International Journal of Life Science and Agriculture Research

ISSN (Print): 2833-2091, ISSN (Online): 2833-2105

Volume 02 Issue 08 August 2023

DOI: https://doi.org/10.55677/ijlsar/V02I08Y2023-10

Page No : 261-270

Preliminary Assessment of Phytochemicals and Free Radical Scavenging Activity of Different Plants Collected from the Western Region of Nepal

Ananda Lamichhane[1,2], Bijaya Tripathi[1], Mamata Dhungana[1], Namrata Bhattarai[1] , Susmita Khatri[1,3], Dr. Nirmala Jamarkattel Pandit[1*]

[1]School of Health and Allied Science, Pokhara University P.O. Box 427, Pokhara-30, Kaski, Nepal

[2]Department of Pharmacy, Kyungpook National University, Daegu, 41566, South Korea

[3] Department of Business Development, Kaplan Business School, 28 Bridge Street, Sydney, Australia

ABSTRACT: Medicinal plants produce vital secondary metabolites in curing various diseases and pathological conditions. There are multiple tribes and communities where people are using plants from the medicinal aspect to cure different diseases. The aim of the present study was qualitative and quantitative phytochemical screening, determination of total phenol and total flavonoid content, and free radical scavenging activity of different parts of ethnomedicinally used plants collected from the western region of Nepal. The phytochemical screening of most of the extract showed the presence of phenols, carbohydrates, flavonoids, and glycosides. The ethanolic flower extract of *Callicarpa macrophylla* showed a higher phenolic content with a value of 195 ± 7.33 µg GAE/mg. Ethyl acetate extract of *Elephantopus scaber* root revealed the highest amount of flavonoid content with the value of 1692.31 ± 34.79 µg QE/mg of extract, followed by ethanolic flower extract of *Callicarpa macrophylla*. Ethanolic flower extract of *Callicarpa macrophylla* showed potent DPPH free radical scavenging activity with a half inhibitory concentration of 7.28 µg/ml. In contrast, *Elephantopus scaber* ethyl acetate root extract revealed the maximum free extreme scavenging properties among all the ethyl acetate extracts. From the experiment, the free radical scavenging potential of ethanolic flower extract of the *Callicarpa macrophylla* was comparable with ascorbic acid. This comparable activity may be attributed to higher phenols and flavonoid content in ethanolic extract. The result signifies that the ethanolic extract of *Callicarpa macrophylla* acts as an antioxidant and may contain a potent bioactive compound which, with further investigation, may lead to a novel compound.

KEYWORDS: Antioxidant, ethnomedicine, free radical scavenging, phytochemicals, total phenol content, total flavonoid content

Published Online: 19 August 2023

Corresponding Author: Dr. Nirmala Jamarkattel Pandit

1. INTRODUCTION

Free radicals are molecules that generate more than one electron (super oxides, hydroxyls, peroxyls) during cell metabolism and are highly reactive in nature. Free radicals constantly exist with various biological activities like the production and degradation of matter, energy transfer, and signal transmission. They can be generated from biological activity, high-energy triggers (heat, light, radiation), molecular oxidation and drug overdose. Free radicals play an important role in some physiological processes, and excessive free radicals affect ecosystem balance and have many harmful consequences. For example, excessive reactive oxygen species (ROS) damages proteins, nucleic acids, and lipids, which may induce cell degeneration, inflammation and cancer (Deweirdt et al., 2017; Liou & Storz, 2010).

To defend against such free radicals produced during various cell metabolism, our body has natural enzymatic antioxidants like Superoxide dismutase, catalase, glutathione peroxidase, and non-enzymatic antioxidants such as Vitamin C & E, Thiol, Melatonin, and Tocopherols. A higher concentration of such reactive free species inside the cell leads to oxidative stress; as a result, it harms the general function of the cell. Among such free radical species, ROS is one of the profound species inside the human body. Antioxidants can scavenge such free radicals by donating their free electron and forming chelates with the free radicals. As ROS plays an active role in the pathogenesis of several diseases, researchers are more focused on natural plants to discover the potent antioxidants that can cure various pathological conditions (Lamichhane et al., 2023).

