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Phytochemical Studies and Toxicity Evaluation of Selected Medicinal Plants from Sarlahi District, Nepal

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Abstract

Medicinal plants play a vital role in primary health care and the development of herbal drugs at low prices and with fewer side effects. The aim of the present work is focused on the study of antioxidant activity, cytotoxicity, phytochemical screening, and estimation of total phenolic and flavonoid contents of *Achyranthes aspera, Azadirachta indica*, *Cascabela thevetia, Catharanthus roseus*, *Clerodendrum indicum*, *C. infortunatum*, *Oxalis latifolia*, *Paederia foetida* and *Tinospora cordifolia* from Sarlahi district, Nepal. Total phenolics and flavonoids were estimated by Folin-Ciocalteu and aluminum chloride methods respectively. The antioxidant activity and toxicity were evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method and brine shrimp lethality method respectively. Among the plants studied, *A. indica* contained the highest phenolic content (250.08 ± 0.319 mg GAE.g^{-1} of dry extract) and *O. latifolia* showed the highest flavonoid content (112.47 ± 0.07 mg QE.g^{-1} dry extract). Methanolic extract of the bark of *A. indica,* the root of *Clerodendrum infortunatum*, and the stem of *C. indicum* showed potent *in vitro* antioxidant activity with IC_{50} values of 14.84 ± 2.250 μg.mL^{-1}, 23.94 ± 2.245 μg.mL^{-1}, and 29.93 ± 0.993 μg.mL^{-1} respectively as compared to the standard ascorbic acid with an IC_{50} value of 9.44 ± 0.902 μg.mL^{-1}. All nine selected medicinal plants showed low toxicity towards the larvae of *Artemia salina* in dose dependent pattern. The results of this study approve the traditional use of the medicinal plants by the local people.

Keywords: Antioxidant activity, *Azadirachta indica,* Brine shrimp, Folin-Ciocalteu reagent

Introduction

Natural products obtained from plants are the secondary metabolites that are produced in various plant parts, such as the stem, root, leaf, flower, seed, rhizome etc. They are not involved in primary metabolism of the plants but aid in survival of living beings by repelling or attracting other species (Gurnani et al., 2014). About 75-80% of the world population either directly or indirectly rely on plants for their primary health care, because of their cultural appropriateness or lower side effects (Cragg et al., 2009; Newman & Cragg, 2012) The sacred Vedas, dating back to 4500-1600 BC, provide a crucial reference to medicinal plants (IUCN Nepal, 2000). The excess concentration of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced in living cells cause various metabolic disorders leading to cancer, aging, neurodegenerative illnesses, pulmonary sicknesses, diabetes, skin problems, heart diseases, liver ailments, etc. Natural antioxidants donate electrons to neutralize these free radicals and prevent the damage of the vital biomolecules (Sen et al., 2010).

Phenolic compounds stop the initiation or propagation of oxidizing chain reactions with free radical species to prevent the oxidative damage of the tissues. Such oxidative damages may be significant causative factors of chronic diseases such as cancer, cardiovascular diseases and inflammatory diseases and have a major role in ageing (Ismail et al., 2004; Torres de Pinedo et al., 2007). The phenolic compounds scavenge the reactive free radical species and exhibit antitumor, antiviral, antimicrobial and antibacterial activities, and prevent AIDS, mutagenesis and ulcer. The flavonoids exhibit antitumor, anti-inflammatory, anti-allergic, anti-carcinogenic, antibacterial and antiviral activities due to their capacity to scavenge reactive free radicals (Cao et al., 1997; Rice-Evans et al., 1996). It has always been a challenge to ascertain the bioactive compounds that can selectively destroy cancerous

cells without hampering normal cells. The cytotoxic analysis is a preliminary step towards finding the plant extract having a significant antineoplastic property for additional works (Hossain et al., 2013).

Nepal is a landlocked country with substantial variations in soil, altitude and climate over a relatively limited area. The varied topography and climatic conditions have endowed it with a rich biodiversity that accounts for 2.8% of overall flowering plant diversity amounting to around 6000 (5309-6973) species (Jha, 2021). The local people of different indigenous communities use plants and plant-derived products for their primary health care. The native inhabitants of the Sarlahi district of Nepal use various plants for their primary health care. The most commonly used plant including *Achyranthes aspera* L.*, Azadirachta indica* A. Juss.*, Cascabela thevetia* (L.) Lippold*, Catharanthus roseus* (L.) G. Don*, Clerodendrum indicum* (L.) Kuntze*, Clerodendrum infortunatum* L.*, Oxalis latifolia* Kunth*, Paederia foetida* L. and *Tinospora cordifolia* (Willd.) Miers are the focus of this study.

