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Research Article

Antioxidant and Antidiabetic Potential of Extracts from Selected Species of *Smilax* L.

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Abstract

Smilax is a potential medicinal plant of the family Smilacaceae, distributed in Nepal's tropical, sub-tropical, and temperate zones. The present work focuses on preliminary phytochemical analysis, antioxidant properties, and antidiabetic activity of methanolic extracts from four different species of *Smilax*. Preliminary qualitative analysis had shown the presence of almost all tested secondary metabolites in methanolic extracts. Although, antioxidant activity was shown by all species of *Smilax*, the lowest IC_{50} value was found in methanolic extract of *S. ferox.* Percentage α-amylase inhibition activity was highest in the methanolic extract of *S. perfoliata* (79.13%) and lowest in *S. lanceifolia* (50.0%). Similarly, the highest percentage of α-Glucosidase inhibition activity was shown by the methanolic extract of *S. lanceifolia* (98.08%) and the lowest by *S. perfoliata* (29.71%). The present findings pave the way for a further comprehensive phytochemical investigation to validate their medicinal properties.

Keywords: α-amylase, α-glucosidase, antioxidant, flavonoids, phenol

Introduction

Smilax L., with about 255 known species (Xu & Chang, 2017), is the only genus of the family Smilacaceae. It is well-known in both temperate and tropical habitats. Species of *Smilax* are among the most abundant and easily recognized climbing plants in many ecosystems (Dong et al., 2021). They are distributed mostly in the forest areas of the Central and Eastern parts of China, Thailand, Vietnam, Myanmar, India and Nepal.

The species of *Smilax*, locally known as 'kukurdaino' are among the most important medicinal plants. In Nepal, there are 16 species of *Smilax* distributed mostly from tropical, to temperate zones, while a few species like *S. menispermoidea* DC and *S. minutiflora* A.DC are also found in the sub-alpine zone (Shrestha et al., 2022). These are among the multi-potential medicinal plants and are used by different tribes and communities of various parts of Nepal as well as other countries. The roots, leaves, and tender shoots are variably used in treating diseases like jaundice, skin problems, toothache, urinary complaints, muscular sprain, stomach pain, rheumatic arthritis, infertility, as a sexual stimulant, abnormal semen discharge, uterine diseases, dysentery, malaria, tuberculosis, wound healing, and also used as antibiotic, antifungal, antiseptic and blood purifier

(Sedai, 2010; Uprety et al., 2012; Kunwar et al., 2010). The plants are also used as vegetables in different countries. A single species has been used in various treatments for example *S. ovalifolia* root decoction is used in venereal disease, to increase appetite, and to cure different types of gastric disorders and sexual diseases (Shah, 2015). Besides, leaves and plants (Harba et al., 2009), shoots are also used as vegetables and unripe fruits are eaten (Acharya & Acharya, 2010).

Various authors have investigated the chemical constituents of *Smilax*. Preliminary phytochemical analysis of *S. zeylanica* revealed the presence of alkaloids, flavonoids, tannins, triterpenoids, and sterols in leaf and fruit extracts (Hossain et al., 2013). Additionally, several biological activities of *Smilax* extracts including anti-inflammatory (Hirota et al., 2016), antifungal (Belhouchet et al., 2008), anti-hyperuricemic (Chen et al., 2011), and antioxidant activities (Shah, 2015) have been also pointed out. Despite valuable medicinal properties, several species belonging to the genus *Smilax* are still unexplored phytochemically as well as for their bioactivity. The present study aimed to determine major phytochemicals in different species of *Smilax* and evaluate their biological activity to verify their ethnomedicinal potential and prospective pharmaceutical applications.

Materials and Methods

Collection and identification of plant materials

Plant leaves were collected from different parts of Kathmandu Valley during the flowering season. Identification was done with the help of comparison with sample species from the National Herbarium and Plant Laboratory (KATH), Godawari, Lalitpur.

Herbarium specimens were prepared and deposited in Tribhuvan University Central Herbarium (TUCH) of the Tribhuvan University for further reference. Details of collected sites and GPS coordinates are given below (Table 1, Figure 1).

Table 1: Collection sites of plant sample.

