Nepal J Biotechnol. 2023 Dec; 11 (2): 86-92 Research article DOI: https://doi.org/10.54796/njb.v11i2.271

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: www.nepjol.info/index.php/njb ISSN (Print): 2091-1130

Exploring the Potency of Medicinal Plants in Central Nepal's Highlands: A Comprehensive Analysis of Antioxidant, Antibacterial Properties, and Toxicity

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Received: 22 May 2023; Revised: 30 Nov 2023; Accepted: 08 Dec 2023; Published: 31 Dec 2023

Abstract

The current research study aimed to assess the antibacterial, antioxidant, and toxicity effectiveness of eight locally accessible medicinal plants found in the Kavre District of Nepal: *Schima wallichii, Woodfordia fruticosa, Cuscuta reflexa, Bombax ceiba, Drymaria diandra, Psidium guajava, Myrica esculenta,* and *Urtica dioica*. Plant extracts were prepared in absolute methanol. The qualitative phytochemical analysis of basic classes of secondary plant metabolites; saponin, sterol, , tannin, quinone, alkaloids, terpenoids, reducing sugar, protein, cardiac glycoside and carbohydrate was carried out. Further, antimicrobial activities, free radical scavenging potential, and toxicity bioassays of the crude extracts were performed. The basic secondary metabolites were detected in all plant extracts. Among the studied plants, *W. fructicosa*, *S. wallichi*, *M. esculenta*, and *P. guajava* showed noteworthy antimicrobial activities against inspected pathogens. A significant 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was found in *P. guajava*, *M. esculenta*, *B. ceiba*, *W. fructicosa*, and *U. dioca*. Likewise, the highest toxicity potential was observed in *C. reflexa* followed by *U. dioca*, *B. ceiba*, and *S. wallichi* against *Artemia salina*. These signifies the potential of plant extracts in herbal medicine. Further work on screening of other biochemical properties will enhance the potential of these plant extracts in modern phytotherapy.

Keywords: Medicinal plants, Radical Scavenging, Antibacterial, Phytochemicals, Toxicity

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Introduction

Medicinal plants are a safe therapeutic paradigm that have been employed in many cultures around the world. They are the biological supply of pharmaceuticals for traditional medical systems [1]. Additionally, they are a vast supply of contemporary medications, dietary supplements, herbal remedies, pharmaceutical intermediates, nutraceuticals, and chemical entities for manufactured pharmaceuticals. The way that medicinal plants work is derived from the extensive knowledge that has been gathered over ages by many healers; it can also be passed down from ancestors, transferred from healer to healer, or evolved through time by individual experiences [2] . Now, the therapeutic effects of medicinal plants have been found effective for the up keeping human health and remedies for various ailments [3]. Even though synthetic medications became more and more common with industrialization, traditional cultures' usage of plant-derived medications is becoming more and more admired as a substitute for synthetic medications. [4]. The plants' potential as sources of various bioactive chemicals has long been known, making them appropriate for usage in pharmaceutical, cosmetic, and nutraceutical products in the future. [5].

Plants have the potential to heal a variety of ailments [6], which is mainly driven by some important secondary metabolites such as flavonoids, phenols, etc. [7]. Different bioactive compounds derived from medicinal plants can be used as alternative therapies, either directly or as models for future synthetic molecules [8]. Plant-based natural substances serve as attractive reservoirs for antimicrobial agents due to their inherent natural qualities and cost-effectiveness. Particularly beneficial for rural communities in underprivileged countries, these compounds have historical roots in diverse civilizations, often integrated into medicinal practices through various formulations. [9]. Furthermore, excessive free radical generation and/or poor antioxidant defense cause oxidative stress, which can lead to a variety of pathophysiological disorders in the human body [10]. Various antioxidants found in plants have the ability to neutralize free radicals, providing protection to the organism against oxidative damage by counteracting the production of reactive oxygen species. Moreover, studies on the cytotoxicity of medicinal plants are being

identified, that can act against carcinogens and tumors and can be developed into novel anti-cancer drug candidates [11].