The research work is aimed to evaluate *in vitro* antioxidant activity and *in vivo* toxicity of some of these commonly applied plants in traditional medicine. This research is intended to provide scientific evidence to the traditional medical practice done by the local people since ancient times.

Materials and Methods

Collection of plant sample

The plant samples were collected from Bishnu-4, Vishwanathpur of Sarlahi district of Nepal in May 2017 based on an ethnobotanical practice. Among them, seven plants were identified at the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, one from National Herbarium and Plant Laboratories, Godawari, Lalitpur and one from Ayurveda Campus, Kirtipur. The list of plants with local names, parts used, collected sites and traditional uses are shown in Table 1.

Preparation of plant extract

The collected plant samples were washed with clean water and air-dried in shade for about three weeks. The dried samples were ground into fine powder and used to prepare crude extracts by cold percolation method using 80% methanol as a solvent. The powdered materials were immersed in methanol in the conical flasks and left for 2-3 days at room temperature with shaking at intervals. They were than filtered and the filtrate was concentrated using a rotatory evaporator. The process was repeated 6-7 times. The concentrated filtrate was dried to get a solid or semisolid residue and stored at 4°C.

Phytochemical screening

The phytochemical analysis was carried out by adopting standard protocols. The different phytochemical constituents were identified by the

Table 1: List of medicinal plants collected for the study

Plant samples	Local name	Other names	Collected part	Traditional use
Achyranthes aspera L.	Chirchiri	Datwan, Rough chafftree, Apamarga	Root	Diarrhea and anemia
Azadirachta indica A. Juss.	Neem	Aristha	Bark	Toothache, blood purification, skin disease
Cascabela thevetia (L.) Lippold	Jharkanai	Kaner	Leaves	Joint pains
Catharanthus roseus (L.) G. Don	Naitara	Madasgascar periwinkle Sadabahar	Leaves	Blood cancer
Clerodendrum indicum (L.) Kuntze	Agiyakhar	Bhargi, Angiyaah, Bhaargee	Stem	Wounds
Clerodendrum infortunatum L.	Bhat	Bhate	Root	Toothache
Oxalis latifolia Kunth	Khattibuti	Chariamilo	Whole parts	Digestive problem
Paederia foetida L.	Ganpasar	Skunk vine Gandhaprasarni	Whole part	Cough, fever
Tinospora cordifolia (Willd.) Miers	Gurgus	Gurjo, guduchi	Vine	Digestive problems

color reaction with different reagents (Singh et al., 2022).

Determination of total phenolic content

The total phenolic contents of the extracts of different plant samples were determined by the Folin–Ciocalteu method with slight modifications (Pawar & Dasgupta, 2018; Rover & Brown, 2013). A 0.5 mL of each extract (1 mg.mL^{-1}) was mixed with 2.5 mL Folin–Ciocalteu reagent (1:10 v/v distilled water) and 2 mL of 7% sodium carbonate. The mixture was then vortexed for the development of color and was allowed to stand for 30 min. at 40°C in the dark. Then the absorbance was measured at 765 nm by using a spectrophotometer against a blank. The phenolic content was calculated as mg of gallic acid equivalent per gram of the dry extract by using a standard gallic acid calibration curve.

Determination of total flavonoid content

The total flavonoid contents of plant extracts were determined using the aluminum chloride colorimetric method. The plant extract (0.5 mL) was mixed with water (1.5 mL) followed by 10% aluminum chloride (0.1 mL), 1M potassium acetate (0.1 mL) and distilled water (2.8 mL). The resultant mixture was incubated at 27°C for 30 min. in the dark. The absorbance of the mixture was recorded by using a spectrophotometer at 415 nm against a blank. The flavonoid content was calculated using the standard calibration curve of quercetin. The result is expressed as micrograms of quercetin equivalent (QE)/g of the weight (Sembiring et al., 2018).

