S. S. Kellerby and E. A. Decker, Antioxidant activity of proteins and peptides., Critical Reviews in Food Science and Nutrition, 2008, **48**(5), 430-441.