We chose a few readily available plants from the Kavre area of Nepal for this study in order to examine their antioxidant, antibacterial and toxicological efficacy—aspects of medicinal plant usage that have not gotten much attention up to this point. The study employed conventional methods to conduct phytochemical analysis, the dics diffusion technique for antibacterial activity, the DPPH method for antioxidant activity, and the brine shrimp toxicology test for cytotoxicity. Among the analyzed plants, *Schima wallichi* is being used as an antipyretic and anthelmintic as well as a wound-healing agent in traditional medicine practice [12]. Similarly, *Woodfordia fructicosa* is being used in treating problems regarding stomach and blood [13]. Further, *Cuscuta reflexa* is used for the treatment of knee pain, arthritis, and kidney problem [14]. *Bombax ceiba* is used as an anti-inflammatory and hepatoprotective agent and against dysentery [15]. *Drymaria diandra* is traditionally used for gastritis problems [16], whereas plant juice is used to cure indigestion, conjunctivitis and fever[17]. In addition, *Psidium guajava* is used in curing diseases like diabetes, dysentery, rotaviral enteritis, etc. [18]. *Myrica esculenta* is being used for anti-asthmatic activity [19]. *Urtica dioca* is used to cure various diseases, namely nephritis, hematuria, jaundice, arthritis, and rheumatism [20].

Materials and Methods

Plant collections and extract preparation

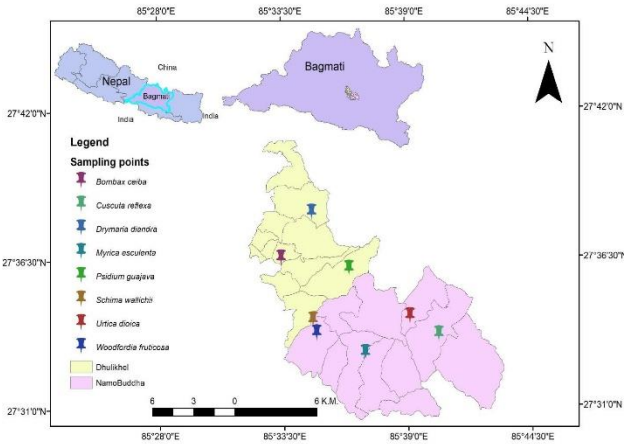

Figure 1. Geographic information system (GIS) map of the collection site of plants collected in Nepal.

Plant specimens were gathered from two locations, Dhulikhel Municipality and Namobudhdha Rural Municipality, situated in the Kavre District of Nepal (Table 1). The collection site of each plant was established using a geographic information system (GIS) (**Figure 1**). The collected plant parts were recognized by a taxonomist, from the Department of Pharmacy and Department of Biotechnology, Kathmandu University, Nepal. The collected plant material was crushed into a fine powder after air drying. Each powder sample, weighing five grams, underwent maceration with 50 mL of absolute methanol over a 24-hour period. The following day, the entire sample was sieved through Whatman filter paper and then evaporated to dry utilizing a vacuum evaporator. The methanol extract of each plant was utilized for pharmacognostic study such as antioxidant effect, antibacterial activity, and toxicity evaluation.

Phytochemical qualitative analysis

The phytochemical qualitative analysis of fundamental secondary plant metabolites, including saponin, alkaloids, sterol, quinone, glycoside, terpenoids, tannin, reducing sugar, and polyphenols was conducted according to established standard protocols [21-23].

Test for Basic Alkaloids (Mayer's Test)

A 5ml portion of the extract was reduced to obtain a residue. This residue was then mixed in 3ml of 2% (v/v) hydrochloric acid . Subsequently, 3 drops of Mayer's reagent were introduced. The formation of a dull white precipitate served as an indication of the presence of basic alkaloids.

Test for coumarin

A 1ml portion of the extract was thoroughly dried, and the resulting residue was dissolved in hot water. Ammonium hydroxide (NH4OH) was then added once the solution reaches a basic pH. The detection of green fluorescence signifies the presence of coumarins.

