

Full Length Research Paper

Antioxidant and alpha amylase inhibitory activity of Nepalese medicinal plants from Gorkha district

Junita Rai¹, Khaga Raj Sharma^{1*} and Yuba Raj Pokharel^{2*}

¹Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

²Faculty of Life Science and Biotechnology, South Asian University, New Delhi-110021, India.

Received 12 February, 2020, Accepted 2 April, 2020

The aim of this study is to evaluate the antioxidant activity, α -amylase inhibition activity, estimation of total phenolic and flavonoid content and the toxicity in ten medicinal plants *Woodfordia fruticosa*, *Tectaria coadunata*, *Prunus cerasoides*, *Abrus precatorius*, *Eclipta prostrate*, *Poranopsis paniculata*, *Chenopodium album*, *Oroxylum indicum*, *Curcuma caesia*, and *Butea monosperma* collected from Gorkha District of Nepal. Methanolic extracts of all the plants showed the presence of different phytoconstituents such as alkaloids, polyphenols, flavonoids, terpenoids, saponins, glycosides, and quinones. The highest radical scavenging was observed in methanol extract of *P. cerasoides* with $IC_{50} = 7.54 \pm 0.223 \mu\text{g/ml}$. The potency of the radical scavenging effect of *P. cerasoides* was about six times greater than standard ascorbic acid ($39.85 \pm 0.025 \mu\text{g/ml}$) taken. *P. cerasoides* showed high phenol content ($805.48 \pm 0.024 \text{ mg GAE/g extract}$) whereas total flavonoid content varied from *O. indicum* ($16.96 \pm 0.015 \text{ mg QE/g extract}$) to *W. fruticosa* ($722.76 \pm 0.108 \text{ mg QE/g extract}$). The methanol extract of *E. prostrate* was found to be toxic against brine shrimp as shown in the LC_{50} value of $6.3 \mu\text{g/ml}$. *T. coadunata* and *A. precatorius* showed effective results with an IC_{50} value of 80.89 and 70.29 $\mu\text{g/ml}$ respectively in α -amylase inhibition test. This study provides some scientific support for traditional uses of plants for diabetes management and other ailments. Since extracts of *W. fruticosa* and *P. cerasoides* are rich sources of bioactive chemical constituents, further *in-vitro* and *in-vivo* bioactivity of these extracts need to be studied for their exact mechanism of action.

Key words: Antioxidant, 1,1-diphenyl-2-picrylhydrazyl (DPPH), phenolic content, flavonoid content, inhibition.

INTRODUCTION

Nepal is home to more than fifty nine culturally rich ethnic and indigenous groups. Many of them have their medical practices handed down orally from generation to generation. One approximation puts that up to 70-80% of the rural population fully depends on medicinal plants for their primary healthcare (Ghimire et al., 2011). Nepal is a country known for abundant natural resources, endowed

with enough medicinal plants that have been consumed by local people since time immemorial without knowing its biological potential and chemical composition (Young and Woodside, 2001). So these medicinal plants are needed to be explored significantly in a scientific basis which would provide a new dimension to the pharmaceutical or therapeutic field. These medicinal plants play a

*Corresponding author. E-mail: ypr@sau.ac.in and khagaraj_sharma33@yahoo.com.

significant role in the management of several illnesses caused by the radicals formed in the body by different chain reactions. Formation of free radicals in human body causes oxidative damage to the biomolecules which ultimately lead to health disorders such as cancer, diabetes, inflammation, asthma, cardiovascular diseases, neurodegenerative diseases, and premature aging (Parajuli et al., 2013). At present most of the synthetic antioxidants are commercially available but are known to have carcinogenicity and other side effects when taken *in vivo*. Hence, their use is being restricted nowadays, and there is increasing interest in finding out safer and bioactive natural antioxidants from medicinal plant sources (Sharma et al., 2018). DPPH free radical scavenging assay has been primarily used by various researchers as a quick, easy, reliable, and reproducible parameter in search of *in vitro* antioxidant activity of a pure natural compound as well as plant extracts. Antioxidant compounds offer hydrogen atom to the free radicals resulting in neutral compounds with loss of color (Subedi et al., 2014). The importance of natural phenolic compounds from plant materials show the antioxidant activity and also enhance their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quencher (Rezaeizadeh et al., 2011). Polyphenol compounds are secondary metabolites found in numerous plant species, and they are reported to have multiple functions to counteract the free radicals and inhibit different types of oxidizing enzymes (Kazeem et al., 2013).

Diabetes mellitus (DM) is a complex disease that is characterized by a gross derangement in carbohydrate, protein, and fat metabolism. It is a progressive metabolic disorder characterized by chronic hyperglycemia that eventually causes damage, dysfunction, and failure of different organs, mainly the eyes, kidneys, nerves, heart, and blood vessels (Alagesan, 2014). Type I diabetes results from the inadequate synthesis of insulin by β -cells of the pancreas, while type II diabetes is characterized primarily by insulin resistance; a condition in which peripheral cells do not usually respond to insulin (Arumugam et al., 2013).