Antioxidant activity

The antioxidant activity of methanolic extracts of nine plants and standard (ascorbic acid) was assessed based on the free radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Blois, 1958; Sharopov et al., 2015). Different concentrations (20, 40, 60, 80, and 100 µg.mL^{-1}) of ascorbic acid and methanol extracts were prepared from the stock solutions. Two milliliters of standard and extract solutions of each concentration is mixed with 2 mL of 0.2 mM DPPH solution respectively. Each of the experiments was performed in triplicate with negative control. The reaction mixture was incubated at 37°C for 25 min. in the dark and the absorbance was recorded at 517 nm using a UV-visible spectrophotometer.

The free radical scavenging activity of the sample was calculated as

$$\% \text{ Scavenging} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \text{ X } 100$$

Where, Ac = Absorbance of DPPH solution,

As = Absorbance of test or reference sample

The IC_{50} (concentration exhibiting 50% of inhibition) values were determined from the graph of the free radical scavenging activity (%) against the extract concentration by linear regression.

Brine shrimp bioassay

Brine shrimp [*Artemia salina* (Linnaeus, 1758)] lethality bioassay was carried out to check the cytotoxicity of the plant extracts by adopting a standard method (Abdullah-Al-Emran et al., 2011). The *Artemia salina* eggs were hatched in artificial seawater under constant aeration and were kept in chamber illuminated for 48 h of incubation at room temperature. The phototrophic larvae (nauplii) were attracted toward the lighted part and collected with a pipette for the test. Stock solution was prepared by dissolving 20 mg of plant extract in 2 mL of methanol and was diluted to the concentrations of 1000 mg.mL^{-1}, 100 mg.mL^{-1} and 10 mg.mL^{-1} for the test. After evaporation of the solvent, 5 mL of artificial seawater was added to each test tube with gentle shaking to ensure that the compounds diffused adequately in the aqueous solution. Three replicates were arranged for each treatment and control. Then, 5 mL artificial seawater with ten matured shrimps (nauplii) was transferred to the test tubes containing samples. Similarly, controls were taken with mature naupliis in each test tube. After 24 hours, the number of survivors was counted with the help of a pipette, and the percentage of death from each dose was recorded. The value of the lethal concentration dose required to kill 50% of the shrimp larvae (LC_{50}) was calculated by the Probit method.

Results and Discussion

Percentage yield

Generally, biologically active substances are present in low concentrations in plants. An effective extraction method can produce a high yield with the least quantity of necessary alterations to the functional properties of the extract. Based on sample matrix characteristics, chemical characteristics of the analytes, matrix-analyte interaction, efficiency, and desired features, it is essential to choose the best extraction method and solvent (Dhanani et al., 2017). In this study, cold percolation method was used for the extraction resulting in different percentages of yield varying from 21.48% for *Azadirachta indica* to the minimum yield of 7.86% for *Achyranthes aspera*. Many internal and external factors, including plant organs, phenological stages, genetic profiles, and environmental abiotic and biotic factors, such as growing site, light, temperature, radiation, soil drought and salinity, pathogens, and herbivore attacks, all play a significant role in the content of bioactive compounds in plants (Cirak & Radusiene, 2019).

Phytochemical screening

Phytochemical constituents are the natural bioactive compounds that are found in plants. The qualitative screening of phytochemical constituents like alkaloids, flavonoids, tannins, terpenoids, saponins, steroids, carbohydrates, glycosides and polyphenol were carried out in this study. The result obtained from the phytochemical analysis is shown in Table 2. Plants secrete secondary metabolites for various purposes such as to cope with biotic stresses, attract pollinators, establish symbiosis, be adept with light, repel herbivores, insects, etc. The results exhibited that all the plants contained alkaloids, flavonoids, and polyphenols. Alkaloids are nitrogen-containing secondary metabolites, which protect the middle-aged human and animals from several diseases. On the other hand, flavonoids play a crucial role in the human diet and prevent cancer, cardiovascular disease, inflammatory disease, radiation, and chemical damage (Bertleff-Zieschang et al., 2017; Khan et al., 2019). Tannins were present in all of the plants except *Oxalis latifolia*. These compounds are polyphenolic secondary metabolites with high molecular weight present in most plants. They are considered to prevent plants from microorganisms. In the case of animals, they may help in the digestion of protein and prevent from immediate growth of animals (Bertleff-Zieschang et al., 2017).