Test for Saponins

A 2ml portion of the extract was vigorously shaken in a test tube for 30 seconds. The appearance of a thick foam that lasted for 30 minutes acted as a signal of the existence of saponins.

Test for Glycosides

A 2ml extract was evaporated to 1ml, and then 1-2ml of ammonium hydroxide (NH4OH) was added and agitated. The presence of glycosides was revealed by the appearance of a cherry red tint.

Test for Reducing Sugar (Fehling's Test)

The process involved completely drying a 1ml extract, followed by dissolving the resulting residue in hot water. Subsequently, 1ml of Fehling's reagent was introduced to this solution. The mixture underwent warming over a water bath for half an hour, and the appearance of a

Table 1. Details on plant collection and extract preparation

Site	Scientific name	Nepali vernacular name	Geographic coordinates	Elevation (masl)	Plant part	Methanolic extract (%)
1	*Schima wallichii* Choisy	Chilaune	85°34'48.20"E 27°34'16.24"N	1680	Bark	1.90
2	*Woodfordia fruticosa* Kurz	Dhangero	85°34'57.73"E 27°33'47.03"N	1573	Bark	9.00
3	*Cuscuta reflexa* Roxb.	Aakashbeli	85°40'22.75"E 27°33'41.28"N	1168	Whole Plant	7.66
4	*Bombax ceiba* L.	Simal	85°33'24.81"E 27°36'33.82"N	1587	Flower	3.12
5	*Drymaria diandra* Blume	Abhijalo	85°34'47.68"E 27°38'14.52"N	1058	Whole Plant	1.56
6	*Psidium guajava* L.	Amba	85°36'25.48"E 27°36'9.05"N	1182	Leaves	0.78
7	*Myrica esculenta* Buch.-Ham. ex D. Don	Kaphal	85°37'6.61"E 27°33'1.93"N	1514	Bark	1.66
8	*Urtica dioica* L.	Sisnoo	85°39'6.52"E 27°34'21.97"N	1250	Leaves	1.00

masl = meter above sea level

brick-red precipitate indicated the existence of reducing sugar.

Test for Sterols and Triterpenes

A 5ml extract was dissolved in 3ml concentrated sulfuric acid (H2SO4) and 2ml chloroform. The presence of terpenoids was suggested by the emergence of a reddish-brown hue at the interface, whereas steroids were indicated by green fluorescence. Three layers were observed: an upper dark brown layer, a middle light-yellow layer, and a bottom reddish layer.

Test for Flavonoids

1.5% methanol solution (1.5 ml) is added to 4 ml of extract and heated. Magnesium metal was introduced to the solution, followed by the addition of 5-6ml concentrated hydrochloric acid (HCl). The appearance of a red color indicated the presence of flavonoids, an orange color indicated the presence of flavones, and a violet color indicated the presence of flavanones.

Test for tannins and polyphenols

A mixture of 1ml of extract and 1ml of water was prepared. To this mixture, 3 drops of 1% (w/v) ferric chloride (FeCl3) were added. The appearance of a violet color or blue-black indicated the existence of polyphenols and tannins.

Antibacterial assay

The disc diffusion method was used to evaluate the antibacterial activity of plant extracts with slight modifications [24]. Muller Hinton Agar (MHA) plates with a thickness of about 4 mm were made and dried at a suitable temperature to eliminate excess moisture from the media's surface. Fresh inoculums of *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Staphylococcus aureus* were generated and swabbed thoroughly all over the plate to match the turbidity standard (OD = 0.6 in 600 nm). The inoculation plates were allowed to air dry at room temperature for a few minutes with the lids closed. Whatman filter paper was used to make the discs, which were then sterilized. After sterilization, discs were immersed into the desired plant extracts concentration (25, 50, 100, and 200 mg/mL), which had been obtained in a sterile environment. A control disc was dipped in pure methanol, while other discs A10: Ampicillin-10 mcg/disc, ,T30: Tetracycline-30 mcg/disc and CIP30: Ciprofloxacin-30mcg/disc was used. The plates were then incubated for the whole night at 37°C. After sufficient incubation (12–16 hours), the plates were checked for the zone of inhibition (ZOI) surrounding the discs. Antibacterial activity was assessed and compared with Ciprofloxacin, Tetracycline and Ampicillin as a control [24].