Plant species produce secondary metabolites such as flavonoids, alkaloids, lignans, terpenes, terpenoids, tocopherols, phenolic acids, and phenolics to their basic metabolic processes, which can possess various medicinal properties. Plant-derived antioxidants are effective free radical scavengers used as nutritional supplements to treat various diseases. There are multiple tribes and communities where people use such plants for medicinal purposes. Around 85% of Nepal's rural population is believed to use herbal remedies, owing to indigenous beliefs and an absence of alternatives in rural areas. Knowledge of various ethnic groups contributes to the development and investigations of natural products, which increases knowledge about the close relationship between a compound's chemical and its physiological properties (Sharma & Kumar, 2011: Lamichhane et al., 2023).

Aegle marmelos (L) Correa species of the *Rutaceae* family bears various cultural and religious values in the Hindu community. Traditionally, the fruit of *A. marmelos* is being consumed as an energy drink owing to the nutritional and therapeutic properties due to the presence of vitamins, natural sugars, antioxidants, minerals, flavonoids, organic acids, and fiber, making *A. marmelos* a valuable fruit with medicinal properties. The bark decoction was used to treat fever and cough. The fruit pulp of *A. marmelos* contains tannin, amino acids, lignin, proteins, gallic acid, quercetin, and chlorogenic acid (Patra et al., 2017; Prakash et al., 2011).

Similarly, leaves of *Agave americana* L. species of the *Asparagaceae* family are known for their antidiuretics and anti-inflammatory effect and are recommended for pathogenic bacterial growth. According to Maazoun et al. (2019), leaves extract of *A. americana* revealed the presence of flavonoid glycosides like kaempferol and quercetin derivatives. Also, the leaves of *A. americana are* a rich source of steroidal saponins due to edema and fruit retention, while a paste of leaves and bark was used against wound healing and some sorts of stomach problems (Chaachouay et al., 2022; Singh et al., 2019).

The decoction of the bark and root of *Callicarpa macrophylla* Vahl species (*Lamiaceae* family) have been consumed to cure fever and typhoid. According to Gandhi et al. (2022), *C. macrophylla* is being used in Ayurvedic products like Priyangu and Phalini. Barkatullah et al. (2015) reported a significant phytotoxic activity from the crude extract of leaves. Meanwhile, ethanolic stem bark extracts of *C. macrophylla* showed moderate growth inhibitory activity against some gram-positive and gram-negative strains (Yadav et al., 2012).

Elephantopus scaber L. is widely used in traditional medicine in many countries like India, Nepal, China, Thailand, and Brazil to cure various diseases conditions. The fresh root of the plants was used to treat menorrhagia, menstrual problems, spermatorrhea, ametrorrhagia, leucorrhoea, and dysmenorrhea . Its fresh leaf juice is used to heal wounds, and root juice has been used to treat liver and heart problems (Behera & Misra, 2005).

Ethnomedicinally roots, fruits, and stems of *Premna barbata* were used as insect repellants against fever, eczema, and wound healing**.** According to Adjalian et al. (2015), leaves, fruits, wood, and stem bark of *P. barbata* are used as various sources of medicine in India and Pakistan. The wood paste is applied on wounds, while plant decoction is also used in the treatment of arthritic pain, urinary infection, and heat sickness. The seeds of *P. barbata* are fed to the horses to cure anorexia, and the paste of seeds in sesame oil is messaged on the body to relieve the pain (Bhatia et al., 2011).

This study aimed to determine the preliminary experimental scientific evidence of these selected medicinal plants by using their local use as a guideline for plant selection which can contribute to the further study of such plants. The principal objective of this study is to screen these medicinal plants for phytochemicals and determine the total phenolic, flavonoid, and free radical scavenging activity. The general overview of workflow in this experiment is shown in Figure 1.