Total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of different samples were determined by adopting the standard protocols and the results are presented in Table 3. The highest total phenolic content was found in *Azadirachta indica* (250.08 ± 0.319 mg GAE.g^{-1}) bark extract.

Another study of the *Azadirachta indica* collected from Dhaka revealed a TPC of 285.77 ± 0.99 mg GAE.g^{-1} which was close to the present value (Abdullah-Al-Emran et al., 2011). Similarly, another study had shown the total phenolic content from 80%

Table 2: Phytochemicals present in different plant extracts

Phytochemicals	*Achyranthes aspera*	*Azadirachta indica*	*Cascabela thevetia*	*Catharanthus roseus*	*Clerodendrum indicum*	*Clerodendrum infortunatum*	*Oxalis latifolia*	*Paederia foetida*	*Tinospora cordifolia*
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	+	-	+	+
Tannins	+	+	+	+	+	+	-	+	+
Polyphenols	+	+	+	+	+	+	+	+	-
Glycosides	+	-	+	-	+	-	+	-	+
Steroids	+	+	+	-	+	+	-	+	+
Carbohydrates	+	-	-	-	+	+	+	-	+
Saponins	+	-	-	+	+	+	-	-	-

Note: (+) = present; (-) = absent

ethanolic, ethyl acetate and butanol extracts of *A. indica* 69.17 ± 1.57 mg GAE.g^{-1}, 38.13 ± 1.25 mg GAE.g^{-1} and 24.38 ± 3.13 mg GAE.g^{-1} respectively (Pandey et al., 2014). Total flavonoid content in *A. indica* was 62.26 ± 0.012 mg QE.g^{-1}. Akhtar et al. (2018) reported the comparable values of TPC and TFC of methanol/chloroform extract and aqueous extract of *A. indica* collected from Pakistan. The methanol/chloroform extract had the TPC and TFC of 29.6 ± 4.5 mg GAE.g^{-1} and 16.0 ± 2.5 mg QE.g^{-1} respectively. Similarly, the aqueous extract of the same plant was reported the TPC and TFC of 27.2 ± 2.0 mg GAE.g^{-1} and 14.2 ± 2.6 mg QE.g^{-1} respectively. Among the nine plants studied, we observed the maximum total flavonoid in *Oxalis latifolia* (112.47 ± 0.07 mg QE.g^{-1}) of dry extract. Several studies showed the total phenolic contents to be higher than the total flavonoid content in plant extracts, but sometimes it was reversed. In case of *O. latifolia* such a result was obtained which is comparable to the total phenolic content (63.43 ± 2.62 mg GAE.g^{1}) and total flavonoid content (72.73 ± 2.37 mg QE.g^{1}) reported by Krishnan et al. (2019). The minimum total phenolic content was reported at 54.93 ± 0.315 mg GAE.g^{1} in *Paederia foetida*. The previous study reported TPC of 3.98 ± 0.54 mg GAE.g^{1} in shoots of *P. foetida* (Senapati et al., 2013). This result was supported by another similar study by Osman et al. (2009). Total flavonoid content in *P. foetida* was found to be 83.52 ± 0.091 mg QE.g^{1}. Similarly, the minimum amount of total flavonoid content was 28.04 ± 0.065 mg QE.g^{1} in *Catheranthus roseus*. In the present result, the total phenolic content in *C. roseus* was 73.74 ± 0.140 mg GAE.g^{1} in the plant extract. A similar study showed that the value of TPC was 285 ± 0.3 mg GAE.100 g^{1} which is also comparable to the present study (Kaur & Mondal, 2014). Rani and Kapoor (2019) collected white and pink colored *C. roses* from Panjab, India and evaluated for their TPC and TFC. The pink variety of *C. roses* had the TPC and TFC values of 40.8 ± 0.52 mg GAE.g^{1} and 12.7 ± 0.77 mg GAE.g^{1} respectively which was quite greater than that of the white variety. It shows that the quantity of phytoconstituents greatly fluctuates in the morphological varieties of the plant.

