Estimation of phenolic content, flavonoid content, antioxidant, and alpha-amylase inhibitory activity of some selected plants from Siraha district, Nepal

Numer Alam, Khaga Raj Sharma*

Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal. Email: khagaraj_sharma33@yahoo.com

Received: 28 December 2019, Revised and Accepted: 25 January 2020

ABSTRACT

OBJECTIVES: The purpose of this research was to estimate the phenolic content, flavonoid content, antioxidant, antibacterial, and alpha-amylase inhibitory activity of some selected plants such as Anethum sowa, Trigonella foenum-graecum, Lepidium sativum, Cuscuta reflexa, Eclipta alba, Lesca cephalotes, and Tinospora cordifolia collected from Siraha district of Nepal using in vitro studies.

METHODS: Methanol extracts of these medicinal plants were prepared by cold percolation method. Preliminary phytochemical screening was performed by color differentiation method. Total phenolic and flavonoid content were estimated by Folin–Ciocalteu reagent method and aluminum chloride colorimetric method. Antioxidant potential was evaluated by 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. Furthermore, the alpha-amylase enzyme inhibitory activity was studied using starch as a substrate, pancreatic alpha-amylase as the enzyme, and acarbose as standard.

RESULTS: Phytochemical screening showed that the plant extracts were found a rich source of secondary metabolites. The phenolic content estimation showed T. foenum-graecum 939.76±0.01, L. sativum 551.63±0.02, A. sowa 306.34±0.06, L. cephalotes 233.19±0.03, T. cordifolia 211.76±0.02, E. alba 202.67±0.02, and C. reflexa Roxb. 145.09±0.09 mg milligram gallic acid equivalent per gram. The flavonoid content estimation showed T. foenum-graecum 852.07±0.11, L. sativum 553.81±0.05, E. alba 322.13±0.02, A. sowa 329.02±0.05, L. cephalotes 164.93±0.02, and C. reflexa Roxb. 146.37±0.00 mg milligram quercetin equivalent per gram. The antioxidant potential showed by E. alba LC₅₀ 33.48±0.02 to A. sowa 47.57±1.09 µg/ml. The alpha-amylase inhibitory activity showed by A. sowa 76.78±2.00–E. alba 777.36±9.66 µg/ml. The result of brine shrimp toxicity showed LC₅₀ value >1000 µg/ml. Among the seven plant extracts, only the plant extract of E. alba showed a zone of inhibition 14 mm against Staphylococcus aureus.

CONCLUSIONS: The plant extract of T. foenum-graecum showed the highest phenolic content, and T. cordifolia showed the highest flavonoid content. The highest antioxidant potential exhibited by E. alba and the highest alpha-amylase inhibition activity showed by A. sowa. The plant extract of E. alba showed moderate antibacterial activity against S. aureus. All plant extracts were found non-toxic against brine shrimp larvae although further study is needed to assess its mechanism of action.

KEYWORDS: Antioxidant, alpha-amylase, gallic acid, quercetin, plant extract.

INTRODUCTION

Nepal is rich in all the three levels of biodiversity, namely, species diversity, genetic diversity, and habitat diversity. In Nepal, the use of different parts of several medicinal plants to cure specific diseases has been in vogue since ancient times [1]. The use of herbal medicines in Asia represents a long history of human interaction with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infection diseases. Phytochemical from plants as safe and broadly effective alternatives with the less adverse effect is beneficial for biological activities such as anticancer, antimicrobial, antioxidant, analgesic, and wound healing [2,3]. Oxidation reaction can produce the free radicals, which starts chain reactions that damage the cells. Antioxidants are natural or synthetic substances that may prevent or delay oxidative cell damage [4]. Antioxidant plays the role on prevention of diabetes and other diseases too [5]. The antioxidant from natural sources neutralizes the radicals formed in the human body by oxidative stress. The plants secondary metabolites have pharmacological properties [6,7]. Today, numerous antimicrobial agents exist to treat a wide range of infections. The development of new anticancer and antimicrobial therapeutic agents from natural sources is one of the fundamental goals in medicinal chemistry [8].

