

ANTIOXIDATIVE AND ANTIDIABETIC PROPERTIES OF SKUNK VINE (*Paederia foetida* L.)

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Abstract

This experiment was executed at the research field of Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh during 2019 to 2020. In this study, methanolic extract of different plant parts was used for observing the antioxidant properties such as total phenolic, flavonoids, carotenoids and anthocyanins content in Skunk vine (*P. foetida*). Results showed that, the total phenolic were more or less similar in leaf (357.62 µg/g) and stem (349.42 µg/g) and that were significantly higher than that of root extract (233.56 µg/g). The highest amount of flavonoid content (602.71 µg/g) was recorded in leaf extract and the content was more or less similar in stem (493.16 µg/g) and root extracts (501.86 µg/g). Maximum amount of anthocyanin content was recorded in leaf (42.04 µg/g) that was identical with that of stem extract (39.68 µg/g) and statistically the lowest amount in root extract (27.44 µg/g). The fresh leaf extracts contained higher carotenoid content (41.96 mg/g) than stem (25.58 mg/g). Total antioxidant activity in respect to percentage of DPPH ((2,2-diphenyl-1-picrylhydrazyl) scavenging efficiency, leaf extract showed 63.78% inhibition efficiency which were significantly higher than that of stem (45.75%) and root (24.19%). α -glucosidase activity was greatly inhibited (59.13%) by leaf extract at the concentration level of 1.5 mg/mL which was better than that of stem (45.60%). Inhibition of α -amylase activity was recorded up to 46.23 and 42.57% by leaf and stem extracts, respectively which were statistically similar. The present study suggests that medicinal plant Skunk vine (*P. foetida*) has both antioxidative and antidiabetic properties which would be the good source for producing safe natural drugs.

Keywords: Total phenolic content, flavonoid, carotenoid, anthocyanin, % DPPH, α -glucosidase and α -amylase, Skunk vine (*Paederia foetida*).

Introduction

Human beings greatly depend on medicinal plants for their food and health security. Among the plants present in nature, the medicinal plants are being considered for the most exclusive source of life saving drugs (Reddy and Reddy, 2008). The country like Bangladesh which is enriched with plant resources have long historical background for the therapeutic uses of medicinal plants (Nandkoni 2002; Khare 2007). Skunk vine (*Paederia foetida* L.) 'belonging' to the family

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'Rubiaceae', locally named as 'gandhabadali' and widely distributed throughout the Asia (Reddy and Reddy, 2008). The plant has lot of therapeutic uses such as leaf extract is used for maintaining stomach ailment, allergy, jaundice and physical weakness. However, the plant has been reported to be a reservoir of many of the important secondary metabolites like iridoid glycosides, sitosterol, stigmasterol, alkaloids, carbohydrates, proteins, amino acids and volatile oils (Nandkoni 2002; Khare 2007). In living organisms, Reactive Oxygen Species (ROS) generated during oxidative stress are very responsible for the degradation and degeneration of cellular materials like DNA, RNA, proteins and occurrence of many important diseases like gastric disorder, ulcer, cancer, arthritis hepatic disorder etc. The enzymatic antioxidants such as super oxide dismutase, peroxidase and catalase etc. and non-enzymatic antioxidants like phenols, flavonoids etc. present in the plant that may neutralize oxidative stress generated in plants.

Though having lot of potentials of secondary metabolites, the little efforts were made in ROS scavenging capacity and antioxidant activity of medicinal plant Skunk vine (*P. foetida*). Although, recent investigation reported the antioxidant activity in the leaf of Skunk vine (Nayak *et al.*, 2015), more efforts should be needed to clarify by using different plant parts. However, no report has been made yet in Bangladesh. Besides antioxidant activity, some medicinal plants have

been reported to show the inhibition of α -amylase and α -glucosidase enzymes, interference of which were supposed to cause diabetic disease type II (Mayur *et al.*, 2010; Shai *et al.*, 2010). Both of those enzymes are key regulators for the digestion of starch and increasing blood glucose. Therefore, inhibitory efficiency of those enzymes are very crucial for the management of diabetic type II. Since therapeutic chemical drugs have several side effects (Chakrabarti and Rajagopalan 2002; Kimmel and Inzucchi 2005), the plant products showing inhibitory effect to those enzymes was suggested to use with minimal side effects (Tarling *et al.*, 2008). Therefore, continuous searching of safer plant products in potential medicinal plant like Skunk vine would be very interesting and essential for treating patients having diabetic type II. Considering the facts, the present investigations were made to find out the antioxidative and antidiabetic properties of Skunk vine using different plant parts.

Materials and methods

The fresh and fully expanded leaves, stems and roots of Skunk vine were collected from the research field of the Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh, during 2019 to 2020. After repeated washing, the collected materials were allowed for drying for 4-5 days at room temperature. The dried materials were chopped and crushed into powder using mortar and pestle and stored in air tight containers. Sample of 5 g of powder was mixed into 50 ml methanol and allowed to filtration. The extract

was then concentrated using rotary evaporator and stored at -20°C until further use.