Antioxidant potential

The antioxidant activities of the methanol extracts of different plant species were determined by DPPH free radical scavenging method. The degree of color change (yellow on purple background) denotes the presence of antioxidants in the extract of plant. The dose-dependent variation of percentage radical scavenging of different plant extracts and ascorbic acid as standard are shown in Figure 1. The graph shows the highest antioxidant activity in methanol extracts *Azadirachta indica* and *Clerodendrum infortunatum* close to that of the standard. The linear regression of the percentage of radical scavenging versus concentration was used to calculate the concentration of each plant extract required for 50% inhibition of DPPH radical (IC_{50}). The antioxidant potential is in inverse relation to the IC_{50} value, lower value of IC_{50} indicates high antioxidant potential. The IC_{50} values of the plant extracts

Table 3: TPC, TFC and antioxidant activity of different plant extracts

S.N.	Plants	TPC (mg GAE.g^{-1})	TFC (mg QE.g^{-1})	Antioxidant activity (IC_{50} in µg.mL^{-1})
1	*Achyranthes aspera*	75.70 ± 0.187	40.95 ± 0.130	NC
2	*Azadirachta indica*	250.08 ± 0.319	62.26 ± 0.012	14.84 ± 2.25
5	*Cascabela thevetia*	159.62 ± 0.254	94.16 ± 0.193	30.55 ± 1.87
4	*Catharanthus roseus*	73.74 ± 0.140	28.04 ± 0.066	NC
3	*Clerodendrum indicum*	67.55 ± 0.155	85.34 ± 0.06	29.93±0.993
6	*Clerodendrum infortunatum*	58.87 ± 0.049	52.10 ± 0.109	23.94 ± 2.24
7	*Oxalis latifolia*	61.42 ± 0.065	112.47 ± 0.070	34.02 ± 0.07
8	*Paederia foetida*	54.53 ± 0.315	83.52 ± 0.091	NC
9	*Tinospora cardifolia*	129.89 ± 0.182	81.52 ± 0.092	38.96 ± 1.94
10	Ascorbic acid	-	-	9.44 ± 0.90

Note: Values are mean ± SD; n = 3; NC = not calculated

along with the standard ascorbic acid are shown in Table 3. The antioxidant activity of different plant extracts is influenced by several factors like phenolic, flavonoid, phytochemical constituents, the composition of extract and the environment. The antioxidant activity of ascorbic acid as a standard was found to be quite high with an IC_{50} value of 9.44 ± 0.902 µg.mL^{-1}. The *A. indica* bark extract exhibited significant antioxidant activity having an IC_{50} value of 14.84 ± 2.25 µg.mL^{-1}. This value was near the IC_{50} value of ascorbic acid. The IC_{50} value of *A. indica* is clearly supported by high total phenolic content i.e. 250.08 ± 0.319 mg GAE.g^{-1}. Kiranmai et al. (2011) reported IC_{50} value of 27.3 ± 0.23 µg.mL^{-1} for this species. Similarly, the ethanolic extract of the *A. indica* collected from Bangladesh had a TPC of 238.81 ± 0.98 mg GAE.g^{-1} and IC_{50} value for DPPH radical scavenging test were 13.81 ± 0.06 µg.mL^{-1} (Hossain et al., 2014).

The root extract of *Clerodendrum infortunatum* exhibited significant DPPH radical scavenging activity with an IC_{50} value of 23.94 ± 2.245 µg.mL^{-1}. The antioxidant potential of the plant was supported by the previous result in which IC_{50} values were 13.95 ± 0.44 µg.mL^{-1}, 32.35 ± 0.73 µg.mL^{-1} and 31.0 ± 1.06 µg.mL^{-1} for the leaf, stem, and root extracts respectively (Dey et al., 2012). Swargiary et al. (2019) reported that the leaves of *C. infortunatum* collected from Assam state of India exhibited moderate antioxidant activity with an IC_{50} value of 137.0 µg.mL^{-1} which is more inactive than that of the present sample. The season of collection, maturity, topography and several other factors may have influenced the biological activity of the plant extract. Similarly, the stem bark extract of *C. indicum* was the third active antioxidant among the nine plants evaluated in this study. In DPPH radical scavenging experiment, we observed the IC_{50} value of 29.93 ± 0.993 µg.mL^{-1}. Majumdar et al. (2019) reported IC_{50} value of 7.89 µg/mL for of ethanolic leaf extract of *C. indicum* collected from Bangladesh. The higher DPPH value obtained in that sample might be attributed to the higher TPC and TFC values. Barua et al. (2014) evaluated the antioxidant activity of ethanolic and hydroethanolic extracts of the *C. indicum* collected from Assam, India by the DPPH radical scavenging method and reported that the IC_{50} of ethanolic extract of the plant (49.52 µg.mL^{-1}) was higher than that of hydroalcoholic extract (82.17 µg.mL^{-1}). The aqueous and ethanolic leaf extract of the plant from Myanmar was found to be less potent than our sample. In DPPH method, IC_{50} of the aqueous and ethanolic extracts were 723.18 ± 12.30 µg.mL^{-1} and 430.29 ± 17.32 µg.mL^{-1} respectively (Aye et al., 2020). Here, we evaluated the antioxidant activity of the stem which was found more potent than the leaves of the same plant that was collected from another geographical location.

