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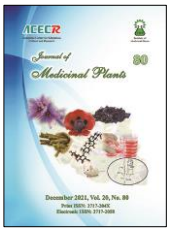

Research Article

Estimation of phytochemicals, antioxidant, antidiabetic and brine shrimp lethality activities of some medicinal plants growing in Nepal

Junu Kapali, Khaga Raj Sharma*

Central Department of Chemistry, Tribhuvan University, Kirtipur Kathmandu, Nepal

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ABSTRACT

Background: Extreme production of free radicals in the human body causes direct damage to biological molecules that leads to the different types of diseases. The natural or synthetic antioxidants inhibit directly the production or restrict propagation or nullify the free radicals produced in the human body to protect the immune system. **Objective:** This study aims to quantify the total phenolic and flavonoid content, antioxidant and antidiabetic activities and toxicity test for the methanol extracts of aerial parts of traditionally used medicinal plants like *Ageratina adenophora* (Spreng.) R.M.King & H.Rob., *Cupressus sempervirens* L. and *Lantana camara* L. **Methods:** The total phenolic content (TPC) was estimated by Folin-Ciocalteu reagent method and the total flavonoid content (TFC) by aluminum chloride assay. The α-amylase inhibition activity was performed to evaluate the antidiabetic activities of plant extracts. **Results:** *Lantana camara* showed the highest phenol content (10.20 ± 0.343 mg gallic acid equivalent /g extract) and flavonoid content (1.87 ± 0.160 mg quercetin equivalent /g extract) among the three plant samples, respectively. The methanol extracts of *Lantana camara* showed the strongest DPPH radical scavenging activity with IC_{50} of 106.18 ± 11.390 µg/ml. In addition, *Ageratina adenophora* methanol extract was found to inhibit α-amylase activity with IC_{50} value of 1.84 ± 0.007 mg/ml. The methanol extract of *Ageratina adenophora* was found to be toxic against brine shrimp with median lethal concentration (LC_{50}) value of 833.68 µg/ml. **Conclusion:** This research shows that the traditionally used medicinal plants are the rich and potent sources of natural antioxidant and antidiabetic compounds which may be the potent natural drug candidates in the future drug discovery process.

1. Introduction

Medicinal plants have been used practically all over the world from the presence of human civilization on an observational basis, without rational knowledge on their pharmaceutical activities or active constituents [1, 2]. These medicines refers to the use of natural products from natural origin for medicinal purposes.

Abbreviations: DPPH, 1,1-Diphenyl-2-Picrylhydrazyl; GAE, Gallic Acid Equivalent; QE, Quercetin Equivalent; TPC, Total Phenol Content; TFC, Total Flavonoid Content; IC_{50}, Median Inhibitory Concentration; LC_{50}, Median Lethal Concentration; FCR, Folin-Ciocalteu Reagent

*Corresponding author: khaga.sharma@cdc.tu.edu.np

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These medicinal plants provides significant advantages for treatment of many human illness. Due to presence of complex chemical constituents in different parts of plants, these medicinal plants provide certain beneficial effects [3, 4]. Plants and plant-based products are the natural sources of different types of secondary metabolites such as phenols, flavonoids, alkaloids, glycosides, lignin, and tannins. Beside their uses as therapeutic agents, these chemical constituents are also used as a starting materials for the development of new drugs or as models for synthesizing pharmacologically active constituents [5, 6, 7].

An antioxidant is a chemical compound that nullify free radical either by accepting or donating electron (s) or an active hydrogen atom to eliminate the unpaired condition of the radical, thus reducing its capacity to damage directly the biomolecules. These antioxidant agents are capable to directly react with the reactive species and eliminate their radical status, while they may itself became a new free radical which are less active than those radicals they have neutralized. These newly formed free radicals is again neutralized by other antioxidant or by other mechanism to terminate the chain reaction [8, 9]. Free radical reactions could be one of the etiologic aspects for many human diseases such as hemochromatosis, diabetes, ischemic heart disease, cardiovascular, atherosclerosis, and neurodegenerative diseases such as Alzheimer's disease and Parkinson, and hepatic disorders and disease conditions such as ageing process, inflammation and immune-suppression [10, 11]. Free radicals are generated on exposure to UV light, ionizing radiation, heavy metal ions, smoking, chemicals and fried foods [12].

Based on the traditional aspects, this study was conducted to evaluate the total phenolic content, total flavonoid content, antioxidant activity, antidiabetic activity and toxicity test of the methanol extracts of aerial parts of *Ageratina adenophora, Cupressus sempervirens* and *Lantana camara* collected from Kathmandu Valley of Nepal. *Ageratina adenophora* (Spreng.) R.M. King & H. Rob. (family: Asteraceae) [syn. *Eupatorium adenophorum* (Spreng) is commonly known as Cat weed, Crofton weed, Sticky snakeroot or Mexican devil is a many stemmed, perennial herbaceous shrub, 1-2 m high, reproduce by seed. *Ageratina adenophora* is used in India as an antiseptic and as a blood coagulant. A decoction of the plant is used to treat jaundice and ulcers. Root of *Ageratina adenophora* is known to possess diuretic and antispasmodic properties. The plant is known to have diuretic and anthelmintic properties and is used for the treatment of diphtheria [13]. Its juice is used to cuts and wounds. Juice of root is useful for fever treatment. A paste of the leaf is applied to cure boils and it also used to treat eyes insomnia (Fig. 1) [14].