The extracted material was used for the determination of total phenolic contents using Folin ciocalteu method as described by Singleton and Rossi (1965). The absorbance of the reaction mixture was measured at 765 nm. The results were expressed as $\mu\text{g/g}$ (Gallic Acid Equivalent, GAE).

The extract of plant materials was used for the determination of flavonoid contents using aluminium-chloride colourimetric assay (Biju *et al.*, 2014). Quercetin at different concentration was used as standard solution. The absorbance of the extracts and standard solutions was measured at 510 nm using a UV/Visible spectrophotometer. The results were expressed as microgram; Quercetin equivalents (QE) per gram of extracts ($\mu\text{g/g}$).

Anthocyanin content was determined using the method followed by Hughes and Smith (2007). The anthocyanin content was expressed as micrograms for cyanidin-3-glucoside equivalent per gram of dry sample ($\mu\text{g/g}$).

Total carotenoid content was determined according to the procedure of Lachman *et al.*, (2003) with slide modification. Total carotenoid content was expressed as mg of lutein equivalent per gram of fresh weight sample (mg/g, LE).

The plant extract was allowed for showing DPPH radical scavenging activity by following the method of Xu *et al.* (2010). Ascorbic acid as antioxidant was used to make reference solution. The reaction mixture of plant extracts and DPPH (2,2-diphenyl-1-picrylhydrazyl) were kept in dark condition at room temperature for 30 min. Then the absorbance of samples was measured at 517 nm using a spectrophotometer. The absorbance of control and blank samples were also determined to make comparison with the absorbance of plant extract. The inhibition percentage was calculated by the following equation:

% DPPH radical scavenging activity = $[(A_0 - A_1)/A_0] \times 100$. Where A_0 = absorbance of the control and A_1 = absorbance of the sample.

The plant extracts was allowed for α -glucosidase inhibitory activity by following the method used by Laoufi *et al.* (2017). Different concentration of leaf and stem extracts (0.05, 0.1, 0.5, and 1.5 mg/mL) were used in reaction mixture containing α -glucosidase enzyme. Acarbose as commercial inhibitor at different concentrations (0.005, 0.01, 0.1 and 0.2 mg/mL) was used as positive control. The absorbance of the reaction mixture will be recorded at 405 nm using spectrophotometer. The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula:

Inhibition of α -glucosidase activity (%) = $((\text{Abs C} - \text{Abs S})/\text{Abs C}) \times 100$, where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (plant extract or acarbose).

Inhibition of α -amylase activity of the plant's extracts was determined by analysing the reducing power of released oligosaccharide from soluble starch by following the method of Bernfeld (1955). Different concentration of leaf and stem extracts (0.05, 0.2, 0.5, 1.0 and 5.0 mg/mL) were used in reaction mixture containing porcine pancreatic α -amylase enzyme. Acarbose as commercial inhibitor at different concentrations (0.005, 0.01, 0.1 and 0.2 mg/mL) was used as positive control. Inhibition of α -amylase activity was determined by measuring the absorbance of the reaction mixture at 540 nm, using spectrophotometer. The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula:

Inhibition of α -amylase activity (%) = $((\text{Abs C} - \text{Abs S})/\text{Abs C}) \times 100$, where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (plant extract or acarbose).

All the experiments were conducted by following CRD (Completely Randomized Design) with at least three replications. Student t-test was used for analyzing the data.

Results and Discussion

Since commercial drugs have long term side effects and lot of complications, searching of antioxidant potentials and plant based natural products in medicinal plant Skunk vine is very imperative for maintaining good health. The results of the experiments were discussed as following subheads.

Total phenolic content in Skunk vine

Higher phenolic content of the plant shows increased ability to the reduction of free radicals produced by the reactive oxygen species (ROS). It was determined of total phenolic in the methanolic extract of dried leaf, stem and roots of Skunk vine. The values of total phenolic were expressed as $\mu\text{g/g}$ (GAE; gallic acid equivalent). Among the plant parts used, the total phenolic was statistically similar in leaf (357.62 $\mu\text{g/g}$) and stem (349.42 $\mu\text{g/g}$) and root contained lowest amount of polyphenols (233.564 $\mu\text{g/g}$) which was significantly different as compared to leaf and stem (Fig. 1). In another investigation, total polyphenol content of the leaf extract was reported in different cultivated and wildy grown Skunk vine and found the ranges of phenolic contents; 0.063-3.044 mg GAE/ml and the data of which are little bit different than that of present investigation (Sahoo and Bhatnagar, 2015). In previous investigation, three different traditional medicinal plants were screened for phytochemical analysis; where Skunk vine showed the highest phenolic content 138.33 ± 6.415 mg GAE/g (Devi *et al.*, 2016). In another experiment Skunk vine showed comparatively lower phenolic content 165.81 mg GAE/100 g FW among seven different medicinal plants (Islam *et al.*, 2018). The variation is due to the age, cultivar,

climatic condition of the plant growing locations, extraction methods, assay techniques and chemical as well.