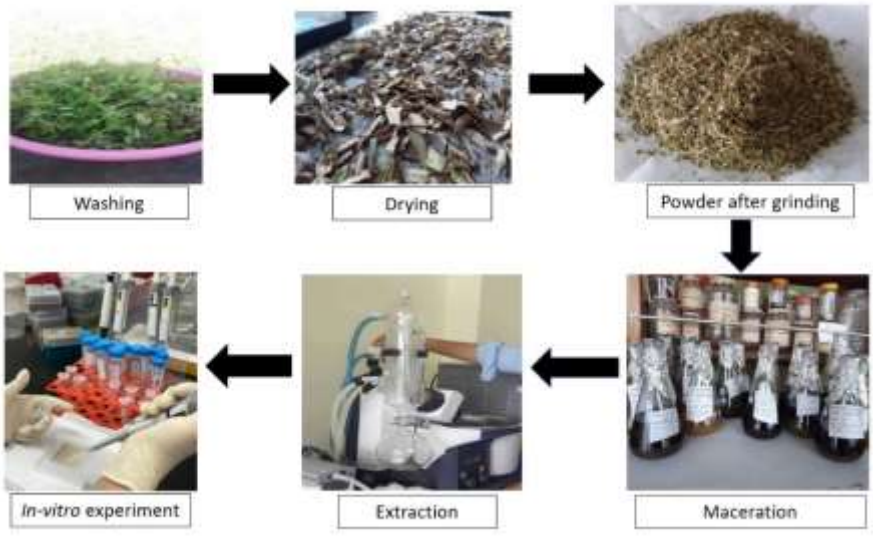

Figure 1: General overview of workflow in this study

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Tokyo Chemical Industry Co., Ltd. in Japan supplied the original 1,1-diphenyl-2 picryl hydrazyl (DPPH). Meanwhile, benedict reagent, sodium hydroxide, sodium nitrite, sodium carbonate, ethanol, lead acetate, ammonium hydroxide, sodium hydroxide tablets, mercury chloride, ferric chloride, hydrochloric acid, and benedict reagent were purchased from Thermo Fisher Scientific, Pvt. Ltd. India. L-ascorbic acid was purchased from Himedia Laboratories. Qualigens Fine Chemicals supplied sulfuric acid, whereas ethyl acetate, pure copper sulfate pentahydrate, sodium, and 1-naptol were purchased from Merck Specialties Pvt. Ltd., Germany.

2.2 Collection and Identification

The experimental plants (Shown in Table 1) were collected from the Annapurna Rural Municipality, Kaski, Western Nepal, in July 2019, located at 4528 feet above sea level. Plants were gently washed, shed dried, herbariums were prepared and identified at the National Herbarium Kathmandu, while the crude samples were stored at the Pharmacognosy Laboratory of Pokhara University, School of Health and Allied Sciences. The moisture in the sample was removed using a hot air oven (40°C), and the amount of moisture was continuously checked using a weight variation test at various time intervals. After complete drying, they were ground into a fine powder.

Table 1: List of plants with their crude drugs voucher number

Scientific Name	Family	Local Name	Plant Parts	Crude Drugs Voucher Number	Sample Number
Aegle marmelos (L) Correa	Rutaceae	Bel	Bark	PUCD-2020-01	CR1
Aegle marmelos (L) Correa	Rutaceae	Bel	Leaves	PUCD-2020-02	CR2
Agava Americana L.	Asparagaceae	Ketuki	Leaves	PUCD-2020-03	CR3
Callicarpa macrophylla Vahl	Lamiaceae	Dahichamle	Flower	PUCD-2020-04	CR4
Callicarpa macrophylla Vahl	Lamiaceae	Dahichamle	Leaves	PUCD-2020-05	CR5
Elephantopus scaber L.	Asteraceae	Sahasrajari	Roots	PUCD-2020-09	CR6
Premna barbata	Lamiaceae	Kalogineri	Leaves	PUCD-2020-12	CR7

2.3 Preparation of Plant Extracts

Successive maceration as an extraction method was used to extract the crude drug as described by Pandey and Tripathi, (2014). For extraction, 50–100 gram of the crude extracts was macerated for 48 hours with 1:5 w/v ethyl acetate. The filtrate was collected and concentrated using a vacuum evaporator. The residue was again macerated for upto 48 hours with sufficient ethanol (1:5 w/v ratio), after which the filtrate and again collected and concentrated using a vacuum evaporator.

2.4 Phytochemical Screening

Phytochemical screening was conducted following the prior reports by Yadav and Agarwala, (2011).

2.5 Total Phenolic Content

Total phenolic content was determined using Folin Ciocalteu Method as described by Sulaiman and Balachandran with minor modification (Sulaiman & Balachandran, 2012). Gallic acid was used as a standard positive control, various concentrations of gallic acid were prepared in ethanol, and a standard calibration curve was plotted. 1 ml of crude extract (1 mg/mL), 5 ml of distilled water and 1 ml of the Folin-Ciocalteu reagent were mixed. After 5 minutes, 1 ml of distilled water and 1 ml of 10% sodium carbonate were added to a mixture and gently shaken. After 60 minutes of incubation, absorbance was measured at 725 nm.