Antioxidant assay

The free radical scavenging activity was determined by using a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay as by previous investigators [25]. One microliter of each plant extract solution (5, 10, 20, 40, 80 µg/mL) was combined with 3 mL of 0.1 mM DPPH and dark incubated for 30 minutes. A UV-Visible Spectrophotometer (Shimadzu UV-1800) was used to measure discolorations of reaction mixture at 517 nm. Ascorbic acid was chosen as the reference and methanolic DPPH as the control. Using the following formula, the percentage of radical scavenging activity (RSA) was calculated.

% RSA= (Absorbance of Control-Absorbance of Sample)*100/Absorbance of Control

Toxicity assay

Toxicity bioassay was conducted on newly hatched brine shrimp (Artemia salina Leach) using the crude extracts [26]. After gathering the extract of the various quantities, 10 milliliters of DMSO were used to dissolve it and create the stock solution. Following that, 50 mg of A. salina eggs were dispersed into a beaker with 300 mL of seawater. Samples of each extract were evaluated in triplicate in vials comprising 5 mL of brine solution and 10 *A. salina* at doses of 10, 100, and 1000 ppm. After 24 hours, survivor *A. salina* was counted.

Statistical tools

All experiments were conducted in triplicate, and the outcomes are expressed as the mean ± standard deviation (mean ± SD). The inhibitory concentration, where the absorbance is 50% (IC50 values) for antioxidant activity, was determined through linear regression analysis of the percentage of radical scavenging. Moreover, the lethal concentration where death is 50% (LC_{50} values) for Brine Shrimp assay was estimated by Probit analysis with 50% confidence intervals. The One-way Analysis of Variance (ANOVA) was employed, followed by Post-hoc Tukey and Multiple Comparison tests, to analyze the significant differences in mean IC_{50} and LC_{50} values of antioxidant and cytotoxicity activities of analyzed plant extracts using IBM SPSS version 26. All graphical representations were built using the Origin 2018.

Results

Phytochemicals

The basic secondary metabolites flavonoids and polyphenols were abundant in all plant extracts. Additionally, the presence of terpenoids, saponin, and coumarin was identified in the majority of plant extracts.Further, alkaloids was present in *W. fructicosa*, *C. reflexa*, and *D. diandra*, gylcosides was present in *S. wallichi*, *W. fructicosa*, and *B. ceiba*, and sterol was present only in *D. diandra* and *U. dioica*. Among the examined plants, the least phytochemicals screened were in *U. dioica*. The overall results on phytochemicals in examined plant extracts are presented in **Table 2**.

Antibacterial activities

Among the studied plants, *W. fructicosa*, *S. wallichi*, *M. esculenta*, and *P. guajava* showed noteworthy antimicrobial activities against inspected pathogens. The highest zone of inhibition was noted in *W. fructicosa* against *Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* with the value of 15.667±1.527 mm, 17.333±0.577 mm and 14.000±1.000 mm respectively, and least was found in *U. dioca* with the value of 6.000±0.000 mm against all three pathogens **(Figure 2)**.

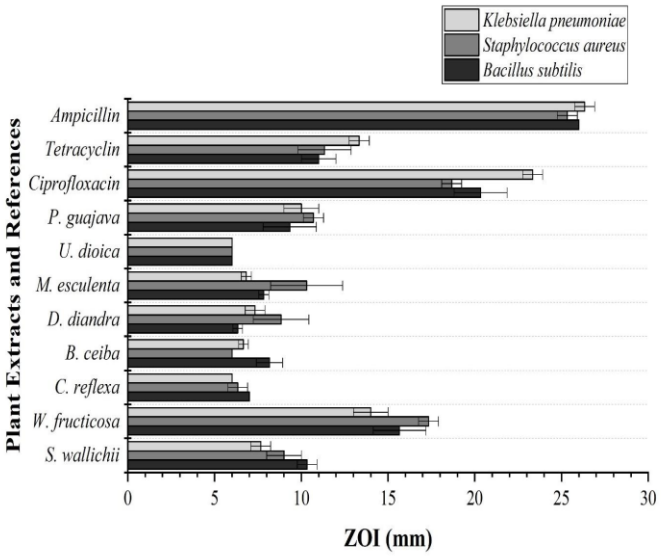

Figure 2. Zone of inhibition of bacterial growth induced by methanolic extract of plants collected in Nepal