The disease, especially Type II DM, has turned into a global health problem and is the third leading cause of death having a high occurrence and mortality rate. In Nepal, 526,000 cases of diabetes were reported in 2015 (Najafian, 2014). Due to becoming a major health problem, multiple therapies have been developed for type 2 diabetes. One of the best therapeutic targets is α -amylase. Alpha-amylase catalyzes the hydrolysis of glucosidic linkage in starch to oligosaccharides, whereas membrane-bound intestinal glucosidase hydrolyze oligosaccharides to glucose (Sales et al., 2012). Inhibition of α -amylase therapy is responsible for delaying the absorption of glucose after a meal. Several α -amylase inhibitory compounds have been isolated from medicinal plants that lead to the development of new drugs with high potentiality and lower adverse effect than the existing

ones (Kwon et al., 2007). Synthetic drugs are reported to cause various side effects such as abdominal distention, flatulence, and diarrhea owing to the excessive inhibition of pancreatic α -amylase. This results in abdominal bacterial fermentation of undigested carbohydrates in the colon. Hence, at present, there is an increasing interest among food scientists to identify natural sources of α -amylase and α -glucosidase inhibitors for the dietary management of type II diabetes (Piette and Kerr, 2006). It is estimated that more than 800 plant species have hypoglycemic activity, and more than 450 plants have been experimentally tested (Ciulei, 1982). Therefore, it is urgent to identify and explore the antioxidant, total phenolic and flavonoid contents and alpha-amylase inhibitors from the medicinal plants of Nepal.

Thus, the present investigation was undertaken to make a comparative study of antioxidant activity, estimation of total phenolic and flavonoid contents and alpha-amylase inhibitory activity from some medicinal plants, that is, *Woodfordia fruticosa*, *Tectaria coadunata*, *Abrus precatorius*, *Prunuscerasoids*, *Eclipta prostrate*, *Eclipta prostrate*, *Poranopsis paniculata*, *Chenopodium album*, *Oroxylum indicum*, *Curcuma caesia*, and *Butea monosperma* used traditionally for the treatment of a number of illnesses including diabetes.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals used in this study were methanol (Merck, Germany), porcine pancreatic alpha-amylase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ascorbic acid (Sigma, Aldrich, USA). All the needed chemicals used in this research work were of the commercially available analytical grade.

Plant collection and extract preparation

Ten medicinal plants were collected from 12 kilo VDC of Gorkha District of Nepal based on their ethnobotanical uses as shown in Table 1. All collected plants were identified by an expert from the Central Department of Botany, Tribhuvan University, Kirtipur Kathmandu, Nepal. 100 g powder of each plant was extracted by cold percolation method in methanol (200-300 ml) at room temperature for 48 h with constant agitation. The mixture was filtered through clean cotton and filtrate was concentrated at the temperature lower than the boiling point of methanol under reduced pressure by rotary evaporator to get the crude extract. Finally, the concentrated extracts were fridge dried to yield a dry powder.

Phytochemical screening

Phytochemical screening is the method of finding the primary, secondary metabolites present in the plant extracts. The phytochemicals as secondary metabolites were investigated by following the standard protocol put forward by Kim et al. (2003). The analysis of the presence of leading groups of natural constituents present in the different plant extracts was done by the color reaction

Table 1. List of selected plants and their therapeutic uses.

| Scientific name | Nepali name | Used Part | Altitude(m) | Therapeutic uses |
|------------------------------|--------------|-------------|-------------|---|
| <i>Woodfordia fruticosa</i> | Dhayero | Flower | 1300-1600 | Asthma, inflammation, liver disorder, constipation (Das et al., 2007) |
| <i>Tectaria coadunata</i> | Kaloniuro | Rhizome | 1000-1900 | Insect bites, cold cough, bronchitis, asthma (Marahatta et al., 2019) |
| <i>Prunus cerasoides</i> | Paiyun | Bark | 1200-2400 | Sprain, ulcer, whooping cough, dyspepsia, diarrhea (Joseph et al., 2018) |
| <i>Abrus precatorius</i> | Laalgedi | Root | Below 1200 | Nervous disorder, eye, skin diseases (Bhatia et al., 2013) |
| <i>Eclipta prostrate</i> | Bhringraj | Aerial part | 180-1990 | Hemorrhage, cough, vomiting, inflammation, fever (Soni and Soni, 2017) |
| <i>Poranopsis paniculata</i> | Sikarilahara | Leaf | Up to 2000 | Bone fracture (Acharya and Rokaya, 2005; Hasan et al., 2013) |
| <i>Chenopodium album</i> | Bethe | Whole plant | Below 4700 | Constipation, joint pains, ulcer, Intestinal worm (Singh and Hamal, 2013; Esmail and Snafi, 2015) |
| <i>Oroxylum indicum</i> | Tatelo | Bark | 300-1800 | Rheumatism, ulcer, diarrhea, dysentery (Padgilwar et al., 2014) |
| <i>Curcuma caesia</i> | Vuinchampa | Rhizome | 500-3000 | Tumors, piles, rheumatic pain (Devi et al., 2015) |
| <i>Butea monosperma</i> | Palaash | Bark | 1500-3000 | Swellings, dysentery, anthelmintic (Burlia and Khade, 2007) |

using different specific reagents.