2.6 Determination of Total Flavonoids Content

The aluminum chloride colorimetry method was used to determine the total amount of flavonoid in plant extract with the previously reported methodology by Sulaiman and Balachandran (2012). 1 ml (1 mg/ml) of each extract solution was added with 4 ml of water and 0.3 ml of 5% Sodium nitrite. After incubation of 5 minutes, 1.3 ml of 20% Aluminum chloride was added and incubated for another 6 minutes. After the addition of 2 ml of 1M Sodium hydroxide, the absorbance at 510 nm was immediately measured. The calibration curve was prepared using quercetin as the standard concentration of 15.63 mg/ml, 31.25 mg/ml, 62.5 mg/ml, 125 mg/ml, 250 mg/ml, and 500 mg/ml. Total flavonoid content was determined with the help of a calibration curve (Figure 2), and results were expressed as mg quercetin equivalent per gram dry extract weight as shown in Table 5.

2.7 DPPH Free Radical Scavenging Method

The reduction of the reaction color assessed the free radical scavenging activity of the different plant samples among DPPH solution and sample extract as described by Jabbari and Jabbari, (2016). Methanol was used to dilute the stock solution to a dilution series (50g - 1000g/ml), where an aliquot of each dilution (2mL) was mixed with a DPPH methanolic solution and incubated at room temperature for 30 minutes. The experiment was then repeated with a control containing a methanolic solution of DPPH (2mL, 0.06mM) and ethanol (2mL). At the wavelength of 512 nm, the absorbance was measured against methanol as a blank, whereas ascorbic acid was used as a standard. DPPH radical scavenging ability was expressed as half maximal inhibitory concentration (IC_{50}), and the inhibition percentage was calculated using Equation I.

DPPH radical scavenging activity (%) = $\frac{Absorbance\ of\ Control - Absorbance\ of\ sample}{Absorbance\ of\ control} * 100$ …… Equation I

3. RESULTS AND DISCUSSION:

3.1 Yield value determination

The extraction yield value of selected medicinal plants in various solvents was calculated and listed in Table 2.

Table 2: Yield value of plant extracts in different solvents

Sample Number	CR1	CR2	CR3	CR4	CR5	CR6	CR7
%Yield (Ethyl acetate extract)	6.14	1.66	1.23	1.78	2.19	3.63	1.38
% Yield (Ethanol extract)	3.08	3.81	3.40	6.13	9.95	4.98	4.28

3.2 Phytochemical Screening

The presence or absence of phytochemicals was evaluated by observing the intensity of color change in the sample. Phytochemical screening of extracts of plants in different solvents showed the presence of alkaloids, flavonoids, glycosides, and carbohydrates as shown in Table 3.

Table 3: Phytochemical analysis of ethyl acetate and ethanolic extract

Phytochemical Constituent	Tests	CR1		CR2		CR3		CR4		CR5		CR6		CR7	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2
Alkaloid	Mayer's test	+	-	+	+	-	+	-	+	+	+	-	-	-	-
Carbohydrates	Molish test	+	+	+	-	+	+	+	-	-	-	-	+	-	-
Phenol	Ferric chloride test	-	-	-	-	+	-	+	+	+	+	-	+	+	+
	Lead acetate test	-	-	+	+	+	-	-	+	+	+	+	+	+	+
Flavonoid	Alkaline test	-	+	-	-	+	-	+	+	+	+	+	+	+	+
Glycoside	Legal's test	-	+	-	-	+	-	-	+	+	+	-	-	+	+

[1]Where; (+): Detected, (-): Not Detected, 1; Ethyl acetate extract, 2; Ethanolic extract

3.3 Total Phenolic Content

The total phenol content for all plant extracts was quantified by the Folin Ciocalteu method, using gallic acid as the standard. Results were expressed in µg gallic acid equivalent per mg of extracts as in Table 4. Among all the ethanolic extracts, the flower extract of *C. macrophylla* showed maximum flavonoid content with a value of 195.25 ± 7.33 µg GAE/mg of extract. Similarly, the ethyl acetate extract of *P. barbata* revealed 40.37 ± 7.53 µg GAE/mg of extract of flavonoid content among other ethyl acetate extracts.