Cascabela thevetia extract contained relatively higher TPC and TFC (Table 3) and exhibited good antioxidant activity (IC_{50} = 30.55 ± 1.87 µg.mL^{-1}). Seetharaman et al. (2017) evaluated chloroform, water, and methanol extracts of the whole plant of this species from Tamilnadu, India for antioxidant activity by the DPPH method. All of the extracts exhibited similar potency with an IC_{50} value of 60.1 µg.mL^{-1} for the methanolic extract. Similarly, *Oxalis latifolia* showed significant antioxidant activity with an IC_{50} value of 34.02 ± 0.07 µg.mL^{-1} which can support the relatively high TPC and TFC of the same plant in an analogous study reports of Krishnan et al. (2019).

The antioxidant activity of *Tinospora cardifolia* was moderate with an IC_{50} value of 38.96 ± 1.94 µg.mL^{-1}. Shrestha and Lamichhane (2021) evaluated the antioxidant activity of *T. cordifolia* from Kavrepalanchok district of Nepal by DPPH method. The methanolic extract showed weak activity with an IC_{50} value of 238.0 µg.mL^{-1}. However, reverse type of result was reported by Upadhyay et al. (2014). It indicates that the antioxidant activity of this plant may be dependent not only on the extracting solvents but also on several factors like maturity, collection season, locality etc.

In this study, we observed low antioxidant activity of *Achyranthes aspera, Catharanthus roseus* and *Paederia foetida* but the literature revealed higher activities of the plants collected from different regions. Mishra and Bisht (2012) reported an IC_{50} value of 21.32 µg.mL^{-1} for the leaf extract of

P. foetida collected from southern Orissa, India. Similarly, another study reported the antioxidant activity of *C. roseus* (IC_{50} = 48.5 µg.ml^{1}) in acetone (Mir et al., 2018) and other studies as 129.91 µg. mL^{1} and 241.86 µg.mL^{1} in root and leaf of *A. aspera* respectively (Kumar & Jat, 2017).