Total polyphenol content determination

Total phenolic contents (TPC) of all selected plant extracts were determined using Folin-Ciocalteu Reagent (FCR) and gallic acid as a standard compound following the procedure put forward by Zhou et al. (2010). Briefly, the stock solution of all the extracts was prepared by dissolving 10 mg in 1 ml of methanol (10 mg/ml). Serial dilutions were carried out to get the concentration of 0.125, 0.25, 0.5 and 1.0 mg/ml. To these diluted solutions, 10% FCR and 7% Na₂CO₃ were added and incubated for 30 min, followed by measurement of absorbance at 760 nm using the UV-visible spectrophotometer. The calibration curve was constructed using the solution of gallic acid as standard in methanol using the concentration ranging from 10-100 µg/ml. Based on this standard calibration curve of gallic acid, the concentrations of the individual samples were calculated. The results of TPC are measured in milligrams of gallic acid equivalent (mg GAE) per gram of dry extract (Kalita and Barman, 2013). TPC in the plant extracts taken under study was calculated by using regression equation:

$$y = 0.0023x$$

R² = 0.9902, obtained from the calibration curve of gallic acid (Piette and Kerr, 2006).

Total flavonoid content determination

Aluminum chloride colorimetric method was adopted with some modification for flavonoid content determination (Process et al., 2013). Serial dilutions of concentration of 0.125, 0.25, 0.5, and 1.0 mg/ml were made from the stock solution of all the extracts. At zero time 0.3 ml 5% NaNO₂ (sodium nitrite) was added. After 5 min, 0.3 ml of 10% AlCl₃ (aluminium chloride) was added and kept to stand for 6 min. Then 2 ml of 1M NaOH was added to the mixture and shaken well. The absorbance was recorded at 510 nm using UV-visible spectrophotometer. The calibration curve was constructed with the help of standard quercetin solution in methanol with the concentration ranging from the 10-100 µg/ml. Total flavonoid content (TFC) in the plant extract was expressed in terms of the milligram of quercetin equivalent per gram of the dry mass (mg QE/g) (Jamuna et al., 2012). TFC in the plant extracts was calculated by using regression equation $y = 0.0174x$, R² = 0.9974, obtained from the calibration curve.

Antioxidant activity

The ability of different plant extracts to scavenge DPPH free radicals was performed following the standard protocol adopted by Jamuna et al. with some modification (Jamuna et al., 2012). In brief, 2 ml of different extract solution (20, 40, 60, 80, and 100 µg/ml) of each plant samples was mixed with 2 ml of DPPH solution. The mixture was kept to stand for 30 min. Then the absorbance was measured at 517 nm using UV spectrophotometer. Radical

Table 2. Phytochemical screening of plant extracts.

| Groups of compounds | J ₁ | J ₂ | J ₃ | J ₄ | J ₅ | J ₆ | J ₇ | J ₈ | J ₉ | J ₁₀ |
|---------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| Basic alkaloids | - | + | - | - | - | - | - | - | - | + |
| Coumarins | + | + | + | + | - | - | + | - | - | - |
| Flavonoids | + | - | + | + | + | - | + | - | - | - |
| Glycosides | + | + | + | + | + | - | + | + | + | - |
| Polyphenols | + | + | + | + | - | - | + | + | - | - |
| Quinones | + | - | + | + | + | + | - | + | - | - |
| Reducing sugars | - | + | + | - | - | - | + | + | - | + |
| Saponins | - | - | - | + | + | - | + | - | - | - |
| Terpenoids | + | + | + | - | + | + | + | + | + | - |

‘+’ represents presence and ‘-’ represents absence; J₁ = *Woodfordia fruticosa*, J₂ = *Tectaria coadunata*, J₃ = *Prunus cerasoides*, J₄ = *Abrus precatorius*, J₅ = *Eclipta prostrate*, J₆ = *Poranopsis paniculata*, J₇ = *Chenopodium album*, J₈ = *Oroxylum indicum*, J₉ = *Curcuma caesia*, J₁₀ = *Butea monosperma*.

scavenging activity of each sample was calculated by using the formula;

$$\text{Radical scavenging (\%)} = [(A_0 - A_s) / A_0] \times 100$$

Where:

A₀ = Absorbance of the control (DPPH solution + methanol), A_s = Absorbance of the test sample

Control was the test solution without sample, and ascorbic acid was used as the positive control. The absorbance was measured at least in triplicate. The inhibition curve was plotted for the triplicate and represented as a percentage of mean inhibition ± standard deviation, and the IC₅₀ values were obtained. The linear regression of the rate of radical scavenging versus concentration was used for the calculation of the concentration of each plant extract required for 50% inhibition of DPPH activity (IC₅₀).