Cupressus sempervirens L. (family: Cupressaceae) is a tree that grows up to 30 m tall with dark green and obtuse leaves and obtusely spiked scales [15]. The core and young branches of *Cupressus sempervirens* were reported to have antiseptic, aromatherapeutic, astringent, balsamic and anti-inflammatory activities. Cypress is also described to exert antispasmodic, astringent, antiseptic, deodorant, and diuretic effects, to promote venous circulation to the kidneys and bladder area, and as a coadjuvant in therapy of urinary incontinence and enuresis (Fig. 1) [16, 17].

Lantana camara L. (family: Verbenaceae) is a perennial aromatic shrub that grows up to 2 to 3 meters and can spread about 2.5 meters in wide [18]. *Lantana camara* has been reported to be used in traditional medicine system for the treatment of itches, cuts, ulcers, swellings,

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bilious fever, cataract, eczema and rheumatism. Various parts of the plants are used in the treatment of cold, headache, uterine haemorrhage, chicken pox, eye injuries, whooping cough, asthma, bronchitis and arterial hypertension. The fruits are useful in fistula, pustules, tumors and rheumatism (Fig. 1) [19].

Several phytocomponents such as alkaloids, flavonoids, terpenoids, phenols, steroids, phytosterols, polysaccharides, tannins, saponins, coumarins, hydroxy anthraquinones, anthraquinone glycosides, have been extracted from different parts of the plant [20-23]. These phytocomponents are responsible for several pharmacological activities such as antiseptic, analgesic, antipyretic, antibacterial, antifungal, antihelminthic, antioxidant, anti-inflammatory, hepatoprotective, cytotoxic, larvicidal, and antispasmodic [16, 19, 24].

This research was carried out to evaluate the total phenolic, flavonoid contents, the antioxidant and antidiabetic activities in methanol extracts of the aerial parts of *Ageratina adenophora*, *Cupressus sempervirens* and *Lantana camara* collected from Kathmandu valley of Nepal. The correlation between them could help in the assessment and development of safer and inexpensive new drugs candidate and establishment of new drug for advance level of research.

Ageratina adenophora

Cupressus sempervirens

Lantana camara

Fig. 1. Photographs of plant samples used in the study

2. Materials and Methods

2.1 Chemicals

All reagents and solvents used were of laboratory grade from E. Merck, T. Fisher Scientific and Qualigens Chemical Companies, India, purchased from the local vendors. Folin-ciocalteu reagent (FCR) for TPC was purchased from local vendor.

2.2 Equipments

Buchi RE111 Rotavapor was used for the evaporation of solvents. Absorbance for DPPH assay, TPC, TFC and antidiabetic activity were measured using 96 well plate reader. Grinder, Digital weighing machine (4 digit), Micropipettes (Erba BIHOT), water bath (Physilab Scientific Industries, Ambalacantt, India) and others like filter paper (Whatmann), forceps, round bottom flask, conical flasks, funnels, glass rods, measuring cylinder, pipettes, test-tubes and glassware were used during these work.

Other chemicals and reagents like DPPH, ascorbic acid, gallic acid, quercetin required for antioxidant were used of analytical grade provided at the research laboratory of Central Department of Chemistry, Tribhuvan University. Chemicals like aqueous Na_2CO_3, $NaNO_2$, NaOH, $AlCl_3$, methanol, etc. were used of analytical grade.

[DOR: 20.1001.1.2717204.2021.20.80.9.6]
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2.3. Collection and identification of plant samples

Aerial parts of the plants *Ageratina adenophora, Cupressus sempervirens* and *Lantana camara* were collected from Kathmandu Valley, Nepal during the month of April and June, 2019. The plants were collected with the permission of Department of Plant Resources, Ministry of Forests and Environment, Government of Nepal, in accordance with article no. 10 (B) of Plant Resource Research Procedure 2013 and revised 2016. Then proper identification of plant was done from National Herbarium and Plant Laboratories (NHPL)/KATH, Godawari-5, Lalitpur, Nepal. The voucher specimen 1(KATH), 2(KATH) and 3(KATH) were submitted to the same department.

2.4. Extraction of the samples

The aerial parts of plant were cleaned and dried under dry shade for two weeks before the extraction procedure. The dried samples were then powdered separately in a grinder. 100 g of each dried sample of aerial parts of plants were dissolved in 250 ml methanol and thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 36 hours with frequent agitation for more interaction between the powdered particles and the solvent. This process is termed as cold percolation. After complete extraction, the plant extracts was filtered with sterilized filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using filter paper. The filtrate was kept in rotary evaporator for evaporation of the solvent. The solution was also kept in the water bath at the temperature of 35 ºC for solvent evaporation. After running this procedure, a gummy concentrated extraction was obtained which were preserved in refrigerator at 4 ºC for further use. The percentage yield of *Ageratina adenophora, Cupressus sempervirens* and *Lantana camara* methanol extracts were 19.75 %, *25*.53 % and 23.59 % respectively. [25].