Antioxidant and toxicity activities

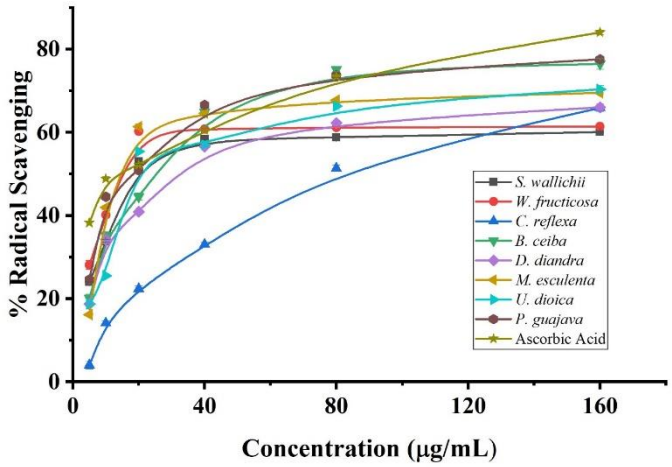

Figure 3. Percentage of DPPH scavenging by plant extracts collected in Nepal.

Our results revealed the astounding potential of examined plants on antioxidant activities. The highest DPPH scavenging was found in *P. guajava* (77.55±0.33%) with an IC_{50} value of 37.581±0.427 µg/mL. Moreover, *M. esculenta*, *B. ceiba*, *W. fructicosa*, and *U. dioca* also show the astonishing DPPH scavenging potential (**Table 2, Figure 3**). Also, examined plant extracts show the noteworthy cytotoxicity potential with a considerable lethal concentration range. The highest cytotoxicity potential was observed in *C. reflexa* (100% death in 1000 ppm concentration) with an LC_{50} value of 6.45 µg/mL. Additionally, *U. dioca*, *B. ceiba*, and *S. wallichi* show the worthy toxicity potential against Brine Shrimp (**Table 2, Figure 4**).

Table 2: Classes of organic compounds detected in the studied plant species through pharmacognostic methods

Plants	Alkaloids	Coumarin	Saponin	Glycosides	Reducing Sugar	Sterols	Terpenoids	Flavonoids	Polyphenols
S. wallichii	-	-	+	+	+	-	+	+	+
W. fruticosa	+	+	+	+	-	-	+	+	+
C. reflexa	+	+	+	-	+	-	+	+	+
B. ceiba	-	+	+	+	-	-	+	+	+
D. diandra	+	+	-	-	-	+	-	+	+
P. guajava	-	+	-	-	+	-	+	+	+
M. esculenta	-	+	+	-	+	-	+	+	+
U. dioica	-	-	-	-	-	+	-	+	+