Alpha-amylase inhibition assay

Alpha-amylase inhibition assay was performed using a standard where the undigested starch due to enzyme inhibition was detected at 630 nm (blue, starch-iodine complex) described by Meyer et al. (1982). The stock solution of all the plant extracts was made by dissolving 10 mg in 10 mL of dimethylsulphoxide (DMSO) (1000 µg/ml). The substrate was prepared by dissolving 200 mg starch in 25 ml of NaOH (0.4 M) by heating at 100°C for 5 min. The pH of the mixture was adjusted to 7.0 after cooling, and the final volume was made up to 100 ml using distilled water. Acarbose was used as Positive control. 400 µl of substrate solution was pre-incubated at 37°C for 5 min with 200 µl of acarbose or plant extract at varying concentrations (40, 80, 160, 320 and 640 µg/ml), followed by 200 µl of 50 µg/ml α-amylase (20mM phosphate buffer with 6.7 mMNaCl, pH 6.9), and incubated at 37°C for 15 min. Termination of the reaction was performed by adding 800 µl of HCl (0.1M). Then, 1000 µl of iodine reagent (2.5 mM) was added, and absorbance was measured at 630 nm. The assay was carried out in triplicates using a spectrophotometer. Percentage of inhibition was calculated using the formula;

$$\% \text{ Inhibition} = (1 - [Abs_2 - Abs_1 / Abs_4 - Abs_3]) \times 100$$

Where:

Abs1 is the absorbance of the incubated mixture containing plant sample, alpha-amylase, and starch. Abs 2 is the absorbance of the incubated mixture of sample and starch. Abs3 is the absorbance of

the incubated mixture of starch and α-amylase. Abs4 is the absorbance of the incubated solution containing starch.

Statistical analysis

Data were recorded as the mean of (±) standard deviation of three determinations of absorbance for each concentration, from which the linear correlation coefficient (R²) value was calculated using MS Office Excel 2007. The linear regression for a straight line is:

$$y = mx + c;$$

The concentration of the extract was calculated using the regression equation.

Toxicity

The toxicity test was performed by using brine shrimp assay following the standard protocol put forward by Joseph et al. (2016) 20 mg of the sample to be tested was dissolved in 2 ml methanol. The solution thus prepared was used as stock solutions. The newly hatched brine shrimp nauplii (*Artemia selina*) was introduced to the solution of crude plant extracts with different concentrations: 1000, 100 and 10 µg/ml. The control was performed by adding the solvent used to dissolve the extracts. The entire test was performed in a temperature controlled room at 20°C, under continuous light. After 24 h, the numbers of survivors were counted with disposable pipettes, and the number of deaths at each dose was recorded. This method evaluates the toxicity of plant extracts towards the nauplii by determining the LC₅₀ (µg/ml). Compounds of LC₅₀ value less than 1000 ppm are considered as pharmaceutically active.

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical screening are shown in Table 2. The result shows that glycosides and terpenoids were present in most of the extract, whereas some extracts showed the presence of reducing sugars and saponins. Flavonoids were present only in *W. fruticosa*, *P. ceraoids*, *A. precatorius*, *E. prostrate* and *C. album*.

Table 3. Total phenolic content, flavonoid content and free radical scavenging (IC₅₀).

| Plant extract | Total phenolic mg GAE/g \pm SD | Total flavonoid mg QE/g \pm SD | Radical scavenging (IC ₅₀) |
|-----------------------|-------------------------------------|-------------------------------------|---|
| <i>W. fruticosa</i> | 794.97 \pm 0.194 | 722.76 \pm 0.108 | 24.40 \pm 0.383 |
| <i>T. coadunata</i> | 728.03 \pm 0.062 | 69.89 \pm 0.167 | 15.17 \pm 0.180 |
| <i>P. cerasoides</i> | 805.48 \pm 0.024 | 330.75 \pm 0.732 | 7.54 \pm 0.223 |
| <i>A. precatorius</i> | 709.84 \pm 0.168 | 45.32 \pm 0.157 | 107.05 \pm 0.174 |
| <i>E. prostrate</i> | 746.34 \pm 0.089 | 508.74 \pm 0.022 | 10.78 \pm 0.307 |
| <i>C. album</i> | 747.57 \pm 0.173 | 103.53 \pm 0.326 | 11.69 \pm 0.362 |
| <i>O. indicum</i> | 720.79 \pm 0.139 | 16.96 \pm 0.015 | 59.76 \pm 0.969 |
| <i>C. caesia</i> | 399.32 \pm 0.100 | 68.45 \pm 0.867 | 542.22 \pm 0.464 |
| <i>B.monosperma</i> | 277.39 \pm 0.108 | 53.05 \pm 0.986 | 170.19 \pm 2.02 |
| <i>P. panniculata</i> | 364.69 \pm 0.055 | 88.79 \pm 0.011 | 475.36 \pm 0.125 |

Alkaloids were present only in *T. coadunata* and *B. monosperma*. The absence of alkaloids in most of the plant extracts may be due to the decomposition of alkaloids while concentrating the extract by the rotatory evaporator. The results presented in the Table 2 are slightly different from the data present in the literature of some plants. The outcome of phytochemical screening shows the presence of different phytochemicals of similar plants is due to variation in altitude, different environmental conditions, method and time of sample collection, extraction procedure and also due to lab setup and chemical grades.