2.5. Determination of total phenol content (TPC)

2.5.1. Preparation of standard gallic acid for calibration curve

Total phenol contents (TPC) of the methanol extracts were determined by Folin-Ciocalteu reagent using the method as described by Ainsworth et al. (2007) & Lu et al. (2011) [26, 27] with a slight modifications. Standard gallic acid stock solution was prepared by dissolving 1 mg of it in 1 ml of methanol (1 mg/ml). Various concentrations of gallic acid solutions in ethanol (10, 20, 30, 40, 50, 60, 70, and 80 µg/ml) were prepared from the stock solution. The total assay mixture containing 20 µl of 10-80 µg/ml standard gallic acid, 100 µl Folin-Ciocalteu reagent followed by 80 µl 1 M Na_2CO_3 separately were incubated in dark for 15 min in dark and absorbance was measured at 765 nm using micro-plate reader.

2.5.2. Preparation of samples for total phenol content

The procedure as described for standard gallic acid was followed to 50 µl of each sample (three replicates). The results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g). The total phenol contents in all the samples were calculated by the using the formula:

$$C = \frac{cV}{m}$$

where C = total phenol content mg GAE/g dry extract,

c = concentration of gallic acid obtained from calibration curve in mg/ml,

V = volume of extract in ml, and m = mass of extract in gram.

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2.6. Determination of total flavonoid content

2.6.1. Preparation of standard quercetin for calibration curve

Total flavonoid contents in the extracts were determined by aluminum chloride method as described by Chang et al. (2002) [28] with some modifications. Stock solution of 1 mg/ml was prepared by dissolving 1 mg of quercetin in 1 ml of methanol. Various concentrations of quercetin such as 10, 20, 40, 80, 160, 320 & 500 µg/ml were prepared from the stock solutions. The whole assay mixture containing 130 µl of 10-500 µg/ml standard quercetin, 60 µl ethanol, 5 µl of 10 % $AlCl_3$ and 5 µl of 1M potassium acetate separately were incubated in dark for 30 min and absorbance was measured at 415 nm using micro-plate reader.

2.6.2. Preparation of samples for total flavonoid content

The procedure as described for standard quercetin was followed to the each sample of 50 µl (three replicates). The results was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g) of the extract using the linear equation by plotting the standard calibration curve [28].

2.7. Determination of antioxidant activity

2.7.1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity

The *in-vitro* antioxidant activities of the extracts were determined by using the DPPH free radical scavenging assay as described by Demirkiran et al. (2013) [29]. It is a rapid, simple and inexpensive method. DPPH in oxidized form gives a deep violet color in methanol. An antioxidant compound donates the electron to DPPH thus causing its reduction and in reduced form its color changes from deep violet to yellow.

2.7.2. Preparation of DPPH solution (0.1 mM)

DPPH solution (0.1 mM) was prepared by dissolving 0.39 mg of DPPH in a volumetric flask, dissolved in methanol, and the final volume was made 100 ml and kept in dark place for further use.

2.7.3. Measurement of DPPH free radical scavenging activity

The extracts were dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 1 mg/ml (1000µg/ml). The antioxidant activity of the extracts was measured at a concentration of 500 μg/ml. The capacity of plant extracts (500 μg/ml) to directly react with and quench free radicals was evaluated as described by Demirkiran et al. (2013) [29]. The assay was performed in 96-well plates. Different concentrations of test samples of 0.01 mg/ml, 0.02 mg/ml, 0.04 mg/ml, 0.08 mg/ml, 0.16 mg/ml, 0.32 mg/ml were prepared from stock solution. The reaction mixture, containing 100 μL of DPPH and 100 μL of the diluted test sample, was incubated at 37 ^{o}C for 30 min in dark. After 30 minute, absorbance of the samples was measured at 517 nm using micro-plate reader. Ascorbic acid of the concentration 0.01 mg/ml, 0.02 mg/ml, 0.04 mg/ml, 0.08 mg/ml, 0.16 mg/ml, 0.32 mg/ml and 0.5mg/ml was prepared as standard and its absorbance was also taken at 517 nm. For DPPH test, ascorbic acid of 20 µg/ml was used as positive control and 50 % DMSO was used as negative control.

The percentage of the DPPH free radical scavenging activity was calculated by using the following equation:

$$\% \text{ Inhibition} = \left(\frac{A_{Control} - A_{Sample}}{A_{Control}}\right) \times 100$$

Where, $A_{Control}$ = Absorbance of the DPPH

A_{Sample} = Absorbance of the DPPH + sample

% Inhibition = Percentage of inhibition.