Table 4: Total phenolic content expressed as µg GAE/mg of extract

Sample Number	Ethyl Acetate (µg GAE/mg of extract)	Ethanol (µg GAE/mg of extract)
CR1	23.36 ± 2.99	129.61 ± 14.57
CR2	18.38 ± 1.73	71.63 ± 2.77
CR3	18.32 ± 2.67	27.27 ± 0.12
CR4	24.30 ± 2.81	195.25 ± 7.33
CR5	30.53 ± 1.96	149.55 ± 20.27
CR6	23.49 ± 0.74	60.55 ± 2.52
CR7	40.37 ± 7.53	58.97 ± 2.04

[1]Data are expressed as Mean ± SD (n=3)

3.4 Total Flavonoid Content

Total flavonoid content was quantified and presented in Table 5. Results were expressed as µg quercetin equivalent per mg of extracts. Among the ethyl acetate extracts, *E. scaber* showed the maximum flavonoid content, while the flower extract of *C. macrophylla* revealed the maximum among other ethanolic extracts. The calibration curve of different concentrations of gallic acid is shown in Figure 2.

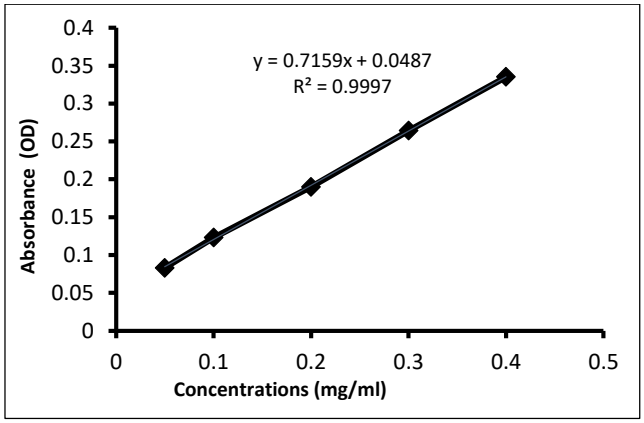

Figure 2: Calibration curve of a standard quercetin

Table 5: Total flavonoid content expressed as µg QE/mg of extract

Sample Number	Plants (Parts)	Ethyl Acetate (µg QE/mg of extract)	Ethanol (µg QE/mg of extract)
CR1	*Aegle marmelos* (Leaves)	128.67±74.15	411.18±43.60
CR2	*Aegle marmelos* (Bark)	86.71±21.16	276.45±20.99
CR3	*Agave americana* (Leaves)	32.63±9.09	274.12±26.64
CR4	*Callicarpa macrophylla* (Flower)	819.58±12.58##	1602.33±17.76##
CR5	*Callicarpa macrophylla* (Leaves)	386.01±28.9	993.93±37.14##
CR6	*Elephantopus scaber* (Root)	1692.31±34.79##	400.46±13.72
CR7	*Premna barbata* (Leaves)	146.38±25.87	271.79±0.80

[1] ## represents that the initial concentration of an extract falls out of the range, so they were reduced to half and the final value was calculated as equivalent to the original concentration. Data are expressed as Mean ± SD (n=3)

3.5 Antioxidant Activity Analysis

From the DPPH free radical scavenging experiment, the most potent antioxidant activity was revealed by the ethanolic extract of *C. macrophylla* (Flowers) i.e., IC_{50} 7.28 µg/ml which is comparable to that of standard ascorbic acid IC_{50} of 3.562 µg/ml. The result of antioxidant activity is expressed in Table 6 and graphically presented in Figure 3 and Figure 4.