Total phenolic content (TPC)

The TPC of different plant extracts (mg gallic acid equivalent per g dry extract) are tabulated in Table 3. The results demonstrate that the total phenolic content was found high in *P. cerasoides* (805.48 \pm 0.024 mg GAE/g extract) and low in *B. monosperma* (277.39 \pm 0.108 mg GAE/g extract) while the rest have moderate values. The total phenolic content was compared with the previous result in which the total phenolic content of *M. oleifera* flowers were recorded as 19.31 mg/g of the gallic acid equivalent of total phenolic content in the dry extract (Hasan et al., 2013). The present study showed that plants are the good sources of polyphenols as secondary metabolites.

Total flavonoid content (TFC)

The TFC of different plant extracts is shown in Table 3. The antioxidant property of flavonoid depends on their structure, particularly hydroxyl position in the molecule and their ability as an electron donor to a free radical (Sharma et al., 2018). Among the studied plant extracts, there is variation in total flavonoid contents ranging from *O. indicum* (16.96 \pm 0.015 TFC mg QE/g extract) to

fruticosa (722.76 \pm 0.108 TFC mg QE/g extract). Also, the extract of *T. coadunata*, *P. cerasoides*, *E. prostrate*, *C. album* showed high total flavonoid content while the rest had a significantly low value of total flavonoid content. Previous results showed that total flavonoid content was found to be 96.15 \pm 4.26 mg of quercetin equivalent/100 g (aqueous extract), 85.18 \pm 4.21 mg of quercetin equivalent/ 100 g (hydro-alcohol extract), 96.01 \pm 2.99 mg of quercetin equivalent/ 100 g (ethanol extract) (Das et al., 2007). This study shows plants are rich in flavonoid content. Although, the 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.

DPPH free radical scavenging activity

DPPH free radical scavenging activities of selected medicinal plants are presented in Figure 1. The percentage scavenging effect on the DPPH radical was concomitantly increased with the increase in concentration of the plant extracts from 20-100 μ g/ml. The antioxidant potential is in an inverse relation with IC₅₀ value; the lower value of IC₅₀ indicates high antioxidant potential. The IC₅₀ value of the plant extracts along with the standard ascorbic acid is presented in Table 3. Effective results were obtained from methanolic extract of *W. fruticosa* (24.40 \pm 0.383 μ g/ml), *T. coadunata* (15.17 \pm 0.180 μ g/ml), *P. cerasoides* (7.54 \pm 0.223 μ g/ml), *E. prostrate* (10.78 \pm 0.307 μ g/ml) and *Chenopodium album* (11.69 \pm 0.362 μ g/ml) which showed the strongest DPPH radical scavenging activity as their IC₅₀ value was close to standard ascorbic acid (39.85 \pm 0.025 μ g/ml). The previous result showed the IC₅₀ value of *P. cerasoides* was 56.00 \pm 0.242 μ g/ml that is the value close to IC₅₀ value of ascorbic acid as standard IC₅₀ value 25.55 \pm 0.156 μ g/ml (Joseph et al., 2016).