[DOR: 20.1001.1.2717204.2021.20.80.9.6]

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The radical scavenging activities of the extracts are expressed in terms of their IC_{50} values. The data were presented as mean values ± standard deviation (n = 3).

2.8. Determination of antidiabetic activity

2.8.1. Preparation of samples for antidiabetic activity

The stock solution of all plant extracts was prepared by dissolving 500 mg in 1 ml of dimethylsulphoxide (DMSO). Different concentrations of test samples of 1.56 mg/ml, 3.12 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml were prepared from stock solutions.

2.8.2. Pancreatic α-amylase inhibition assays (Starch-Iodine colour assay)

Screening of plant material for α-amylase inhibitors was carried out in a 96 well microtiter plate according to Xiao et al. (2006) [30] based on the starch-iodine test with a slight modification. The total assay mixture composed of 20 µl 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 20 µl 4 units of (Porcine pancreatic α -amylase) PPA solution and 20 µl plant extracts at concentration from 1.5625-25 mg/ml (w/v) were incubated at 37 °C for 10 min. Then 20 µl soluble starch (1 %, w/v) was added to each reaction well and incubated at 37 °C for 15 min. 1 M HCl (20 µl) was added to terminate the enzymatic reaction, followed by the addition of 100 µl of 5 mM iodine reagent. The colour change was noted and the absorbance was recorded at 620 nm on a 96 well microplate reader. The control reaction representing 100 % enzyme activity did not contain any plant extract. A dark-blue colour indicates the presence of starch; a yellow colour indicates the absence of starch i.e. complete degradation of starch while a brownish colour indicates partially degraded starch in the reaction mixture. The known PPA inhibitor, acarbose, was used a positive control at a concentration range of 1.56-25 mg/ml. In the presence of inhibitors from the extracts the starch added to the enzyme assay mixture is not degraded and gives a dark blue colour complex whereas no colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolysed by α-amylase.

The other quantifiers were calculated as follows:

$$\% \text{ Relative enzyme activity} = \left(\frac{\text{Enzyme activity of test}}{\text{Enzyme activity of control}}\right) \times 100$$

% Inhibition in the α-amylase activity = (100 - % Relative enzyme activity)

Where, Enzyme activity of test = Total starch – Remaining Starch

Enzyme activity of control = Starch only – (Starch + Enzyme)

2.9. Brine shrimp toxicity bioassay

The toxicity test was performed by using brine shrimp assay following the standard protocol given by Meyer et al. (1982) [31]. The artificial sea water was prepared by dissolving 15 mg of sodium chloride, 0.45 g of potassium chloride, 0.55 g of calcium chloride and 1.76 g of magnesium sulphate in distilled water to make 500 ml solution. Brine shrimp nauplii hatching tank was filled with water and the eggs sprinkled in to the covered part of the tank. The bioactive compounds present in the extracts show toxicity towards brine shrimp larvae. This method evaluates the toxicity of extracts towards the nauplii by determining the LC_{50} (µg/ml). Compounds of LC_{50} less than 1000 ppm are considered as pharmaceutically active. LC_{50}

[DOR: 20.1001.1.2717204.2021.20.80.9.6]
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value is the lethal concentration required to kill 50 % of the shrimps. It is calculated as,

$$\alpha = \frac{1}{n}[\Sigma\, y - \beta\, \Sigma\, x]$$

$$\beta = \frac{\Sigma xy - \frac{\Sigma x\, \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

where, 'n' is the number of replicate (here three), 'x' is the log of constituents in mg/ml (log10, log100, and log1000 for three dose level respectively). 'y' is the prohibit for average survivor of all replicates.

From probit regression,

$$Y = \alpha + \beta X$$

$$X = \frac{(Y - \alpha)}{\beta}$$

Where Y is constant having value 5 calculating LC_{50} values

Thus,

$$LC_{50} = \text{antilog } X$$

Also,

Percentage mortality (% M) = $\frac{\text{Number of dead nauplii}}{\text{Total number of nauplii}} \times 100$

3. Results

3.1. Total Phenol Contents (TPC)

Total phenol contents in different methanol extracts were determined by Folin-Ciocalteu (F-C) method using gallic acid as the standard. The absorbance values of each extract at different concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 60 µg/ml, 70 µg/ml, 80 µg/ml) were recorded at 765 nm by using micro-plate reader. The TPC in the plant extracts taken under study was calculated by using the regression equation y = 0.0374x, R^2 = 0.9641 of the curve obtained from the above graph followed by the formula cV/m and expressed as mg GAE per g of extract in dry weight.

The total phenol content of all selected medicinal plant extracts showed varied data ranging from 4.31 ± 0.147 to 10.2 ± 0.343 mg GAE/g respectively. The total phenol content of all extract lied between these two extremes. TPC values were higher in *Lantana camara* and lowest in *Cupressus sempervirens.* TPC value of *Lantana camara* was 10.20 ± 0.343 mg GAE/g. Similarly, TPC value of *Ageratina adenophora* was 4.70 ± 0.059 mg GAE/g and that of *Cupressus sempervirens* was 4.31 ± 0.147 mg GAE/g (Table 1).