Table 6: Percentage of free radical scavenging activity

Plants	Solvents	% DPPH scavenging activity				IC 50 (µg/ml)
		0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	
CR1	Ethyl Acetate	2.89±1.22	11.31±1.66	18.52±0.98	46.63±2.41	>100
	Ethanol	4.10±5.24	12.15±20.13	17.24±8.78	72.71±5.81	61.14
CR2	Ethyl Acetate	0.16± 0.02	4.26±4.20	7.29±4.01	25.90±3.05	>100
	Ethanol	1.03±0.55	4.63±1.16	13.50±2.74	90.23±0.54	54.26
CR3	Ethyl Acetate	3.29± 1.57	12.21±2.06	13.68±1.42	33.60±2.11	>100
	Ethanol	0.56±0.02	4.88±2.41	9.41±1.24	23.41±1.45	>100
CR4	Ethyl Acetate	6.57±1.03	13.52±2.27	18.52±2.94	49.51±4.87	>100
	Ethanol	1.03±0.57	5.93±0.94	68.03±4.93	91.2±0.18	7.28
CR5	Ethyl Acetate	10.08±1.42	15.57±4.82	17.21±2.34	57.86±1.82	82.64
	Ethanol	1.57±0.77	11.82±1.93	51.41±5.20	91.00±0.65	34.88
CR6	Ethyl Acetate	4.35±1.34	12.47±3.80	23.03±4.89	27.45±2.44	>100
	Ethanol	0.87±0.54	1.97±5.74	17.40±1.84	91.77±0.51	52.26
CR7	Ethyl Acetate	11.31±0.68	13.77±1.53	19.26±1.08	56.14±2.5	85.20
	Ethanol	4.96±1.65	6.62±2.37	18.69±4.67	73.18±7.33	63.62
Ascorbic Acid		13.26±0.5	44.28±0.55	95.37±0.21	96.47±0.21	3.562

[1]Data are expressed as mean ± standard deviation (n=3)

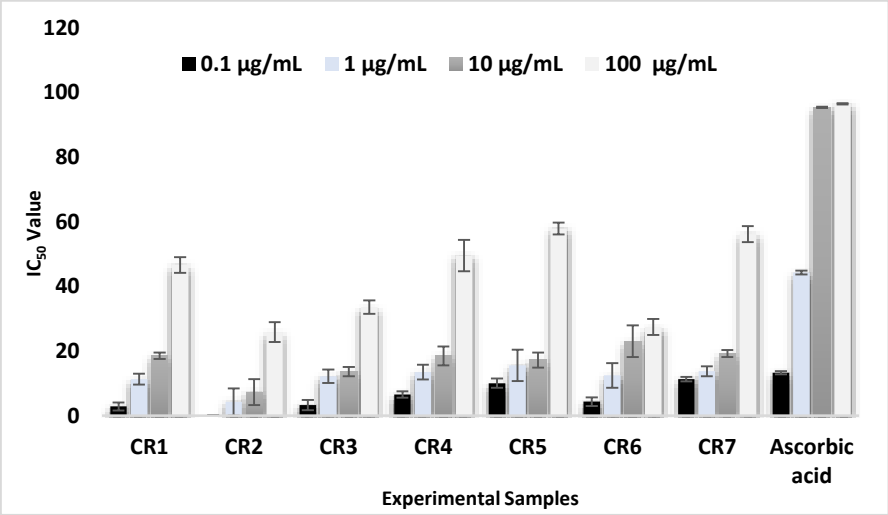

Figure 3: Graphical presentation of % DPPH scavenging activity in ethyl acetate extract

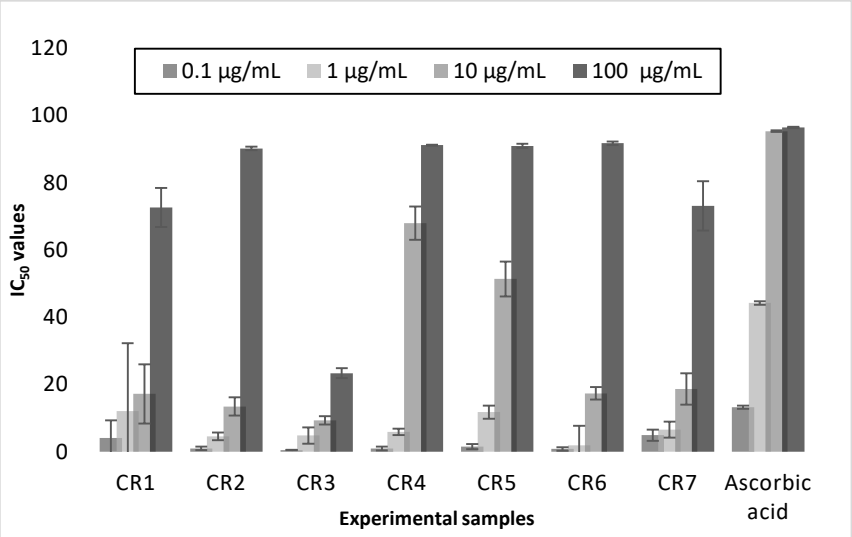

Figure 4: Graphical presentation of % DPPH scavenging activity in ethanolic extract