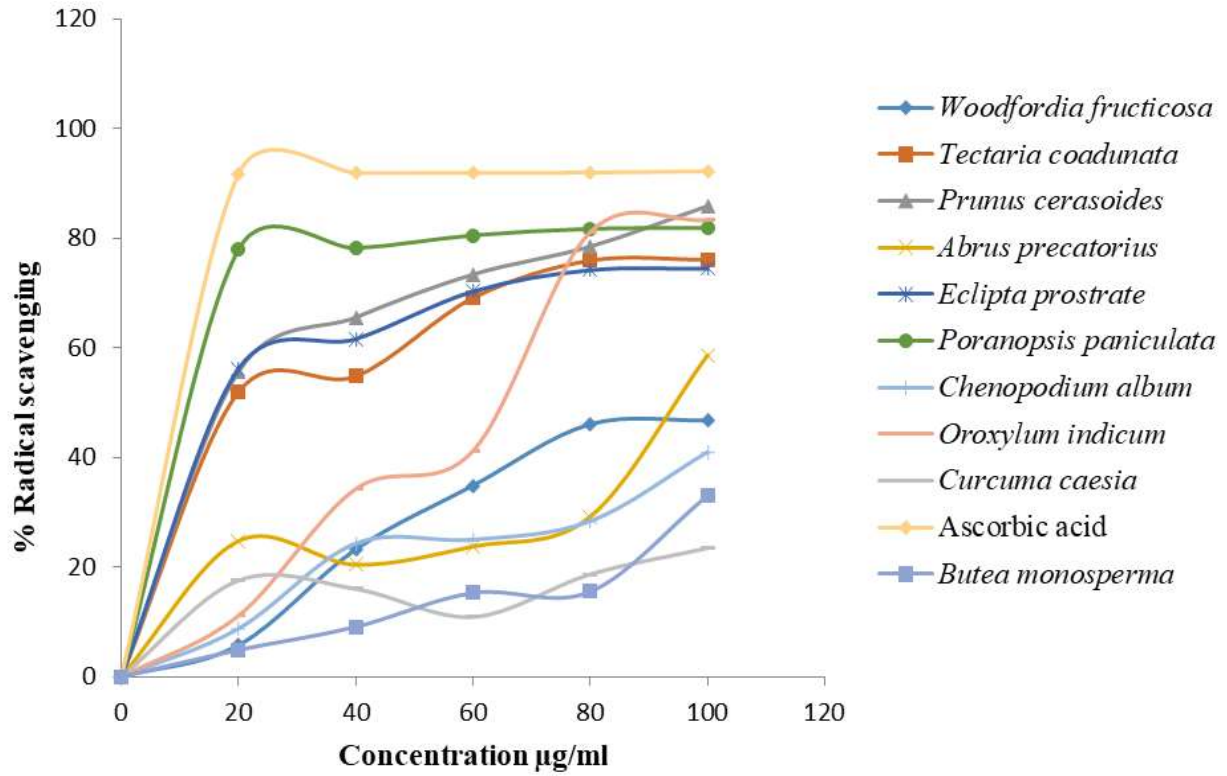


Figure 1. Radical scavenging against the concentration of plant extracts.

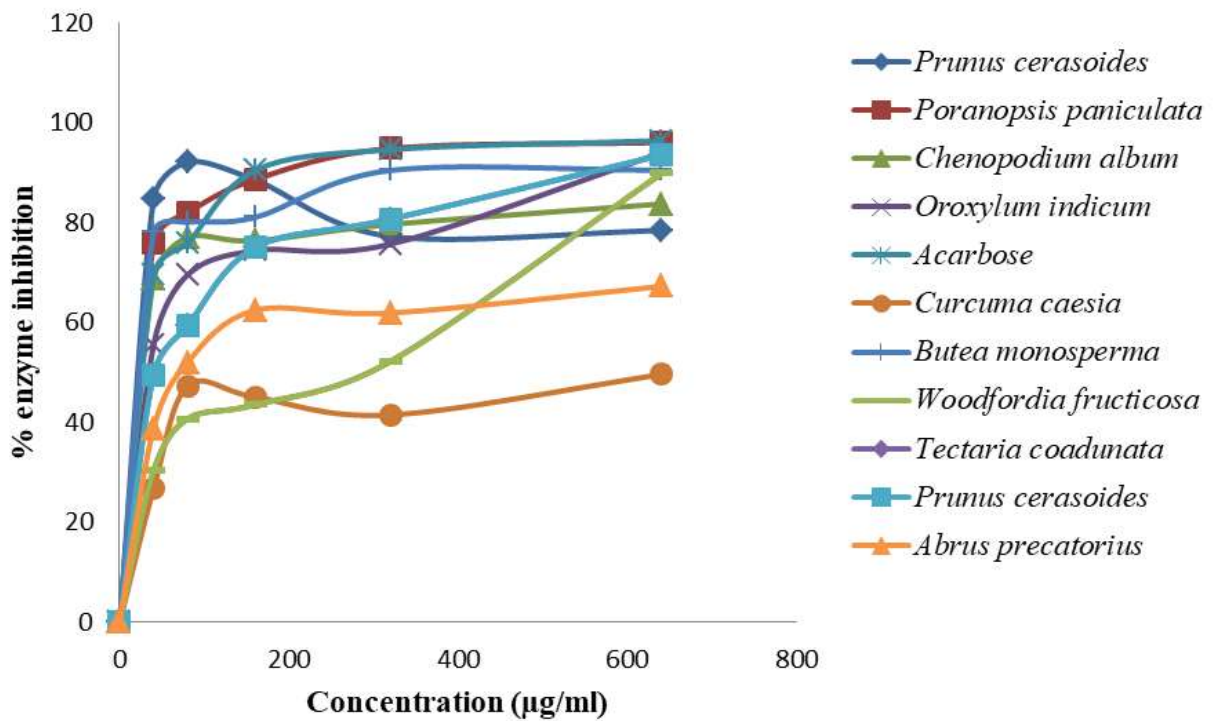


Figure 2. Inhibition of alpha-amylase activities by plant extracts.