Table 1. Total phenol content (TPC) of different plant extracts

S.N.	Name of plants	TPC (mg GAE/g Extract)			Mean ± SD
		C1	C2	C3	
1	*Ageratina adenophora*	4.77	4.66	4.67	4.70± 0.059
2	*Cupressus sempervirens*	4.15	4.34	4.44	4.31 ± 0.147
3	*Lantana camara*	9.86	10.55	10.19	10.20 ± 0.343

3.2. Total flavonoid contents (TFC)

Total flavonoid content of the extracts was calculated from the regression equation of the calibration curve y = 0.005x + 0.0626, R^2 = 0.9996 and expressed as mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g). The TFC values also showed similar trends with that of TPC values. TFC value of *Lantana camara* was 1.872 ± 0.16 mg QE/g. Similarly, TFC value of *Ageratina adenophora* was 0.645 ± 0.122 mg QE/g and that of *Cupressus sempervirens* was 0.379 ± 0.046 mg QE/g (Table 2).

[DOR: 20.1001.1.2717204.2021.20.80.9.6]
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Table 2. Total flavonoid content (TFC) of different plant extracts

S.N.	Name of plants	TFC (mg QE/g Extract)			Mean ± SD
		C1	C2	C3	
1.	*Ageratina adenophora*	0.51	0.75	0.67	0.65 ± 0.122
2.	*Cupressus sempervirens*	0.43	0.35	0.35	0.38 ± 0.046
3.	*Lantana camara*	1.71	2.03	1.87	1.87 ± 0.160

3.3. Antioxidant activity

Antioxidant activity of the methanol extracts was determined by DPPH free radical scavenging assay. The reducing power of the plant extracts was evaluated on the basis of IC_{50} values, the amount required to scavenge 50 % DPPH free radicals. The IC_{50} value of the plant extract along with the standard ascorbic acid is shown in Table 3, Fig. 2.

In the present research, ascorbic acid was used as standard and different extracts showed variable antioxidant properties. The IC_{50} values of standard ascorbic acid was 17.46 ± 0.822 µg/ml. The antioxidant potential is in an inverse relation with IC_{50} value, lower value of IC_{50} indicates high antioxidant potential. Among these, methanol extracts of *Lantana camara* showed the strongest DPPH radical scavenging activity. The IC_{50} values of *Lantana camara* was 106.18 ± 11.390 µg/ml. Similarly, the IC_{50} values of methanol extract of *Ageratina adenophora* and *Cupressus sempervirens* were found to be 422.20 ± 18.580 µg/ml and 218.07 ± 4.405 µg/ml.

3.4. Antidiabetic activity

α-Amylase inhibitory activity of plant extract was determined using quantitative starch-iodine method. The IC_{50} value of the plant extract along with the standard acarbose is shown in Table 3 and Fig. 3. Yet several studies showed the antidiabetic potential of these plants, no previous reports has been given on the mechanism by which it exerts this effect. In the present study methanol extract of different plants were investigated for *α*-amylase activity. IC_{50} value of standard Acarbose was found to be 1.25 ± 0.007 mg/ml. Among these plants, methanol extracts of *Ageratina adenophora* showed highest α-amylase inhibitory activity with IC_{50} value of 1.84 ± 0.007 mg/ml. Similarly, methanol extracts of *Lantana camara* and *Cupressus sempervirens* has the IC_{50} value of 2.75 ± 0.046 mg/ml and 4.86 ± 0.209 mg/ml respectively. Among the selected medicinal plants, *Ageratina adenophora* showed the highest percentage inhibition of α-amylase.

Table 3. Comparison of antioxidant activity IC_{50} values for different plants extracts with standard ascorbic acid

S.N.	Sample	IC_{50} ± SD (µg/ml)
1.	Standard (ascorbic acid)	17.46 ± 0.822
2.	*Ageratina adenophora*	422.20 ± 18.580
3.	*Cupressus sempervirens*	218.07 ± 4.405
4.	*Lantana camara*	106.18 ± 11.390