Phenolic and flavonoid compounds are the main class of secondary metabolites in the plants and are divided into phenolic acids and polyphenols. The antioxidant activity of phenolic and flavonoid compounds is attributed to the capacity of scavenging free radicals, donating hydrogen atoms, electrons, or chelate metal cation [9]. Medicinal plants have been using since many years for the primary cure of different diseases in all most all countries of the world. Among the several diseases, diabetes is the seven leading causes of death. Hence, the search of potent natural compounds with high antioxidant potential is a urgent need. Due to the chronic hyperglycemic disturbances of carbohydrate, fat, and protein metabolism resulting from defect in insulin secretion, its action, and both causes diabetes mellitus [5]. The number of diabetes patients will be increased from 171 million from the year 2000 to 366 million or more by the year 2030 [5]. Mainly diabetes is of two types, Types 1 and II. Type 1 diabetes is due to insulin-deficient causes when pancreatic beta-cell does not produce insulin, and Type II is insulin resistance. There are several factors of causing diabetes, although both genetic and environmental factors such as obesity, changing lifestyle eating habits, and lack of exercise play a significant role [5]. Long-term using oral hypoglycemic agents and insulin secretion agents lead to increase blood sugar level, drug resistance and affects the immune system of the body. To avoid such problems, the natural antidiabetic agents can be used for good management of diabetes [10]. The phytoconstituents reported to
have antidiabetic activity, including carbohydrate hydrolyzing enzyme inhibition and antioxidant activity. α-amylase secreted by the pancreas and salivary gland have a significant role in breaking down starch and glycogen [5]. The α-amylase is also found in microorganisms, plants, and higher organisms. α-amylase catalyzes the hydrolysis of starch into a mixture of several oligosaccharides. Rapid breakdown of starch by α-amylase may lead to increase blood sugar level, i.e., post-prandial hyperglycemia [5]. Inhibition of α-amylase activity decreases the blood glucose level by delaying the carbohydrate hydrolysis and absorption in the blood.

The aim of the present study was to evaluate the antioxidant activity, estimation of phenolic and flavonoid content, antibacterial activity, α-amylase inhibition activity, and toxicity of some plants collected from Sinha district of Nepal.

METHODS

Collection and identification of plant materials

The plant material was collected from Madar Village Development Committee of Sinha district Nepal. The plants were identified at the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu. The list of plants with common name, family name, and parts used is shown in Table 1.

Extract preparation

The collected parts of plants were washed with tap water to remove the contaminants. The shade dried plant parts were grinded into powder form in an electric grinder and stored in a clean plastic bag at a low temperature until the used. The phytochemicals present in the powdered were extracted by cold percolation method using methanol as a solvent. Powdered plant parts (150 g) were kept separately in the clean and dry conical flasks. Methanol (400 ml) was added to each flask and kept for each 72 h with frequent shaking. The mixtures were decanted and filtered with the help of cotton plug and thus obtained filtrates were concentrated with the help of rotary evaporator. The concentrated filtrates were kept in a beaker wrapping with aluminum foil containing small pores to facilitate the evaporation of the solvent. These extracts were stored at 4°C until performing the biological activities.

Preliminary phytochemical analysis

The preliminary phytochemical analysis was performed by color differentiation method adopting the protocol of Harbone with some modifications [11].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The ability of different plant extracts to scavenge DPPH free radicals was estimated by the protocol given by Jamuna et al. [12]. The percentage of DPPH free radical scavenging activity was calculated using the following equation,

\[
\text{Radical scavenging} (\%) = \left(1 - \frac{A_s}{A_o}\right) \times 100
\]

where, \(A_o\) = Absorbance of the control (DPPH solution+methanol), \(A_s\)=Absorbance of test sample.

Table 1: List of some selected medicinal plants

| Plant sample           | Common name | Family            | Parts used |
|------------------------|-------------|-------------------|------------|
| Anethum sowa           | Suwa        | Umbelliferae      | Leaf, stem |
| Trigonella foenum-graecum | Methi saag   | Fabaceae          | Leaf       |
| Lepidium sativum       | Chamsur saag | Brassicaceae      | Leaf       |
| Cuscuta reflexa         | Akash beli  | Convolvulaceae    | Whole part |
| Eclipta alba            | Bhringraj   | Asteraceae        | Stem, leaf |
| Leucas cephalotes       | Guma        | Lamiaceae         | Stem, leaf, flower |
| Tinospora cordifolia    | Gario       | Menispermacae     | Stem, leaf |

The \(IC_{50}\) value is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals. \(IC_{50}\) values were calculated using the inhibition curve by plotting extract concentration versus the corresponding scavenging effect.

Total phenolic content (TPC) estimation

The total phenol content of all selected plant extracts was estimated using Folin-Ciocalteu reagent using gallic acid as standard based on oxidation-reduction reaction. The protocol adopted for the estimation of total phenol content was Kim et al. 2012 [13]. TPC content was expressed in milligram gallic acid equivalent per gram of dry weight (mg GAE/g) of extract using the formula, \(C=cV/M\) where, \(C=\)total phenolic content, \(c=\)concentration of gallic acid established from the calibration curve in mg/ml, \(V=\)Volume of extract in ml, \(M=\)Weight of plant extract.