4. DISCUSSION

Plants contain a wide variety of organic compounds as secondary metabolites that exhibit a range of therapeutic properties. In this study, different plant species were collected based on their ethnomedicinal uses, and their phytochemical screening, free radical scavenging, total phenolic content, and total flavonoid content were evaluated. From the phytochemical result, it was found that ethyl acetate contains the least phytochemical constituents compared to the ethanolic extract. Most plants revealed the presence of phenol, flavonoid and glycosides, which are further correlated with the potent free radical scavenging activity.

There are different types of flavonoid derivatives namely glycone, aglycone, and methylated flavonoids. Glycone types of compounds show more affinity towards the polar solvents than aglycone with the least polar solvents (Tapas et al., 2008). In this study, a specific extract shows the higher TFC with lower free radical scavenging potency because there are various antioxidant activity mechanisms, among which only DPPH free radical scavenging activity is evaluated. Thus, the extracts showing a higher TFC value can show potent antioxidant activity through the other mechanism (Muniyandi et al., 2019).

Phytochemical screening illustrates that almost all plant extracts contain significant phytoconstituents, including phenols, flavonoids, and glycosides as secondary metabolites. Phenolic compounds are among the most abundant groups of plant metabolites. They possess various health benefits antioxidants through a mechanism like reactive oxygen species scavenging and inhibition, electrophile scavenging, and metal chelation. Phenolic and glycosidic flavonoids have been linked to antioxidative action on biological entities since they are scavengers of singlet oxygen and free radicals (Shahidi and Ambigaipalan, 2015).

In this experiment, ethanolic extract of *A. marmelos* leaves showed the presence of alkaloids and phenols, whereas the bark extract showed the presence of carbohydrates, flavonoids, and glycosides which is supported by Rajan et al. (2011) . According to Rajan et al. (2011), the flavonoid content in the fruit pulp aqueous and alcoholic extract of *A. marmelos* was 129.00 and 166.33 mg/g which is much higher than that of our ethanolic extract of both bark and leaves indicating that the fruit pulp contain the highest amount of the flavonoids in *A. marmelos.* Meanwhile, they reported a higher IC_{50} value of aqueous and alcoholic leaf extract (92.648 and 106.158 µg/ml) than our ethanolic leaf and bark extract, which may be due to the collection site, season, and other environmental conditions. According to Karumaran et al. (2016), the antioxidants present in *A. marmelos* are better extracted in methanol or polar solvent, which can be related to our study since both the ethyl acetate extract of leaf and bark has a minimum scavenging activity. Patra et al. (2017), revealed the presence of *β*-carotene, lycopene and ascorbic acid in the fermented fruit juice of *A. marmelos* with 81% DPPH inhibition percentage which is comparable to our ethanolic extract. In contrast, Prakash et al. (2011) reported antioxidant activity and TPC value of fruit pulp to be 58.1% and 26.3 respectively, which is lower than our result. So, it can be said that our extract may also contain Carotene and Lycopene , resulting in the comparable percentage scavenging of free radicals.

Phytochemical screening of *A. americana* leaves extracts revealed the presence of phytochemicals like phenols, flavonoids and saponins correlating the results of Maazoun et al. (2019). TPC, TFC and free radical scavenging activity of *A. americana* are moderate in this study. Pandey et al. (2019) reported the IC_{50} value of methanolic leaf extract was 7.68 µg/ml which is comparable to standard ascorbic acid but also mentioned that it needs a broader study to prove its antioxidant properties. The variation with our result may be due to the collection time, environmental condition and polarity of extracted solvent, analysis methods etc (Lamichhane et al. 2023). Thus, we can say that potent bioactive components like polyphenols and flavonoids may be extracted more in a polar solvent.