S.N.	Species	Collection site	Elevation (m)	GPS coordinates
1	*Smilax aspera* L	Lakuri bhanjyang, Lalitpur	1250	27° 61' 78" N, 85° 41' 43" E
2	*S. ferox* Wall. ex Kunth	Champadevi, Kathmandu	1610	27° 38' 71" N, 85° 15' 83" E
3	*S. lanceifolia* Roxb	Champadevi, Kathmandu	1650	27° 38' 71" N, 85° 15' 83" E
4	*S. perfoliata* Lour.	Champadevi, Kathmandu	1730	27° 38' 71" N, 85° 15' 83" E

Figure 1: Herbarium specimens of *Smilax* species; *S. aspera* (a), *S. ferox* (b), *S. lanceifolia* (c), *S. perfoliata* (d).

Preparation of plant extract and extract dilution

The collected plant materials were cleaned and powdered with the help of a grinder. Four-gram fine powder of each plant sample was taken separately and dissolved in 40 ml of 99% methanol. These samples were subjected to sonication for two hours and centrifuged in falcon tubes then the solvent was filtered and subjected to evaporation at reduced pressure in a rotatory evaporator. The condensed

extract thus obtained was transferred to a clean weighed glass petriplate and allowed to dry at room temperature. Then dried plant extract was removed from a petriplate with the help of a sterile blade and placed in small tubes. From the stock, 100 mg of crude plant extract was weighed accurately and dissolved in 1 ml methanol to make methanolic extracts and was used for the quantification of total phenolic content and total flavonoid content, and also for the evaluation of antioxidant and antidiabetic potential of plant extracts.

Qualitative phytochemical analysis

The methanolic extracts were used to screen for the presence of various secondary metabolites such as flavonoids, glycosides, steroids, terpenoids, alkaloids, tannins, and saponins by following the protocols suggested by Harborne & Baxter (1995) and Todkar et al. (2010).

Antioxidant activity via DPPH free radical scavenging assay

The antioxidant activity of the extract of four species of *Smilax* and standard (ascorbic acid) was evaluated based on the radical scavenging effect of the stable 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical activity following the protocol of Singh et al. (2002). Different concentrations of plant extract and ascorbic acid (10-100 µg/ml) were prepared in methanol in the clean test tubes. A 0.5 ml sample of plant extract as well as ascorbic acid of each concentration was taken separately in clean test tubes. To this sample, 0.5 ml of the 0.2 mM DPPH solution was added. The tubes were shaken uniformly for proper mixing and incubated in the dark for half an hour. The control was prepared as above but without the plant extract or ascorbic acid, and methanol was taken as blank. Then absorbance was taken on a spectrophotometer at 517 nm. The radical scavenging activity was calculated using the following formula:

Percentage of DPPH radical scavenging activity = [(control abs. - sample abs.) / control abs.] * 100

A standard graph was plotted by the concentration of ascorbic acid on the X-axis and the percentage of DPPH scavenging activity on the Y-axis. Based on this standard graph IC_{50} was calculated using the linear equation of graph $Y = a* X + b$.

$$IC_{50} = (50 - b) / a$$

Where, X = concentration, Y = percentage of DPPH radical scavenging activity, a and b are the coefficient and constant of the linear equation. The IC_{50} value of the different species was compared. The species having the lowest IC_{50} was considered to have the best antioxidant properties.

Evaluation of antidiabetic activity

The antidiabetic activity was measured by α-amylase and α-glucosidase inhibition assays.

α-amylase inhibition assay

The α-amylase inhibition of extracts was assessed following the protocol of Ahmed et al. (2009) with modification. Firstly, the reaction medium was prepared by dissolving porcine pancreatic amylase (Sigma Aldrich, Germany) in 0.1 M potassium phosphate buffer (pH 6.8) to make a final concentration of 0.1 units/ml. Then 10 µl of pure methanol or methanolic solution of Acarbose (ARISTO Pharmaceutical Pvt. Ltd., India) or plant extract were mixed with 390 µl of reaction medium in a clean test tube. The respective negative control was also prepared by adding 200 µl of DNS reagent (Sigma Aldrich, Germany) to the reaction mixture. The tubes were incubated at 37 ˚C for 10 minutes. Then 200 µl of 1% soluble starch (Fisher Scientific, India) was added and the tubes were incubated for another 20 min. Then 200 µl of DNS reagent was added in all the tubes (positive control). The tubes were kept in a boiling water bath for 10 min and allowed to cool. Then 4 ml of distilled water was added in each tube and absorbance was taken at 540 nm in a spectrophotometer.