Total flavonoid content (TFC) estimation

The TFC of the plant extracts was determined according to the aluminum chloride colorimetric method using quercetin as standard [14]. The stock solution of quercetin (10,000 µg/ml) was prepared by dissolving 10 mg of quercetin in 1 ml of methanol. Then, various concentrations of quercetin such as 10, 25, 50, and 100 µg/ml were prepared by serial dilution of the stock solution. An aliquot of 0.5 ml quercetin of each concentration in methanol was pipetted out into 20 ml test tube containing 2 ml of double-distilled water. Then, at the 0 time, 0.15 ml of 5% NaNO₂ was added to the test tube. After 5 min, 0.15 ml of 10% AlCl₃ was added, and after 6 min, 1 ml of 1 M NaOH were added to the mixture. Immediately, the total volume of the mixture was made up to 5 ml by adding 1.2 ml of distilled water and mixed thoroughly. Finally, the absorbance of the pink color mixture was measured at 510 nm using a spectrophotometer at blank solution containing all the reagents except quercetin. All the experiments were carried out in triplicate. The average absorbance values obtained at different concentrations of quercetin were used to plot the calibration curve.

The TFC in extract was expressed as milligrams quercetin equivalent per gram of dry weight (mg QE/g) which was calculated in all the extracts separately using the following equation:

\[
C=cV/m
\]

Where, \(C=\)TFC mg QE/g, \(c=\)Concentration of quercetin established from the calibration curve in mg/ml, \(V=\)Volume of extract in ml, \(m=\)Weight of plant extract.

Statistical analysis

Data were recorded as a mean of three determinations of absorbance for each concentration, from which the linear correlation coefficient (R²) value was calculated. The regression equation is given as;

\[
Y=mx+c
\]

where, \(Y=\)Absorbance, \(x=\)Concentration of plant extracts and \(c=\)Intercept.

Using this regression equation concentration of extracts was calculated. Thus, with the calculated value of the concentration of each extract, the total phenolic content was calculated.

α-Amylase inhibitory activity

The antidiabetic activity of plant extracts was calculated by α-amylase inhibition assay proposed by Kusano et al. [15] with some modification. The undigested starch due to enzyme inhibition was detected through the blue starch iodine complex at 630 nm in spectrophotometer. The enzyme solution was prepared by dissolving 3 µg/ml α-amylase in 20 mM phosphate buffer pH 6.9. 400 µl of the substrate was pre-incubated with 200 µl of varying concentrations (640 µg/ml, 320 µg/ml, 160 µg/ml, 80 µg/ml, and 40 µg/ml) of plant extracts and acarbose separately at 37°C for 5 min. After this, 200 µl of α-amylase solution was added.

\[
\text{Radical scavenging} (\%) = \left(1 - \frac{A_s}{A_o}\right) \times 100
\]
Antibacterial activity

Antibacterial activity of the plant extracts was performed by the agar well diffusion method. Effectiveness of antimicrobial substance was evaluated by determination of zone of inhibition (ZOI) by adopting the protocol Cavalieri et al. [16].

Brine shrimp bioassay

Brine shrimp, *Artemia salina* is a tiny crustacean. The eggs of brine shrimp are readily available at low cost, and they remain viable for years in the dry state. On being placed in a brine solution, the eggs hatch within 48 h providing large number of larvae. These larvae are used in this assay for toxicity test. This method is rapid, inexpensive, simple, and in-house approach for screening and monitoring physiologically active plant extracts. It determines the IC_{50} value (S) (µg/ml) for the crude extract (s). Compounds/extracts having IC_{50} values <1000 ppm (µg/ml) are considered as pharmacological active. The assay was carried out by adopting the standard protocol of Meyer et al. [17].

RESULTS AND DISCUSSION

Quantitative estimation showed that the plant extracts are the rich sources of secondary metabolites with high yield percentage. The results of the yield percentage are shown in Table 2.

Phytochemical screening

The results of the phytochemical analysis are shown in Table 3. Most of the plant extracts are found as the rich source of secondary metabolites such as alkaloids, coumarins, flavonoids, glycosides, polyphenols, flavonoids, quinones, reducing sugars, and saponins.

Antioxidant activity

The antioxidant activity of each plant extract was measured using DPPH free radical. DPPH is scavenged by antioxidants through the donation of proton, forming the reduced DPPH. DPPH solutions show a strong absorbance band at 517 nm, appearing as deep violet color. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm.

The DPPH radical assay was performed for each plant extract using ascorbic acid as standard according to the standard procedure. In this assay, the mixture of DPPH with different concentrations of extract solution and ascorbic acid was separately incubated at room temperature, and absorbance was recorded at 517 nm by a spectrophotometer. The comparison of percentage radical scavenging between different plant extracts and ascorbic acid as standard is shown in Fig. 1.