Table 4. IC₅₀ value for α-amylase inhibition activity.

| Scientific name | IC ₅₀ (µg/ml)±SD |
|-----------------------|-----------------------------|
| Acarbose | 27.93±0.145 |
| <i>W. fruticosa</i> | 236±0.159 |
| <i>T. coadunata</i> | 80.89±0.09 |
| <i>P. cerasoides</i> | 253±0.16 |
| <i>A. precatorius</i> | 70.29±0.142 |
| <i>E. prostrate</i> | 556±0.078 |
| <i>P. paniculata</i> | 1335±0.04 |
| <i>C. album</i> | 1168±0.075 |
| <i>O. indicum</i> | 192±0.085 |
| <i>C. caesia</i> | 950±0.20 |
| <i>B. monosperma</i> | 1360±0.05 |

Alpha-amylase inhibition assay

Alpha-amylase inhibitory activity of plant extract was determined using the quantitative starch-iodine method. The result of α amylase inhibition activity is shown in Figure 2. The result of α-amylase enzyme inhibition activity in terms of IC₅₀ value is shown in Table 4. In the present study, methanolic extract of different parts of plants was investigated for their potential to inhibit α-amylase activity. The IC₅₀ value of standard acarbose was 27.93±0.07 µg/ml. Among the selected plants, methanol extract of *A. precatorius* root showed highest α-amylase inhibition activity with an IC₅₀ value of 70.29±0.14 µg/ml. Along with other methanolic plant extract also showed effective results such as *T. coadunata* rhizome extract (80.89±0.09 µg/ml), *O. indicum* bark extract (192±0.08 µg/ml), *W. fruticosa* flower extract (236±0.15 µg/ml), *P. cerasoids* bark extract (253±0.16 µg/ml). Lowest α-amylase inhibition property was shown by methanolic plant extract of *C. album* (1168±0.07 µg/ml), methanolic rhizome extract of *C. caesia* (950±0.20 µg/ml), methanolic leaf extract of *P. paniculata* (1335±0.04 µg/ml), methanolic bark extract of *B. monosperma* (1360±0.05 µg/ml). The result showed a dose-dependent increase in percentage inhibitory activity of α-amylase by all the ten plant extracts. Reddy et al. (2010) analyzed the α-amylase enzyme inhibition activity by oral administration of ethanolic extract of *A. precatorius*. The serum glucose value of 131.16±1.939 mg/dl was found, whereas standard Glibenclamide value was 96.5±1.607 mg/dl (Wink, 2008).

Brine shrimp toxicity

The degree of lethality was found to be directly proportional to the concentration of the extracts where maximum mortalities of the brine shrimp larvae took place at the concentration of 1000 µg/ml, and least mortalities were at 10 µg/ml. Those having LC₅₀ values

less than 1000 µg/ml are supposed to be pharmacologically active. The extract of *E. prostrate* was toxic against brine shrimps having LC₅₀ value of 6.3 µg/ml. *P. cerasoides* ranks as mild toxic plant species with LC₅₀ value 33.88 µg/ml. Besides, the extracts of *C. caesia* (138.04 µg/ml), *P. paniculata* (177.82 µg/ml), *A. precatorius* (489.78 µg/ml) and *W. fruticosa* (912.01 µg/ml) showed significant lethality.

Conclusions

This study provides some scientific support for their traditional use for the management of several mentioned ailments. Data on biological activities of many medicinal herbs are tremendously increasing. However, it is impractical to specify the performance of multi-component mixture, as that plant extracts comprise a wide range of phytochemical constituents, to only a single component from that extract (Singh and Hamal, 2013). Since extracts of *W. fruticosa* and *P. cerasoides* are the better sources of bioactive chemical constituents that can be attributed through antioxidant, total phenol, and flavonoid content determination, further *in vitro* and *in vivo* bioactivity of these extracts needs to be assayed much to identify the typical modes of action of the extracts and isolated pure compounds that can shape potential drug discovery. Here, it is concluded that further bioassay-guided fractionation and isolation approaches will be required on the active plant extract to identify the compound responsible for the promising *in-vitro* anti-diabetic activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to the Central Department of Chemistry, Tribhuvan University for laboratory facilities.

REFERENCES

- Acharya KP, Rokaya MB (2005). Ethnobotanical survey of medicinal plants traded in the streets of Kathmandu valley. *Scientific World* 3:44-48.
- alpha-amylase inhibitors. *International Journal of Pharmaceutical Sciences* 15:141-183.
- Bhatia M, Siddiqui NA, Gupta S (2013). *Abrus Precatorius* (L.): An Evaluation of Traditional Herb. *Indo American Journal of Pharmaceutical Research* 3:1-21.
- Burlia DA, Khade AB (2007). A comprehensive review on *Butea monosperma* (Lam.). *Pharmacognosy Reviews* 1: 94-98.
- Ciulei I (1982). Methodology for analysis of vegetable drugs. *Practical manuals on industrial utilization of medicinal and aromatic plant*, Bucharest. *Phytochemistry* 63:97-104.
- Das PK, Goswami S, Chinniah A, Panda N, Banerjee S, Sahu NP, Achari SB (2007). *Woodfordia fruticosa*: Traditional uses and recent