Percentage of α-amylase inhibition activity = (control abs. – sample abs./ control abs.) × 100

α-glucosidase inhibition assay

To test the α-glucosidase inhibition assay, the protocol of Si et al. (2010) was followed with slight modification. For this, the first reaction medium was prepared by dissolving Maltose (Sigma Aldrich, Germany) in 0.1 M Potassium phosphate buffer (pH 6.4) to a final concentration of 25 mM. Then 1 ml of the reaction mixture was taken in a clean test tube and 20 µl of pure methanol or methanolic solution of acarbose (1 mg/ml) or plant extract solution (1 mg/ml) was added to the previously taken reaction mixture solution. Respective negative controls were

also prepared for each methanol, acarbose, and plant extract. Now 250 µl of Na_2CO_3 was added in all test tubes of negative control. Then tubes were incubated for 5 minutes at 30°C. A 20 µl of α-glucosidase (Sigma Aldrich, Germany) was added in all test tubes including positive and negative control, and incubated for 45 minutes at 30 °C. Then 250 µl Na_2CO_3 was added in all tubes of positive control. At last, 710 µl of distilled water was added to make the volume of 2 ml and absorbance was measured at 405 nm in a UV spectrophotometer.

Percentage of α-glucosidase inhibition activity = (control abs. – sample abs./ control abs.) × 100

Data analysis

All the experiments were performed in triplicates for each sample and values were reported as mean ± S.D. The statistical analysis was done using Microsoft Excel 2013.

Results and Discussion

Qualitative phytochemical analysis

A summary of different tests performed on methanolic extracts from species of *Smilax* is tabulated in Table 2. Methanolic extracts showed the presence of almost all phytochemicals tested in different proportions except the absence of glycosides and the presence of saponin in the extracts of *S. aspera*. The preliminary phytochemical analysis provides a rough outline for the major secondary metabolites of the plant species. Different phytochemicals have been found to possess a wide range of activities, which may help protect against diseases. For example, alkaloids protect against chronic diseases, saponins protect against hypercholesterolemia and antibiotic properties, and steroids and triterpenoids show analgesic properties (Murali et al., 2011). Flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic, etc. (Dai & Mumper, 2010, Uthaman et al., 2024).

Several studies also showed the presence of alkaloids, phenol, flavonoids, tannins, steroids, and saponins in different species of *Smilax* (Dhanya Shree et al., 2018; Chandana et al., 2019; Paneru & Rajbhandari, 2020). Steroids, saponins, and flavonoids were thought to be the characteristic constituents of the genus *Smilax* (Ao et al., 2011). According to Saravanakumar et al. (2014), methanolic extract of *Smilax china* showed higher affinity for various phytochemicals than other solvents like chloroform, acetone, N- hexane, ethanol, and aqueous. As reported by Shah (2015) preliminary phytochemical analysis of the leaf extract showed the presence of varied phytochemicals like carbohydrates, protein, amino acid, saponin, alkaloid, steroid, terpenoid, phenol, glycoside and flavanoid in methanolic extract than aqueous, ethanolic, petroleum ether and chloroform extracts. In the present study, qualitative phytochemical analysis of methanolic extracts of different species of *Smilax* had shown varied phytochemicals like alkaloids, polyphenols, flavonoids, tannin, glycosides, terpenoids, and steroids suggested its potential against ailments.

Antioxidant activity

The antioxidant activity of species of *Smilax* was compared with standard ascorbic acid (Figure 2).

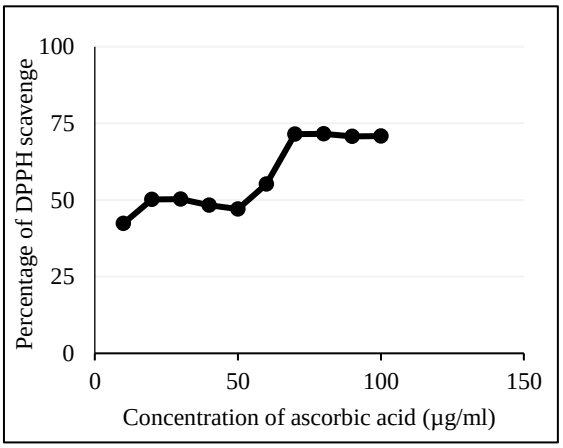

Figure 2: Standard curve of ascorbic acid.