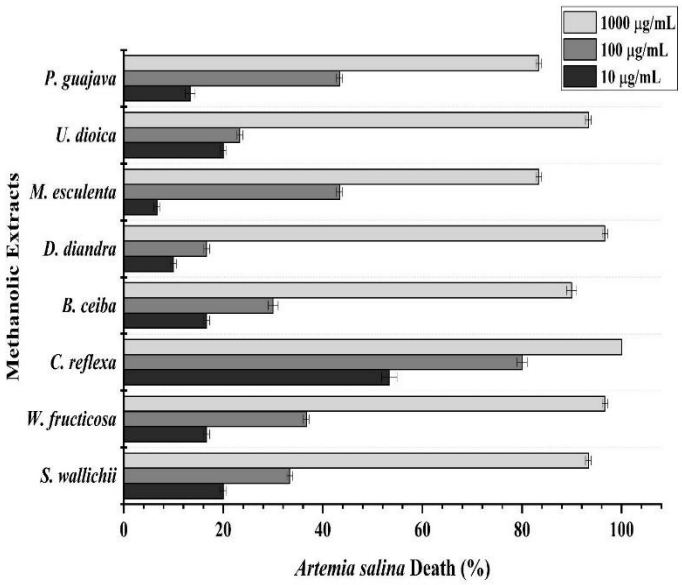

Figure 4. Death percentage of *Artemia salina* by methanolic extracts of plants collected in Nepal.

Table 3: Results on antioxidant activity expressed as the inhibitory concentration at which absorbance is 50% (IC_{50}) and *Artemia salina* toxicity established as 50% lethal concentration (LC_{50}), of methanolic extract of plants collected in Nepal

Methanolic extract	Antioxidant activity (IC_{50}, µg/mL)	Toxicity (LC_{50}, µg/mL)
S. wallichii	57.66 ± 0.07^{d}	107.20 ± 0.45^{b}
W. fruticosa	47.62 ± 0.58^{c}	146.28 ± 0.63^{d}
C. reflexa	96.37 ± 0.34^{f}	6.45 ± 0.33^{a}
B. ceiba	46.82 ± 0.45^{c}	132.19 ± 1.92^{c}
D. diandra	61.83 ± 0.37^{e}	160.38 ± 2.33^{e}
P. guajava	37.58 ± 0.42^{b}	171.14 ± 2.35^{f}
M. esculenta	45.95 ± 0.18^{c}	193.62 ± 1.19^{g}
U. dioca	54.85 ± 0.51^{d}	132.19 ± 0.99^{c}
Ascorbic acid	21.80 ± 0.11^{a}	-

*Different letters in a column represents mean difference of IC_{50} and LC_{50} values of antioxidant and cytotoxicity activities. One-way ANOVA, Post-hoc Tukey, Multiple comparison test ($p<0.05$). Results are presented as the mean ± SD of triplicated experiments. Ascorbic acid used as referential in the antioxidant activity evaluations.

Discussion

The efficacy of medications has diminished in recent years, necessitating the development of innovative, better tolerated, and more effective therapies. Herbal treatments can be seen as an alternative to and complement to allopathic medical procedures as a means to get around this. The aim of this study was to look at current advancements in the study of specific herbal plants as phytotherapy and to show their potential as a therapeutic agent [3]. We examined antioxidant, antibacterial, and cytotoxicity, as well as a qualitative screening of key phytochemicals in eight frequently used medicinal plants in Nepal, to give evidence of plant extracts having therapeutic benefits. In different plant extracts, preliminary phytochemical screening indicated the presence of alkaloids, coumarin, saponin, glycosides, sterols, terpenoids, flavonoids, and polyphenols. The presence of alkaloid, flavonoid, and tannin is well known to be responsible for the cytotoxicity action [27]. Anti-inflammatory and anti-cancer effects are found in flavonoids, terpenoids, and polyphenols [28]. Therefore, the presence of these phytochemicals in our plant extracts is an important factor to consider when using those plants in herbal medicine. However, the cytotoxicity of such plant extracts should be taken into account while evaluating their sound quality and potential in practical applications and medication development.

Antibiotic resistance is on the rise, and novel antimicrobials are in short supply [29]. The need for new, effective, and inexpensive drugs to treat microbial infections is a major concern in global health care, particularly in developing countries, where infectious illnesses account for up to half of all fatalities [30]. The antimicrobial capacity of plant extracts is one of the profound interests of researchers in the field of natural product chemistry. The isolation and identification of potent antimicrobial agents from plant and functional food-based sources have become the one of the alternatives for the discovery of novel antibiotics. From this study, we believe that the *W. fructicosa* extract must be a potent candidate for new drug discovery as it shows significant antibacterial activity against all the organisms. *Woodfordia fruticosa* contain the major pharmaceutical constituents such as tannins, flavonoids, and steroids to be bactericidal, pesticidal, or fungicidal in nature[31]. The findings indicate that the methanol extract derived from *W. fruticosa* flowers contains compounds with modest cytotoxicity and strong anti-inflammatory properties.