The antioxidant potential of the plants extracts is influenced by the various factors and largely depends on both the composition of the extract and the analytical test system. The number of the plant constituents had been proven for the free radical scavenging or antioxidant activities, among the phenolic compounds are very important for the plant constituents. Total phenolic compounds of the plants and antioxidant activities have mutual relationships due to the presence of the hydroxyl group, which has the scavenging ability. By donating an electron, a phenolic compound can also scavenge the hydrogen peroxide and neutralize it to the water. The antioxidant potential of different plants was determined using DPPH assay and ascorbic acid as a standard compound (IC_{50} 39.85±0.025µg/ml). Maximum antioxidant potential was found in *Eclipta alba* (IC_{50} 33.48±0.823) and minimum in *Leucas cephaleotes* (IC_{50} 52.86±1.80). These results are shown in Table 4. Similarly, results found that all the extracts exhibited maximum percentage of free radical scavenging activity at a dose of 100 µg/ml are shown in Table 5 from the literature review it is found that the antioxidant potential radical scavenging of *Cuscuta reflexa* has 76.38% and *L. cephaleotes* has 98.09±0.69 [18,19].

**Table 2: Percentage yield of different plant extracts**

| Plant sample          | Quantity of sample (g) | Quantity of extract (g) | Percentage yield |
|-----------------------|------------------------|-------------------------|-----------------|
| *Anethum sowa*        | 80                     | 11                      | 13.75           |
| *Trigonella foenum-graecum* | 80                 | 9                       | 11.25           |
| *Lepidium sativum*    | 80                     | 12                      | 15              |
| *Cuscuta reflexa*     | 100                    | 12                      | 12              |
| *Eclipta alba*        | 100                    | 14                      | 14              |
| *Leucas cephaleotes*  | 100                    | 10                      | 10              |
| *Tinospora cordifolia*| 120                    | 15                      | 12.5            |

**Table 3: Phytochemical screening of methanol extract**

| Group of compounds | AB1 | AB2 | AB3 | AB4 | AB5 | AB6 | AB7 |
|-------------------|-----|-----|-----|-----|-----|-----|-----|
| Alkaloid           | +   | +   | +   | +   | +   | +   | +   |
| Coumarins         | +   | +   | +   | +   | +   | +   | +   |
| Flavonoids        | +   | +   | +   | +   | +   | +   | +   |
| Glycosides        | +   | –   | +   | –   | –   | +   | +   |
| Polyphenols       | –   | +   | –   | +   | +   | +   | +   |
| Quinones          | –   | +   | +   | +   | +   | +   | +   |
| Reducing sugars   | –   | –   | –   | –   | +   | +   | +   |
| Saponins          | +   | +   | +   | –   | –   | +   | +   |
| Terpenoids        | +   | –   | +   | +   | +   | +   | +   |

| Presence and -- Absence, AB1: *Anethum sowa*, AB2: *T. foenum-graecum*, AB3: *Lepidium sativum*, AB4: *Cuscuta reflexa*, AB5: *Eclipta alba*, AB6: *Leucas cephaleotes*, AB7: *Tinospora cordifolia* |

**Table 4: Comparison of IC_{50} values of different plant extracts with standard ascorbic acid**

| Plant sample/standard | IC_{50} (µg/ml) |
|-----------------------|-----------------|
| Ascorbic acid         | 39.85±0.025     |
| *Anethum sowa*        | 47.62±1.095     |
| *Trigonella foenum-graecum* | 45.71±0.53     |
| *Lepidium sativum*    | 41.80±3.38      |
| *Cuscuta reflexa*     | 47.25±1.44      |
| *Eclipta alba*        | 33.84±0.823     |
| *Leucas cephaleotes*  | 52.86±1.80      |
| *Tinospora cordifolia*| 36.08±3.024     |
Table 5: Percentage of radical scavenging with different concentration of plant extracts