The highest scavenging activity was seen in methanolic extracts of *Smilax ferox* and the lowest in *Smilax aspera* (Figure 3). The percentage radical scavenging activity of other species was found between these two extremes. Higher concentrations of plant extract showed an increasing percentage of radical scavenging activity in DPPH revealing concentration-dependent scavenging properties.

The antioxidant activity of different species of *Smilax* was compared with standard ascorbic acid. The highest IC_{50} value was obtained for the

methanol extract of *Smilax aspera* (188.29 µg/ml) and the lowest was found in *Smilax ferox* (48.05 µg/ml). As the lowest IC_{50} value shows strong antioxidant activity so graph shows *Smilax ferox* with strong antioxidant activity in comparison to other species of *Smilax* (Figure 4).

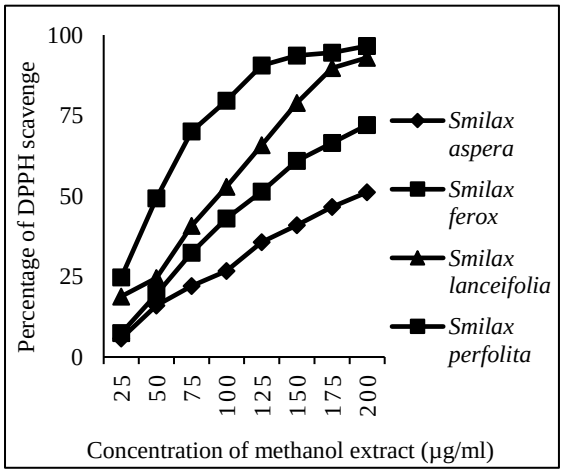

Figure 3: Percentage of radical scavenging activity by methanol extract of *Smilax* species.

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. There is an increasing interest in natural antioxidants, for example, polyphenols and flavonoids that are present mostly in medicinal plants, which might help to prevent oxidative damage (Antolovich et al., 2002). The DPPH is a stable free radical, which has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Sanchez-Moreno, 2002). The methanol extract of *Smilax zeylenica* was found more effective than the aqueous extract (Murali et al., 2011). High amounts of phenol in the leaves resulted in the highest antioxidant capacities, as measured by DPPH, ABTS and FRAP assays in comparison to other samples of seed, stem and root (Acidri et al., 2020). The antioxidant activity depends on the extraction method and solvent used for extraction because the presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent (Barchan et al., 2014). Barchan et al. (2014) found that polar solvents were important for obtaining fractions with high antioxidant activity. In the present study, the methanol extract of *Smilax ferox* showed strong antioxidant activity. Therefore, one of the possible mechanisms of methanol extract's strong antioxidant activity may be the presence of a good amount of phenolic and flavonoid contents as suggested by Uddin et al. (2015).

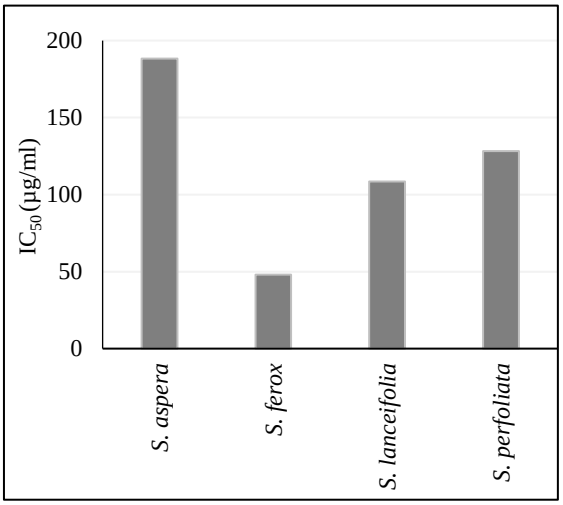

Figure 4: IC_{50} value of DPPH radical scavenging activity of methanol extracts of different *Smilax* species.

Antidiabetic activity

α-amylase inhibition activity

α-amylase inhibition activity of *Smilax* was found highest in the methanol extract of *S. perfoliata* (79.13%) and lowest in *S. lanceifolia* (50.0%) (Figure 5).