Phytochemical screening of *C. macrophylla* flower showed the presence of carbohydrates, phenols, glycosides and flavonoids, whereas leaves extracts contain alkaloids, phenols, flavonoids and glycosides, which is also supported by Mona (2016). Both the ethanolic extract of flowers and leaves revealed a higher phenolic content, which can be visualized in Table 4. In our study, ethanolic extracts of *C. macrophylla* (flower and leaves) show higher TFC than their ethyl acetate extract. Similarly, the ethanolic extract of *C. macrophylla* flower showed potent DPPH free radical scavenging with an IC_{50} value of 7.28 which is supported by Shi et al. (2013). Meanwhile, ethanolic leaves extract of our sample also showed potent free radical scavenging activity but slightly lower than flower extract, which may be due to the presence of the higher amount of phenols as well as flavonoids in an ethanolic flower extract (Shahidi and Ambigaipalan, 2015). According to Shi et al. (2013), *C. macrophylla* contains compounds like citric acid and cinnamic acid which are potent antioxidants, which can be relatable that our extracts may also contain such potent antioxidant compounds

Mohan et al. (2010) reported the presence of flavonoids, phenols, steroids, tannins, and terpenes in the methanolic extract of *E. scaber* which is incoherence with our study. A quantitative study of phenols revealed that the ethanolic root extract contains a higher amount of phenols compared to ethyl acetate extract while the amount of flavonoid content was higher which can be visualized in Table 4. DPPH free radicals scavenging activity is higher in the ethanolic extract of *E. scaber* than the ethyl acetate extract due to the solvent polarity and the presence of glycosidic types of flavonoids on the polar extract. The higher value of the TFC in ethyl acetate extract of *E. scaber* may be due to the presence of the non-glycosidic types of flavonoids without hydroxyl group which possessed higher affinity towards non-polar solvents.

Qualitative determination of the phytochemical screening of *P. barbata* revealed the presence of phenols, flavonoids, and glycosides in both the ethyl acetate and ethanolic extract. The least study has been reported yet on this species, but some studies from another genus *Premna serratifolia*, contains the presence of flavonoid, alkaloid, tannins and saponins (Rajagopal et al., 2014). The inhibitory concentration (IC_{50}) values of both extract were little, expressing the probability of antioxidant activity compared with the standard ascorbic acid. According to Tapas et al. (2008), such a little free radical scavenging activity of the *Premna* species is due to the presence of the glycosidic flavonoids on their extracts. So, further study is necessary to extract detailed scientific evidence of *P. barbata* species behind its potent ethnomedicinal uses.

Research for antioxidant properties has been fundamental in recent times. Antioxidant properties explain the loophole for the discovery of potent anti-aging, anti-inflammation and anti-cancer drugs (Lamichhane et al, 2023). Since the *C. macrophylla* showed potent antioxidant activity via free radical scavenging, we can predict the evidence of this plant being used ethnomedicinally for decades. The result from the study shows that the phenols and flavonoid content in the samples are strongly correlated to show antioxidant potency via DPPH free radical scavenging activity. Also, from the literature, it was found that for any extract to reflect the potent antioxidant properties, it should have polyphenolic as well as glycone types of flavonoids (Hydroxyl group bearing flavonoid) on its extract. This study can be a milestone for the discovery of a novel compound.

5. CONCLUSIONS

The above study suggests that various secondary metabolites such as phenols, flavonoids, glycosides, and carbohydrates were present in the various parts of the plants. This study showed that *E. scaber* (root) ethyl acetate extract showed maximum flavonoid content afterward *C. macrophylla* ethanolic flower extract. The ethanolic flower extract of *C. macrophylla* had a maximum free radical scavenging activity with IC_{50} value of 7.28 µg/ml, comparable to the standard ascorbic acid (IC_{50} 3.56 µg/ml). As the above experimental plants possess such secondary metabolite and activity, they may be utilized in chemical and biological research, which might be very helpful in preventing or slowing the advancement of diverse types of oxidative stress-induced diseases. Our study also demonstrates that different plant parts contain a diverse range of phytochemicals that might be advantageous to human health. Further study may lead to the discovery of new bioactive components.

Funding

The authors did not receive any funding for the submitted work.

Acknowledgments

We are grateful to The School of Health and Allied Sciences, Faculty of Health Science, Pokhara University, Pokhara-30, Nepal, for providing facilities for this study.

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