| Concentration (µg/ml) | Absorbance | Percent scavenging | Mean | Mean±standard deviation |
|-----------------------|------------|--------------------|------|-------------------------|
|                       | Abs1 | Abs2 | Abs3 | S1  | S2  | S3 |                  |                      |
| *Anethum sowa*        |      |      |      |     |     |    |                  |                      |
| 20                    | 1.066| 1.080| 1.072| 59.2507 | 61.46709 | 59.0214 | 59.9133 | 59.91±0.02 |
| 40                    | 1.073| 1.065| 1.059| 58.9831 | 59.28099 | 59.5183 | 59.26±0.26 | 59.26±0.26 |
| 60                    | 1.01 | 1.015| 0.953| 61.39144 | 61.20031 | 63.5703 | 62.05±0.40 | 62.05±1.31 |
| 80                    | 1.007| 0.965| 1.004| 61.50612 | 63.1162 | 61.6208 | 62.07±0.95 | 62.07±0.89 |
| 100                   | 1.012| 0.898| 0.966| 61.31498 | 65.67273 | 63.07339 | 63.35±0.372 | 63.35±2.19 |
| *Trigonella foenum-graecum* |     |      |      |     |     |    |                  |                      |
| 20                    | 1.078| 1.049| 1.050| 58.79205 | 59.9006 | 59.86239 | 59.5138 | 59.51±0.02 |
| 40                    | 1.046| 1.018| 1.034| 60.0152 | 61.0853 | 60.4740 | 60.5247 | 60.52±0.35 |
| 60                    | 0.963| 0.943| 0.975| 63.18880 | 63.952 | 62.7293 | 63.2900 | 63.29±0.7 |
| 80                    | 0.932| 0.920| 0.986| 64.3730 | 64.8318 | 62.3088 | 63.837 | 63.83±1.45 |
| 100                   | 0.907| 0.905| 0.911| 65.3287 | 65.4052 | 65.1785 | 65.30326 | 65.30±0.133 |
| *Lepidium sativum*    |      |      |      |     |     |    |                  |                      |
| 20                    | 1.012| 0.996| 1.032| 61.3149 | 61.9266 | 60.5504 | 61.24 | 61.24±0.12 |
| 40                    | 1.007| 1.074| 0.972| 61.50612 | 58.94493 | 62.84404 | 61.04 | 61.04±3.18 |
| 60                    | 1.06  | 1.059| 0.987| 59.4801 | 59.5183 | 62.27064 | 60.444 | 60.44±2.24 |
| 80                    | 1.049| 0.93  | 1.026| 59.90061 | 64.4495 | 60.77982 | 61.7 | 61.7±2.99 |
| 100                   | 0.969| 0.98  | 1.057| 62.9587 | 62.5382 | 59.5948 | 61.69 | 61.69±2.4 |
| *Cuscuta reflexa*     |      |      |      |     |     |    |                  |                      |
| 20                    | 1.114| 1.065| 1.065| 57.415 | 59.2889 | 59.2889 | 58.66 | 58.66±1.08 |
| 40                    | 1.016| 1.101| 1.015| 61.16208 | 57.9128 | 61.20031 | 60.09 | 60.09±1.88 |
| 60                    | 1.006| 1.038| 1.02 | 61.54434 | 60.3211 | 61.0991 | 60.95 | 60.95±0.613 |
| 80                    | 0.927| 1.023| 1.001| 64.56422 | 60.894 | 61.7354 | 62.398 | 62.39±1.92 |
| 100                   | 0.943| 0.868| 0.862| 63.9526 | 66.8152 | 67.04893 | 65.9403 | 65.94±1.72 |
| *Eclipta alba*        |      |      |      |     |     |    |                  |                      |
| 20                    | 1.046| 1.04  | 1.04 | 60.0152 | 60.2446 | 60.244 | 60.16 | 60.16±0.132 |
| 40                    | 0.817| 0.758| 0.758| 68.7691 | 71.0244 | 71.0244 | 70.27 | 70.27±1.3 |
| 60                    | 0.708| 0.741| 0.741| 72.935 | 71.6743 | 71.6743 | 72.09 | 72.09±0.72 |
| 80                    | 0.485| 0.443| 0.443| 81.46024 | 83.06575 | 83.06575 | 82.53 | 82.53±0.92 |
| 100                   | 0.435| 0.388| 0.388| 83.371 | 85.1682 | 85.168 | 84.56 | 84.56±1.032 |
| *Leucus cephalotes*   |      |      |      |     |     |    |                  |                      |
| 20                    | 1.145| 1.156| 1.156| 56.23089 | 55.8104 | 60.244 | 55.59 | 55.59±0.24 |
| 40                    | 1.146| 1.053| 1.146| 56.19266 | 59.7477 | 71.0244 | 71.35 | 71.35±2.05 |
| 60                    | 1.143| 1.036| 1.037| 56.30724 | 60.397 | 71.6743 | 70.35 | 70.35±2.36 |
| 80                    | 1.123| 1.014| 1.015| 57.07187 | 61.238 | 83.06575 | 59.83 | 59.83±2.39 |
| 100                   | 1.104| 1.013| 1.013| 57.79817 | 61.2767 | 85.1678 | 60.11 | 60.11±2.008 |
| *Tinospora cordifolia*|      |      |      |     |     |    |                  |                      |
| 20                    | 1.145| 1.07 | 0.906| 56.23089 | 59.0976 | 65.3669 | 60.23 | 60.23±4.67 |
| 40                    | 0.859| 1.053| 0.848| 67.1636 | 59.7477 | 67.5841 | 64.83 | 64.83±4.4 |
| 60                    | 0.711| 0.772| 0.715| 72.8211 | 70.489 | 72.682 | 71.99 | 71.99±1.39 |
| 80                    | 0.7  | 0.558| 0.705| 73.24159 | 78.669 | 73.0504 | 74.98 | 74.98±3.19 |
| 100                   | 0.601| 0.057| 0.558| 77.02599 | 97.821 | 78.6697 | 84.50 | 84.50±1.56 |
was not resistant against *Staphylococcus aureus*. Results obtained from the antibacterial screening of different extracts are tabulated in Table 6.