These results support the traditional uses of *W. fruticosa* flowers in treating inflammatory conditions [31]. In this study, we found the higher ZOI against analyzed strains by *S. wallichi, W. fructicosa, M. esculenta*, and *P. guajava* which might be due to the presence of secondary metabolites such as flavonoids, alkaloids , tannins and terpenoids in the plant extracts.

Similarly, the radical scavenging potential of plant extracts is considered crucial due to the ability to destroy reactive species such as oxygen species and reactive nitrogen within the human body that are the source for different life-threatening physical and metabolic complications [5].

Out of all the plants, *P. guajava* has the strongest antioxidant action. Ascorbic acid (98.01%) and the observed 80.13% inhibition are comparable. The current results are similar with those of Ashraf et al. (2016), who discovered that methanol extract possesses the highest level of scavenging free radicals' activity, with an IC50 value of 89.82 µg/mL. Moreover, their findings revealed that the elevated free radical (DPPH) scavenging activity of the methanol extract could be attributed to its higher concentration of polyphenolic compounds. Specifically, the extract exhibited higher levels of total flavonoids and total phenolics, underlining the significant role of these polyphenols in antioxidant effectiveness. Therefore, *P. guajava* has a great deal of promise as a cancer chemo preventive agent when ingested [34].

Going through the brine shrimp results, *C.reflexa* showed LC_{50} value of 6.45ppm that means it has more effect on brine shrimp.(6.45 ppm is needed to kill 50 percent of brine shrimp). Besides that,remaning plant extracts showed LC_{50} value between 100 to 250 ppm. Meyer's toxicity index states that extracts that have an LC_{50} less than 1000 µg/ml are hazardous, whereas extracts that have an LC_{50} more than 1000 µg/ml are non-toxic [32]. Similarly, when assessing the toxicity of plant extracts, according to Clarkson's toxicity criterion, extracts are categorized as follows: those with an LC_{50} of 500–1000 µg/ml are considered low toxic, extracts with an LC_{50} of 100–500 µg/ml fall into the medium toxic category, and extracts with an LC_{50} of 0–100 µg/ml are classified as highly toxic. [33]. Based on this, we may conclude that plant extracts are classified as either highly toxic or medium toxic. Therefore, thorough research must be done before ingestion.

From this study also, our results provide noteworthy evidence of analyzed plant extracts to have radical scavenging and cytotoxic activity which clearly indicates the presence of potent bioactive compounds and their regularity in the extracts. These results reflect the potential of plant extracts in promising therapeutic medication with proper isolation, separation, and purification.

Conclusion

It is evident from the studies mentioned above that a variety of factors have a role in plant activity, and more study is required. Compounds can be analyzed using GC-MS and LC-MS after being separated using thin layer chromatography or column chromatography. This aids in our understanding of the precise chemical exhibiting cytotoxic, antioxidant, and antibacterial properties. Nevertheless, further research into the several substances it contains, their therapeutic significance, and the precise process involved must be done in order to produce a medication soon.

Nepal is a nation rich in natural medicine. There are still many conventional medical practices that need to be supported by research studies. The focus now should be on employing nontoxic herbal medicine to control illnesses in humans, animals, and the environment. Because economically significant medicinal plants are a benefit to humanity, more work should go into their conservation because of the numerous turbulences that these plants face.

Author's contribution

TS: design, lab work, data analysis, manuscript preparation, and revision; SPP: data analysis, manuscript preparation, revision, and editing; PJ: data analysis; AGC: manuscript preparation; SB: laboratory works; JL: supervision of the work

Competing Interests

The authors declare no conflict of interest, financial or otherwise

Funding

None

Acknowledgements

The authors are thankful to the Department of Biotechnology, Kathmandu University for providing laboratory facilities to carry out this work.

Ethics approval and consent

Not applicable

Data availability

Not applicable

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