[DOR: 20.1001.1.2717204.2021.20.80.9.6]
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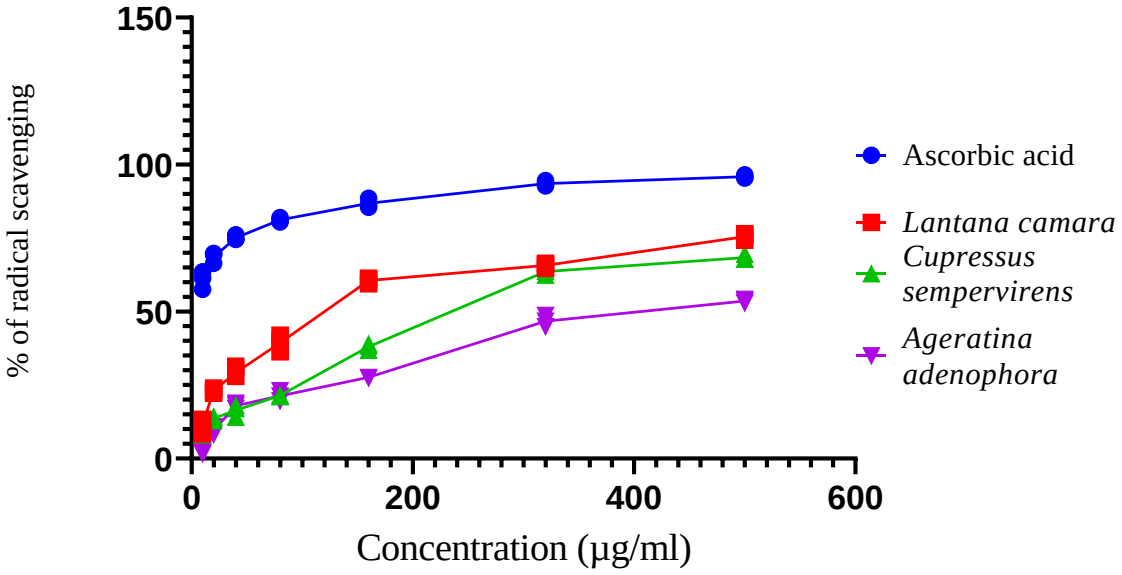

Fig. 2. Plot of percentage radical scavenging versus ascorbic acid (standard) and plant extracts

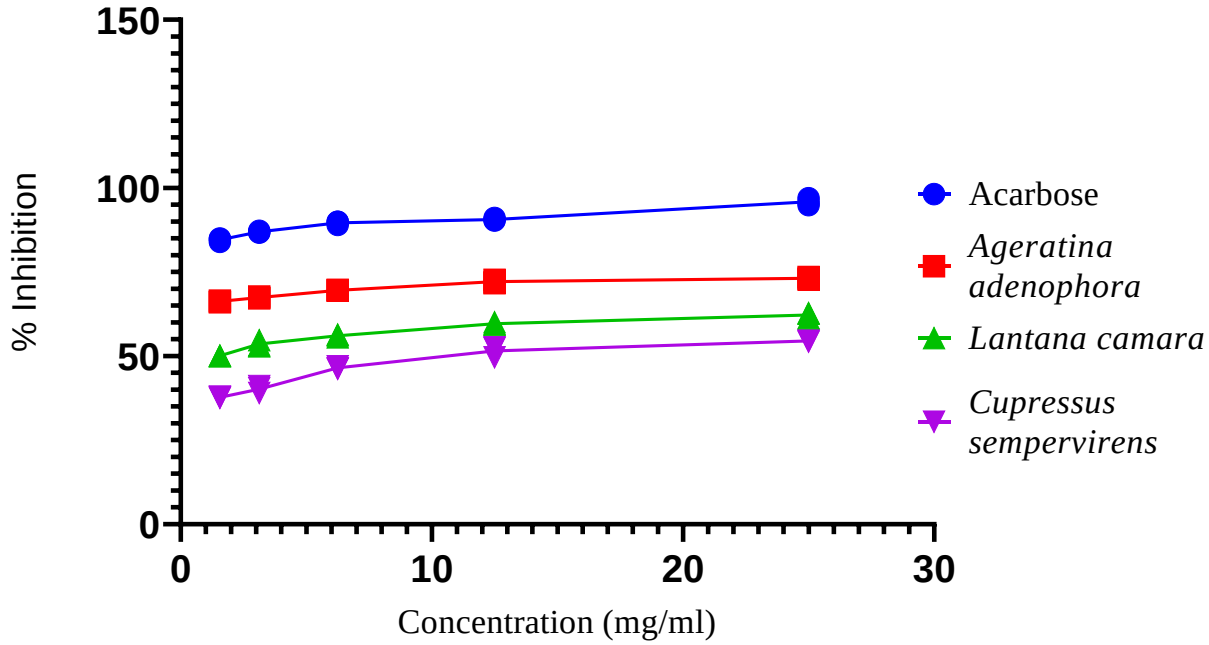

Fig. 3. Plot of α-amylase inhibition activity between Acarbose and different plant extracts

3.5. Brine shrimp toxicity

The methanol extract was screened for *in vitro* cytotoxic activity by brine shrimp lethality test. The result showed that the extract of *Ageratina adenophora* was found to be cytotoxic against brine shrimps as shown by its LC_{50} value of 833.68 µg/ml as its LC_{50} value was found to be lower than 1000 µg/ml. In addition, the extract of

Cupressus sempervirens and *Lantana camara* showed positive results, indicating that the samples are biologically active and do not possess toxicity. Plant extract resulting in LC_{50} less than 1 mg/ml are considered toxic to the larvae [32]. This variation in the results may be due to the altitude variations of collected plant or may be due to laboratory conditions.