The extract of *E. alba* showed high growth inhibition against *S. aureus*, whereas the rest of the plant extract were found inactive against these microorganisms.

### Table 6: Antimicrobial activity of plant extracts

| Plant sample (extracts) | Bacteria          | ZOI (mm) of extracts at concentration 100 mg/ml | ZOI (mm) of chloramphenicol as control at 100 mg/ml |
|-------------------------|-------------------|-----------------------------------------------|--------------------------------------------------|
| Anethum sowa            | *E. coli*         | -                                             | 14                                               |
|                         | *S. aureus*       | -                                             | 18                                               |
| Trigonella foenum-     | *E. coli*         | -                                             | 14                                               |
| graecum                 | *S. aureus*       | -                                             | 18                                               |
| Lepidium sativum        | *E. coli*         | -                                             | 14                                               |
|                         | *S. aureus*       | -                                             | 18                                               |
| Cuscuta reflexa         | *E. coli*         | -                                             | 14                                               |
|                         | *S. aureus*       | -                                             | 18                                               |
| Eclipta alba            | *E. coli*         | -                                             | 14                                               |
|                         | *S. aureus*       | -                                             | 18                                               |
| Leucas cephalotes       | *E. coli*         | -                                             | 14                                               |
|                         | *S. aureus*       | -                                             | 18                                               |
| Tinospora cordifolia    | *E. coli*         | -                                             | 14                                               |
|                         | *S. aureus*       | -                                             | 18                                               |

(-) = No effective antibacterial activity; ZOI: Zone of inhibition.

### Table 7: Phenolic content in different plant extracts

| Plant sample (extract) | TPC mg GAE/g |
|------------------------|--------------|
| Anethum sowa           | 306.3±34     |
| Trigonella foenum-graecum | 939.7±0.01 |
| Lepidium sativum       | 551.6±0.02   |
| Cuscuta reflexa         | 145.0±0.01   |
| Eclipta alba            | 202.6±0.02   |
| Leucas cephalotes       | 233.1±0.03   |
| Tinospora cordifolia    | 211.7±0.02   |

**Total phenolic and flavonoid content**

The result of phenolic content is displayed in Table 7. The total phenolic content of all plant extracts showed varied data ranging from 145.0 to 939.7 mg GAE/g in *C. reflexa* and *T. foenum-graecum*, respectively. The *T. foenum-graecum* has the highest total phenolic content, followed by *L. cephalotes* and *T. cordifolia*. The result showed similar results, as reported earlier 269.5±0.5 mg GAE/100 g of total phenolic content in the *E. alba* [21].

The result of flavonoid content is shown in Table 8. Among the seven plants extract *T. cordifolia* showed the presence of the highest flavonoid contents, whereas the *C. reflexa* showed the least TFCs and other plants showed the flavonoids contents in between them. The results of the present study are found comparable to that of previously reported 142±0.15 mg QE/100 g of total flavonoid in *E. alba* [22]. Antioxidant properties of flavonoids depend on their structure, particularly hydroxyl position in molecule although quantitative determination of flavonoid compounds in plant extracts is influenced by their structural complexity, diversity, nature of analytical assay method, selection of standard, and presence of interfering substances.

### In vitro α-amylase inhibition activity

The mode of α-amylase inhibition of plant's extract was evaluated by determining the IC₅₀ values, adopting the standard protocol given by Bernfeld [22]. Alpha-amylase inhibitory properties are presented in Fig. 2.

The result of IC₅₀ of α-amylase inhibition activity of plant extract is shown in Table 9. The IC₅₀ values of different plant extract along with standard acarbose were evaluated and found that the value range from 76.78 µg/ml to 777.36 µg/ml. The extract of *A. sowa* having IC₅₀ value 76.78±200 µg/ml was found to be potent toward the antidiabetic activity. The previous study reported that the aqueous leaves extracts of *P. americana* possess hypoglycemic activity [23]. Similarly, different fractions of *R. ellipticus* fruits were reported for its antidiabetic activity on alloxan-induced diabetes and glucose tolerance tests in rats [24].