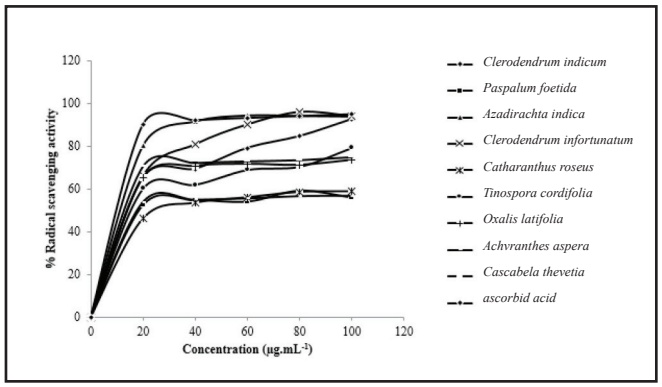

Figure 1: Dose-dependent variation of % scavenging with concentration

Brine shrimp bioassay

The toxicological activities of plant extracts were evaluated based on their toxicity towards *nauplii*. In the method, the LC_{50} value of different plant extracts was determined and those having LC_{50} values less than 1000 µg.mL^{1} were considered pharmacologically active. The results of this study are shown in Table 4. The leaf extract of *Catharanthus roseus* showed the highest LC_{50} value (2163.5 ± 12.56 µg.mL^{1}) among the tested plants. This value was found to be quite low than that obtained by Khairani et al. (2021). In another test, the LC_{50} values of methanol extract and aqueous extracts of the plant were 261.36 µg.mL^{1} and 150 µg. mL^{1} respectively (Narwade & Marathe, 2021). The cytotoxic activity of the plant extracts is due to the presence of flavonoids, tannins and steroids so it may be the source of cytotoxic compounds (Hossain et al., 2013). In another study, the same plant of Bangladeshi origin exhibited strong toxicity (LC_{50} = 20 µg.mL^{1}) in the Brine shrimp lethality test (Harun-or-Rashid et al., 2017). The methanol leaf extract of *Paederia foetida* of Bangladeshi origin exhibited strong cytotoxicity with an LC_{50} value of 65.31 µg.mL^{1} on the Brine shrimp lethality assay which is quite stronger than our observation (Ahmed, 2014).

Table 4: Brine shrimp lethality assay results

Plant	LC_{50} values (µg.mL^{-1})
Azadirachta indica	5014.84 ± 8.25
Cascabela thevetia	8048.7 ± 14.87
Catharanthus roseus	2163.5 ± 12.56
Clerodendrum indicum	3189.77 ± 11.99
Clerodendrum infortunatum	4123.94 ± 18.45
Tinospora cardifolia	3028.96 ± 21.94

Note: Values are the mean ± SD (n=3)

In *Clerodenrum indicum*, the LC_{50} value was found to be 3189.77 ± 11.99 µg.mL^{1}, this value was third least among nine selected plants in this study. No previous reports were found but the another species of the same genus (*C. inerme*) has been reported to have LC_{50} values of 36.5 µg.mL^{1}, 10.0 µg.mL^{1}, and 9.1 µg.mL^{1} in methanol, ethanol and chloroform extracts of leaf respectively (Uddin et al., 2014). Another species like *C. infortunatum* had LC_{50} values of 30.702 mg.mL^{1}, 32.907 mg.mL^{1}, and 42.559 mg.mL^{1} in the root, leaf, and stem in chloroform extract and 20.845 mg.mL^{1}, 24.017 mg.mL^{1}, and 31.379 mg.mL^{1} in the root, leaf and stem for ethyl alcohol extract respectively (Waliullah et al., 2015). The present result of *Cascabela thevetia* for LC_{50} value was 8048.7 ± 14.87 µg. mL^{1}. Similarly, *Achyranthes aspera, Azadirachta indica, Clerodendum viscosum, Oxalis latifolia* and *Tinospora cardifolia* were found to exhibit high LC_{50} values indicating very weak toxicity. Abdullah-Al-Emran et al. (2011) reported that the ethanolic extract of leaves of *Azadirachta indica* collected from Dhaka exhibited moderate toxicity against Brine shrimp larvae with an LC_{50} value of 37.15 µg. mL^{1} which is quite lower than that of the present study. Their results showed that the plant extract had fewer bioactive chemical constituents. The degree of lethality was found to be directly proportional to the concentration of plant extract. At 1000 µg.mL^{1} concentration, maximum lethality was observed. A plant extract with an LC_{50} value of less than 1000 µg. mL^{1} is poisonous and one with a value of more than 1000 µg.mL^{1} is considered non-toxic (Nguta et al., 2012). The majority of the outcomes in the present study were found to be less harmful than those in the earlier studies. It may be due to the variations in environments of the collection sites, laboratory

conditions, seasons, maturity, process, genetics etc. (Hussain et al., 2008; Sampaio et al., 2016)

Conclusion

Phytochemical screening of the methanolic extracts of all nine selected plants showed the presence of different chemical constituents such as alkaloids, flavonoids, polyphenols, tannins and terpenoids. *Azadirachta indica* exhibited the highest phenol content while the second highest was observed in *Cascabela thevetia*. The total flavonoid contents of *Oxalis latifolia* and *C. thevetia* were the highest. In addition, the extract of *Clerodendrum indicum* showed substantial total flavonoid content. The methanol extract of *A. indica* exhibited good antioxidant properties among all nine selected plant extracts with IC_{50} value close to the standard ascorbic acid. Similarly, *Clerodendrum infortunatum* and *C. indicum* showed significant antioxidant activity. The methanol extracts of all nine selected plant species were found to be inactive against brine shrimps.

Author Contributions

Surya Kant Kalauni conceptualized the study. Sushil Kumar Mahato did lab work and prepared the first draft, Lekh Nath Khanal overall review and finalized of the manuscript.

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