Table 4. Toxicity of plant extracts

S.N.	Sample	LC_{50} (µg/ml)
1	*Ageratina adenophora*	833.68
2	*Cupressus sempervirens*	1963.36
3	*Lantana camara*	2624.22

4. Discussion

The TPC value is lower than that of the reported by Swamy et al. [33], where the TPC value of methanol extracts of *Lantana camara* was found to be 92.8 ± 1.7 mg GAE/g. Similarly, according to Tripathi et al. [34], the TPC value of methanol extract of *Ageratina adenophora* were found in the range of 30.71 ± 0.16-68.51 ± 0.58 mg GAE/g. Similarly, in another study, the TPC value of methanol extract of *Cupressus sempervirens* was 4.35 mg GAE/g [35]. Similarly, Phuyal et al. [36], has reported TPC in the cultivated fruit extracts of *Z. armatum* was 226.3 ± 1.14 mg.GAE/g and that for wild fruit was 185.02 ± 2.15 mg GAE/g. The results was found higher as compared to the results of present study. The difference between the values could be due to the variations in genetic backgrounds, environment factors, cultural factors, agronomic practices as well [37].

Phenol based compounds have been known to have antioxidant properties because of their free radical scavenging properties, hence act as a free radical terminator in chain reaction. It has been informed that extract containing large amount of polyphenol content possesses a greater antioxidant activity. These H-donating phenol compounds react with ROS and RNS and breaks the free radical generation cycle in a termination reaction [38].

The total flavonoid content was lesser than (26.5 ± 0.5 mg RE/g) were observed in the methanol leaf extract of *Lantana camara* [33]. In a previous study, the total flavonoid content of methanol extracts of *Ageratina adenophora* were found in the range of 18.93 ± 1.76 to 32.25 ± 1.15 mg QE/g [34]. The total flavonoids content of *Cupressus sempervirens* was 9.5 (mg QE/g extract) [35], which is quite higher than that of the present study.

The antioxidant potential of *Ageratina adenophora, Cupressus sempervirens,* and *Lantana camara* has been evaluated by various previous studies [39-44]. The IC_{50} values of methanol extract of *Lantana camara* (106.179 ± 11.390 µg/ml) was much lesser than the quantity (165 µg/ml) as reported by Swamy et al. [33] from the methanol extract of *Lantana camara* collected from the tropical region of Malaysia.

The excessive formation of free radicals in the body might cause various cell damage and incites different issues. However, antioxidants from the natural origin like plant and plant based products can repair and neutralize these free radicals and thereby, these antioxidants can be very useful in preventing various disorders. The results reflect that the plant studied above can act as a very good alternative in the field of medicine based on antioxidant property of natural products chemistry.

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As reported by Boussoussa et al. [42], the phenol and saponins extracts for both seeds and cones inhibited enzymatic activity of α-amylase under *in vitro* starch digestion bioassay with the IC_{50} values ranged from 0.49 to 1.12 mg/ml. In the previous study, methanol extracts at 100 µg/ml exhibited α-amylase inhibitory potential of 36.3 ± 0.6 % in comparison to standard drug, acarbose with 62.4 ± 0.4 % [45]. Previously it has not been well reported for α-amylase inhibitory activity in *Ageratina adenophora* extract.

α-Amylase inhibitors delay the breakdown of carbohydrate in the small intestine and lower the postprandial blood glucose excursion in a diabetic patient. One of the strategies adopted to lower the levels of postprandial hyperglycemia involves the inhibition of carbohydrate digestive enzymes such as α-amylase in the gastrointestinal glucose absorption. This is an effort to search for alternative source of drug from medicinal plants with increased potency and lesser side effect than existing drugs [46].

Synthetic drugs like acarbose, miglitol, metformin, etc. are used for the clinical treatment of type 2 diabetes. However, these drugs are reported to cause various side effects such as abdominal distention, flatulence and diarrhoea owing to the excessive inhibition of pancreatic α-amylase, which results in abdominal bacterial fermentation of undigested carbohydrates in the colon. Hence, at present there is an increasing interest among the scientists to find out the natural sources of *α*-amylase and *α*-glucosidase inhibitors for the dietary management of type 2 diabetes [47].

5. Conclusion

Findings of this study provides some scientific support for the use of traditional medicinal plants in the treatment of several ailments. Total phenol contents, total flavonoid contents, and antioxidant properties of *Ageratina adenophora, Cupressus sempervirens* and *Lantana camara* were considerable. Among these plants, *Ageratina adenophora* showed a little cytotoxic effect even though it was found potent for α-amylase inhibitor with inhibition near to standard acarbose. The difference in TPC, TFC and antioxidant properties is attributed to altitude and geographical condition along with various environmental and habitat conditions such as sunlight, UV radiation, growing temperature and season, altitude, maturity at harvest, stress, and many microenvironmental conditions. This factor in turn would affect the quality of plant material and hence influence the data. The findings of this study suggested that these plants can be used as the potential sources for natural antioxidant and bioactive chemical constituents. It also provides scientific support to the traditional claims. Similarly, the plant extract with low IC_{50} value can contribute for the effective treatment of diabetes by decreasing high blood sugar. Further studies can be directed towards isolation, purification, characterization and standardization of the bioactive constituents responsible for antioxidant and antidiabetic properties to authenticate their further use as well as validate their traditional uses. Similarly, the more *in-vitro* and *in-vivo* biological activities of these plant extracts will support to isolate the active natural chemical compound that acts as drug candidate in future drug discovery process. This is the first attempt that we added some scientific validation in these traditionally used medicinal plants from the aspect of antioxidant and antidiabetic property.