### Toxicity test

All the plant extracts showed LC₅₀ > 1000 µg/ml indicating the non-toxic effect. This method provides preliminary screening data that can be backed up by more specific bioassays once the active compounds have been isolated.

![Fig. 2: Percent α-amylase inhibition of plant extract in comparison to standard acarbose](image-url)
The moderate and mild antidiabetic activity was shown TFC mg QE/g 852.07±0.011 329.023±0.050 322.138±0.025. J, and 164.9306±0.020 231.3817±0.0413 antioxidant and 146.372±0.00936 antifungal activity of secondary metabolites from plants-a review. Int J Pharmaco 2015;2:60-5.

Sridevi P, Budde S, Neeraja T, Raju BM, Adapa B. Antioxidants and their role in disease management. Int J Med Res Health and Sci 2018;7:175-90.

Kamtekar S, Keer V, Patil V. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. J Appl Pharm Sci 2014;4:61-5.

Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α-amylase inhibitory activity of seatude medicinal plants. BMC Complement Altern Med 2011;115.

Paul T, Banerjee S. In vitro evaluation of α-amylase inhibitory activity and antioxidant potential of Periss vita. L with special reference to its herbal profile. J Complementary Med 2013;4:504-503.

Ganesan A. The impact of natural products upon modern drug discovery. Curr Opin Chem Biol 2008;12:306-17.

Kasote DM, Kattare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 2015;11:982-91.

Mishra LC. Scientific Basis for Ayurvedic Therapies. 1st ed. Boca Raton, USA: CRC Press; 2003. p. 102.

Harbone JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, New York: Chapman and Hall Ltd., 1973.

Jammuna S, Paulsamy S, Karthika K. Screening of in vitro antioxidant activity of methanolic leaf and root extract of Hypocharis radicula. J (Asteraceae). J Appl Pharm Sci 2012;2:149-154.

Khan MS, Yusufzai SK, Rafatullah M, Sarjadi MS, Raalain M. Determination of total phenolic content, total flavonoid content and antioxidant activity of various organic crude extracts of Licuala Spinosa Leaves from Sabah, Malaysia. ASM Sci J 2018;11:53-8.

Pallab K, Tapan B, Tapas K. Estimation of total flavonoid content (TFC) and antioxidant activities of methanolic whole plant extract of T. cordifolia. Asian J Pharm Clin Res 2018;11:53-8.

Ram Prasad Chaudhary

Dr. Khager Raj Sharma analyzed the data and wrote the manuscript, whereas Numer Alam carried out the laboratory work. Both the authors read and approved the final manuscript.

AUTHORS’ CONTRIBUTIONS

The authors are thankful to the Central Department of Chemistry, Tribhuvan University, for providing laboratory services to conduct this research work. We would like to thank Prof. Dr. Ram Prasad Chaudhary and Prof. Dr. Mohan Shwakoti, Central Department of Botany, for the identification of collected plants.

AUTHORS’ FUNDING

The authors themselves bear the publication fees of this paper.

REFERENCES

1. Joshi B, Lekhak S, Sharma A. Antibacterial property of different medicinal plants: Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana. Kath Univ J Sci 2009;5:143-50.

2. Subedi A, Amaya DP, Shrestha TM, Mishra SK, Pokharel BM. Antioxidant and antibacterial activity of methanolic extract of Machilus odoratissima. Kath Univ J Sci Eng Tech 2012;8:73-80.

3. Gokhale M, Wadhawani M. Antimicrobial activity of secondary metabolites from plants-a review. Int J Pharmaco 2015;2:60-5.

4. Sridevi P, Budde S, Neeraja T, Raju BM, Adapa B. Antioxidants and their role in disease management. Int J Med Res Health and Sci 2018;7:175-90.

5. Kamtekar S, Keer V, Patil V. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. J Appl Pharm Sci 2014;4:61-5.

6. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α-amylase inhibitory activity of seatude medicinal plants. BMC Complement Altern Med 2011;115.

7. Paul T, Banerjee S. In vitro evaluation of α-amylase inhibitory activity and antioxidant potential of Periss vita. L with special reference to its herbal profile. J Complementary Med 2013;4:504-503.