- findings. *Journal of Ethnopharmacology* 110:189-199.
- Devi HP, Mazumder PB, Devi LP (2015). Antioxidant and antimutagenic activity of *Curcuma caesia* Roxb. rhizome extracts. *Toxicology Reports* 2:423-428.
- Esmail A, Snafi A (2015). The chemical constituents and pharmacological effects of *Chenopodium album* - an overview. *International Journal of Pharmacological Screening Method* 1:10-17.
- Ghimire BK, Seong ES, Kim EH, Ghimire AK, Yu CY, Ghimire BK, Chung IM (2011). A comparative evaluation of the antioxidant activity of some medicinal plants popularly used in Nepal. *Journal of Medicinal Plants Research* 5(10):1884-1891.
- Hasan MK, Gatto P, Jha PK (2013). Traditional uses of wild traditional medicinal plants and their management practices in Nepal- A study in Makawanpur district. *International Journal of Medicinal and Aromatic Plants* 3:102-112.
- Jamuna S, Paulsamy S, Karthika K (2012). Screening of in vitro antioxidant activity of methanolic leaf and root extracts of *Hypochoeris radicata* L. (Asteraceae). *J. Appl. Pharm. Sci.* 2: 149-154.
- Joseph N, Anjum N, Tripathi YC (2018). *Prunus cerasoides* D. Don: A Review on Its Ethnomedicinal Uses, Phytochemistry and Pharmacology. *International Journal of Pharmaceutical Sciences Review and Research* 48:62-69.
- Joseph N, Anjumand N, Tripathi YC (2016). Phytochemical screening and evaluation of polyphenols, flavonoids and antioxidant activity of *Prunus cerasoids* D. Don leaves. *Journal of Pharmacy Research* 10: 502-508.
- Kalita P, Barman TK (2013). Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Bophytum sensitivum* Linn. *J. Drug Delivery Therapeutics* 3:33-37.
- Kazeem MI, Adamson JO, Ogunwande IA (2013). Modes of inhibition of alpha-amylase and alpha-glucosidase by aqueous extract of *Morinda lucida*. *International Journal of Biomedical Research* 2:1-2.
- Kim DO, Jeong SW, Lee CY (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 81:321-326.
- Kwon YI, Apostolidis E, Shetty K (2007). Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. *Journal of Food Biochemistry* 31:370-385.
- Marahatta AB, Poudel B, Basnyat RC (2019). The Phytochemical and Nutritional analysis and biological activity of *Tectaria coadunata* Linn. *International Journal of Herbal Medicine*. 7:42-50.
- medicinal plants from Western Nepal. *Journal of Traditional Chinese Medical Sciences* 34:584-590.
- Meyer BN, Ferrigni NR, Putham JE, Jacobsen LB, Nicholas DE, Mclaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica* 45:31-34.
- Najafian M (2014). The effects of Curcumin on alpha-amylase in Diabetics rats. Zahedan. *Journal of Research in Medical Sciences* 15:29-34.
- Padgilwar S, Manwar J, Vohrab M, Banginwarc Y (2014). Traditional uses, phytochemistry and pharmacology of *Oroxylum indicum*: a review. *International Journal of Pharmacology and Clinical Sciences*. pp. 1-13.
- Parajuli S, Pun NT, Parajuli S, Pandit JN (2013). Antioxidant activity, total phenol and total flavonoid contents in some selected medicinal plants. *Journal of Health and Allied Sciences* 2:27-31.
- Piette JD, Kerr EA (2006). The impact of comorbid chronic conditions and diabetic care. *Diabetes Care* 29:725-731.
- Process C, Lytoudi K, Mavromelanidou O, Zoumpoulakis P, Sinanoglou V (2013). Antioxidant capacity of selected plant extracts and their essential oils. *Institute of Biology, Medicinal chemistry, and Biotechnology, Technological, Educational Institution of Athens* 2:11-22.
- Rezaeizadeh A, Zuki ABZ, Abdollahi M (2011). Determination of antioxidant activity in methanolic and chloroformic. *African Journal of Biotechnology* 10:4932-4940.
- Sales PMD, Souza PMD, Simeoni LA, Megalhaes PDO, Silveria D (2012). Pharmacophore mapping and virtual screening on human
- Sharma G, Sapkota B, Lamichhane G, Adhikari M, Kunwar P (2018). Screening of antioxidant and antimicrobial property of leaves of *Acacia rugata* (Lam). *International Journal of Biomedical Science* 1: 93-98.
- Singh AG, Hamal JP (2013). Traditional phytotherapy of some medicinal plants used by Tharu and Magar communities of western Nepal, against dermatological disorders. *Scientific World* 11:81-89.
- Soni KK, Soni S (2017). *Eclipta alba* (L.) An Ethnomedicinal Herb Plant, Traditionally Use in Ayurveda. *Journal of Horticulture* 4:1-2.
- Subedi L, Timalisina S, Duwadi P, Thapa R, Paudel A (2014). Parajuli K. Antioxidant activity and phenol and flavonoid contents of eight
- Wink M (2008). Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. *Current Drug Metabolism* 9: 996-1009.
- Young IS, Woodside JV (2001). Antioxidants in health and disease. *Journal of Clinical Pathology* 54:176-186.
- Zhou D, Ruan J, Cai Y, Xiong Z, Fu W, Wei A (2010). Antioxidant and hepatoprotective activity of ethanol extract of *Arachniodes exilis* (Hance) Ching. *Journal of ethnopharmacology* 129(2):232-237.