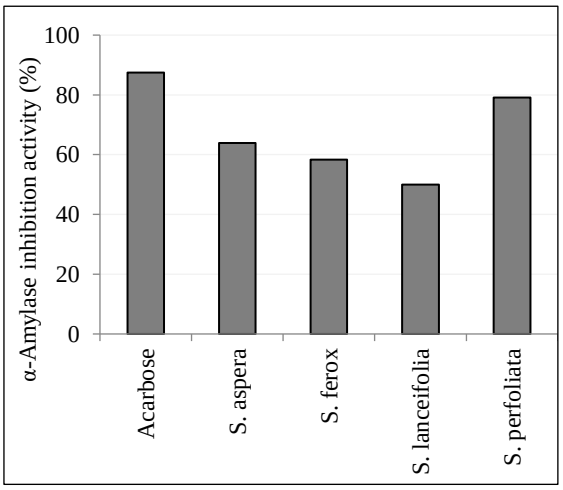

Figure 5: α-amylase inhibition activity of methanol extracts of *Smilax* species.

α-glucosidase inhibition activity

The highest percentage of α-glucosidase inhibition was shown by the methanolic extract of *S. lanceifolia* (98.08 %) and the lowest by *S. perfoliata* (29.71%) (Figure 6).

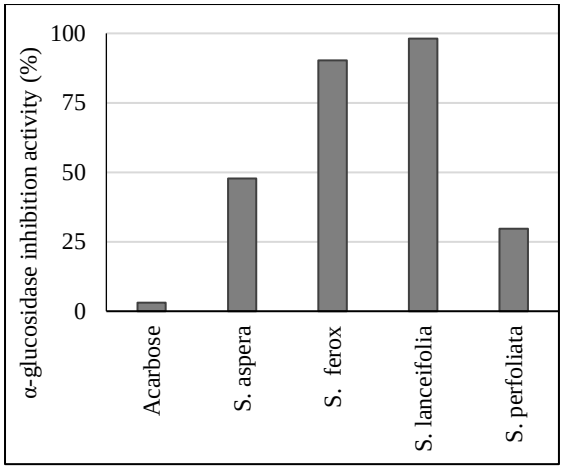

Figure 6: α-Glucosidase inhibition activity of methanol extracts of *Smilax* species.

Non-insulin-dependent diabetes mellitus or type-II diabetes mellitus is one of the most common and serious metabolic disorders with abnormally high blood glucose levels (hyper-glycaemia) due to defects in insulin secretion, action, or both (Rajesh & Perumal, 2014). Glucosidase and pancreatic amylase play a critical role in carbohydrate digestion and glycoprotein processing. So, the inhibitors of these enzymes might be used to treat diabetes (Perez-Najera *et al*., 2018). These inhibitors generally lower blood sugar levels by slowing or decreasing carbohydrate breakdown in the intestine (Nguyen et al., 2020). In *Smilax excelsa* different extract solvents had shown different inhibition activity. Among the solvents used, the methanol extract of leaves showed the highest (98.5%) α-amylase inhibition activity whereas α-glucosidase inhibition was the lowest (1.5%). Similarly, the ethanol extract of the stem also showed the highest (98.5%) α-amylase inhibition activity and α-glucosidase inhibition activity (58.9%) (Dehghan et al., 2016). According to Bhati et al. (2011), hydroalcoholic and aqueous extracts of *S. china* showed a significant reduction in blood glucose levels in comparison with the standard drug (gliclazide) in alloxan-induced diabetic rats. In the present research, *S. lanceifolia* and *S. ferox* had shown good α-glucosidase inhibition activity but α-amylase inhibition activity showed the least activity. The anti-diabetic effect of the extracts of *Smilax* species may be due to the presence of compounds like flavonoid, phenol, alkaloid, and other related compounds which could be beneficial to carbohydrate metabolism. This required further exploration and study to identify the detailed molecular mechanism of action.

Conclusion

The present study revealed variations in phytochemical constituents, antioxidant potential and antidiabetic activities among the methanolic extracts of different species of *Smilax*. For example, extracts of *S. ferox* possess the highest antioxidant potential than others. Similarly, extracts of *S. perfoliata* show the highest α-amylase inhibition activity comparable to the acarbose (control). Furthermore, the extracts of all tested species show higher α-glucosidase inhibition activity than acarbose. This is preliminary information that came out from in vitro studies. However, further in vivo scientific work will be recommended to ensure the medicinal properties of these plants.

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