Author contributions

JK: Study, design, data analysis and interpretation, experimental analysis and

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preparation of manuscript draft; KRS: Original idea presentation, study supervision, final approval of the version to be published.

Conflicts of Interest

The authors declare that there is no conflicts of interests regarding the publication of this paper.

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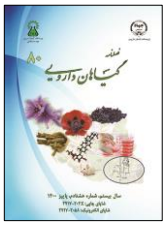

مقاله تحقیقاتی

ارزیابی ترکیبات فیتوشیمیایی و فعالیت آنتی‌اکسیدانی، ضددیابتی و کشندگی میگوی آب‌شور برخی از گیاهان دارویی روییده در نپال

جونو کاپالی، خاگا راج شارما*

گروه مرکزی شیمی، دانشگاه تریبووان، کرتیپور کاتماندو، نپال

اطلاعات مقاله

گل‌واژگان:
Ageratina adenophora
زربین
شاه‌پسند درختچه‌ای
آنتی‌اکسیدانی
ضددیابتی
فیتوشیمیایی

چکیده

مقدمه: تولید شدید رادیکال‌های آزاد در بدن انسان باعث آسیب مستقیم به مولکول‌های بیولوژیکی می‌شود که منجر به انواع مختلف بیماری‌ها می‌گردد. آنتی‌اکسیدان‌های طبیعی یا مصنوعی مستقیماً یا از تولید رادیکال‌های آزاد در بدن انسان جلوگیری می‌کنند و یا با محدودکردن انتشار و خنثی کردن آن‌ها باعث محافظت از سیستم ایمنی می‌گردند. **هدف:** هدف از مطالعه بررسی کمی محتوای تام فنلی و فلاونوئیدی، فعالیت آنتی‌اکسیدانی و ضددیابتی و آزمون سمیت عصاره متانولی قسمت‌های هوایی گیاهان دارویی استفاده شده در طب سنتی مانند *Ageratina adenophora*، زربین و شاه‌پسند درختچه‌ای می‌باشد. **روش بررسی:** محتوای تام فنلی با روش فولین- سیوکالتیو و محتوای تام فلاونوئیدی با سنجش کلرید آلومینیوم اندازه‌گیری شد. فعالیت مهاری آلفا- آمیلاز، برای ارزیابی فعالیت ضددیابتی عصاره گیاهان مورد استفاده قرار گرفت. **نتایج:** از بین سه گونه مطالعه شده محتوای تام فنلی و فلاونوئیدی شاه‌پسند درختچه‌ای به ترتیب با ۱۰/۲۰ ± ۰/۳۴۳ میلی‌گرم گالیک اسید در گرم عصاره و ۱/۸۷ ± ۰/۱۶۰ میلی‌گرم کوئرستین در گرم عصاره از سایر گیاهان بیشتر بود. فعالیت روبش رادیکالی DPPH عصاره متانولی شاه‌پسند درختچه‌ای با IC_{50} برابر با ۱۰۶/۱۸ ± ۱۱/۳۹۰ میکروگرم در میلی‌لیتر از دیگر گیاهان قوی‌تر بود. به علاوه، عصاره متانولی *Ageratina adenophora* فعالیت مهار آلفا- آمیلازی بیشتری با IC_{50} برابر با ۱/۸۴ ± ۰/۰۰۷ میلی‌گرم در میلی‌لیتر از خود نشان داد. همچنین این گیاه در تست بررسی سمیت میگوی آب‌شور با LC_{50} برابر با ۸۳۳/۶۸ میکروگرم در میلی‌لیتر، سمیت از خود نشان داد. **نتیجه‌گیری:** این تحقیق نشان می‌دهد که گیاهان دارویی که به‌طور سنتی مورد استفاده قرار می‌گیرند، منابع غنی و قوی ترکیبات آنتی‌اکسیدانی و ضددیابتی طبیعی هستند که ممکن است کاندیدای قوی داروهای طبیعی در فرآیند کشف دارو در آینده باشند.

مخففها: DPPH، ۱،۱- دی فنیل-۲- پیکریل هیدرازیل؛ GAE، معادل گالیک اسید؛ QE، معادل کوئرستین؛ TPC، محتوای تام فنلی؛ TFC، محتوای تام فلاونوئیدی؛ IC_{50}، میانگین غلظت مهارکننده؛ LC_{50}، میانگین غلظت کشنده؛ FCR، معرف فولین- سیوکالتیو

* نویسنده مسؤول: khaga.sharma@cdc.tu.edu.np

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