8. Ganesan A. The impact of natural products upon modern drug discovery. Curr Opin Chem Biol 2008;12:306-17.

9. Kasote DM, Kattare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 2015;11:982-91.

10. Mishra LC. Scientific Basis for Ayurvedic Therapies. 1st ed. Boca Raton, USA: CRC Press; 2003. p. 102.

11. Harbone JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, New York: Chapman and Hall Ltd., 1973.

12. Jammuna S, Paulsamy S, Karthika K. Screening of in vitro antioxidant activity of methanolic leaf and root extract of Hypocharis radicula. J (Asteraceae). J Appl Pharm Sci 2012;2:149-154.

13. Khan MS, Yusufzai SK, Rafatullah M, Sarjadi MS, Raalain M. Determination of total phenolic content, total flavonoid content and antioxidant activity of various organic crude extracts of Licuala Spinosa Leaves from Sabah, Malaysia. ASM Sci J 2018;11:53-8.

14. Pallab K, Tapan B, Tapas K. Estimation of total flavonoid content (TFC) and antioxidant activities of methanolic whole plant extract of T. cordifolia. Asian J Pharm Clin Res 2018;11:53-8.

15. Ram Prasad Chaudhary

The authors are thankful to the Central Department of Chemistry, Tribhuvan University, for providing laboratory services to conduct this research work. We would like to thank Prof. Dr. Ram Prasad Chaudhary and Prof. Dr. Mohan Shwakoti, Central Department of Botany, for the identification of collected plants.

AUTHORS’ CONTRIBUTIONS

The authors are thankful to the Central Department of Chemistry, Tribhuvan University, for providing laboratory services to conduct this research work. We would like to thank Prof. Dr. Ram Prasad Chaudhary and Prof. Dr. Mohan Shwakoti, Central Department of Botany, for the identification of collected plants.

AUTHORS’ FUNDING

The authors themselves bear the publication fees of this paper.

REFERENCES

1. Joshi B, Lekhak S, Sharma A. Antibacterial property of different medicinal plants: Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana. Kath Univ J Sci 2009;5:143-50.

2. Subedi A, Amaya DP, Shrestha TM, Mishra SK, Pokharel BM. Antioxidant and antibacterial activity of methanolic extract of Machilus odoratissima. Kath Univ J Sci Eng Tech 2012;8:73-80.

3. Gokhale M, Wadhawani M. Antimicrobial activity of secondary metabolites from plants-a review. Int J Pharmaco 2015;2:60-5.

4. Sridevi P, Budde S, Neeraja T, Raju BM, Adapa B. Antioxidants and their role in disease management. Int J Med Res Health and Sci 2018;7:175-90.

5. Kamtekar S, Keer V, Patil V. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. J Appl Pharm Sci 2014;4:61-5.

6. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α-amylase inhibitory activity of seatude medicinal plants. BMC Complement Altern Med 2011;115.

7. Paul T, Banerjee S. In vitro evaluation of α-amylase inhibitory activity and antioxidant potential of Periss vita. L with special reference to its herbal profile. J Complementary Med 2013;4:504-503.

8. Ganesan A. The impact of natural products upon modern drug discovery. Curr Opin Chem Biol 2008;12:306-17.

9. Kasote DM, Kattare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 2015;11:982-91.

10. Mishra LC. Scientific Basis for Ayurvedic Therapies. 1st ed. Boca Raton, USA: CRC Press; 2003. p. 102.

11. Harbone JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, New York: Chapman and Hall Ltd., 1973.

12. Jammuna S, Paulsamy S, Karthika K. Screening of in vitro antioxidant activity of methanolic leaf and root extract of Hypocharis radicula. J (Asteraceae). J Appl Pharm Sci 2012;2:149-154.

13. Khan MS, Yusufzai SK, Rafatullah M, Sarjadi MS, Raalain M. Determination of total phenolic content, total flavonoid content and antioxidant activity of various organic crude extracts of Licuala Spinosa Leaves from Sabah, Malaysia. ASM Sci J 2018;11:53-8.

14. Pallab K, Tapan B, Tapas K. Estimation of total flavonoid content (TFC) and antioxidant activities of methanolic whole plant extract of T. cordifolia. Asian J Pharm Clin Res 2018;11:53-8.

15. Ram Prasad Chaudhary

The authors are thankful to the Central Department of Chemistry, Tribhuvan University, for providing laboratory services to conduct this research work. We would like to thank Prof. Dr. Ram Prasad Chaudhary and Prof. Dr. Mohan Shwakoti, Central Department of Botany, for the identification of collected plants.

AUTHORS’ CONTRIBUTIONS

The authors are thankful to the Central Department of Chemistry, Tribhuvan University, for providing laboratory services to conduct this research work. We would like to thank Prof. Dr. Ram Prasad Chaudhary and Prof. Dr. Mohan Shwakoti, Central Department of Botany, for the identification of collected plants.