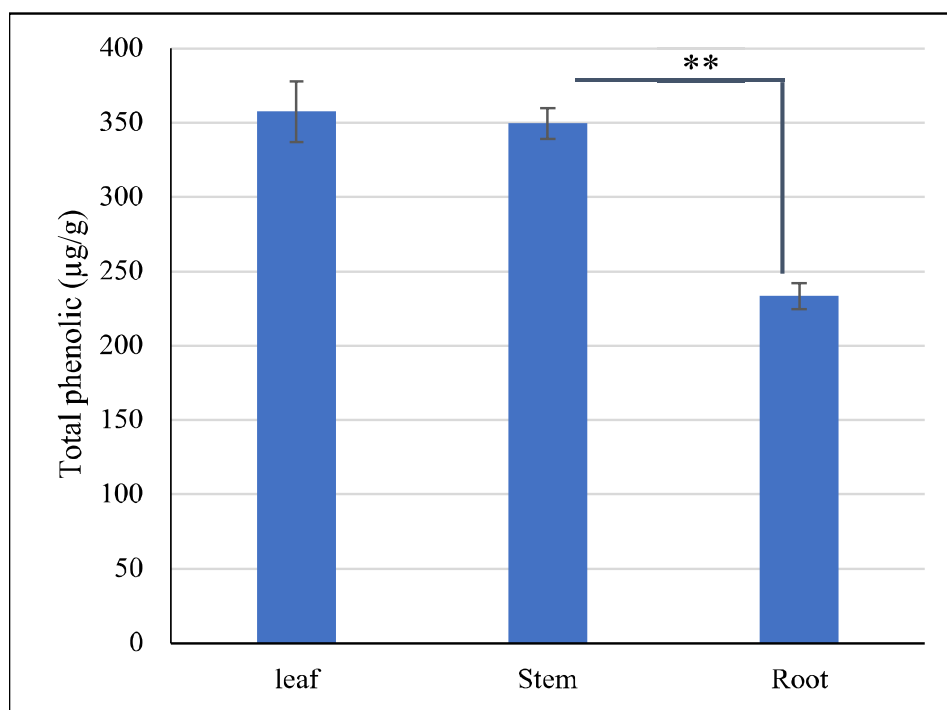


Fig. 1. Total phenolic content of leaf, stem and root extracts of Skunk vine. Error bars indicate the standard deviation ($n = 3$). Asterisks indicate significant difference ($P < 0.01$; t-test).**

Flavonoid content in Skunk vine

The flavonoids are very essential secondary metabolites acting as antioxidant and providing protection to cardiovascular disease by scavenging superoxide and hydroxyl radicals (Harborne, 1988). In this study, flavonoid content in different plant parts of Skunk vine was determined and identified the plant as the potential reservoir of this secondary metabolite. The content of flavonoids was expressed as $\mu\text{g/g}$ (QE; Quercetin equivalent). The highest amount of flavonoid content ($602.71 \mu\text{g/g}$) was recorded in leaf extract and the amount of flavonoid content was significantly lower in stem ($493.16 \mu\text{g/g}$) and root extracts ($501.86 \mu\text{g/g}$) (Fig.2). The flavonoid content of leaf extract of different cultivated and wild species of

Skunk vine were recorded as 0.627 mg QE/ml and $0.647/\text{ml}$ respectively (Sahoo and Bhatnagar 2015). The result is more or less consistent to the flavonoids content of leaf extract in our observation. The results also suggest that leaf is the best source of flavonoid content in Skunk vine.

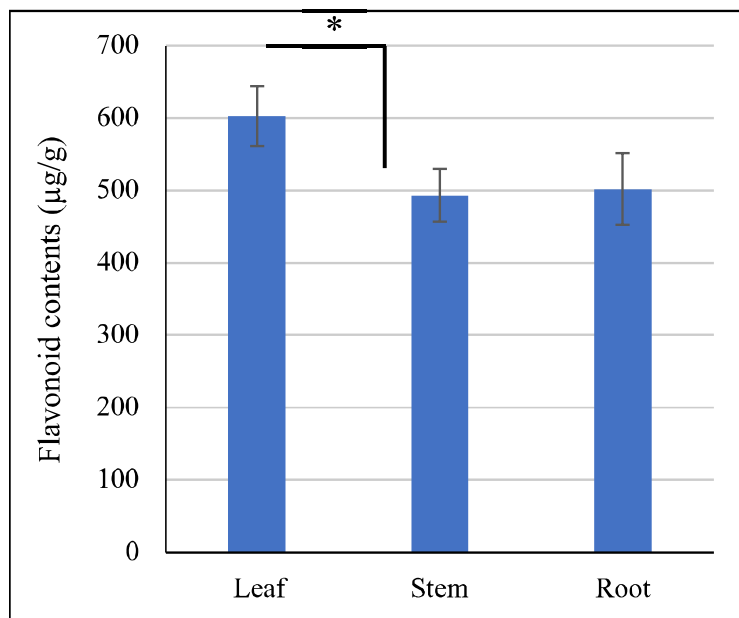


Fig. 2. Flavonoid content of leaf, stem and root extracts of Skunk vine. Error bars indicate the standard deviation ($n = 3$). Asterisk indicates significant difference ($*P < 0.05$, t-test).

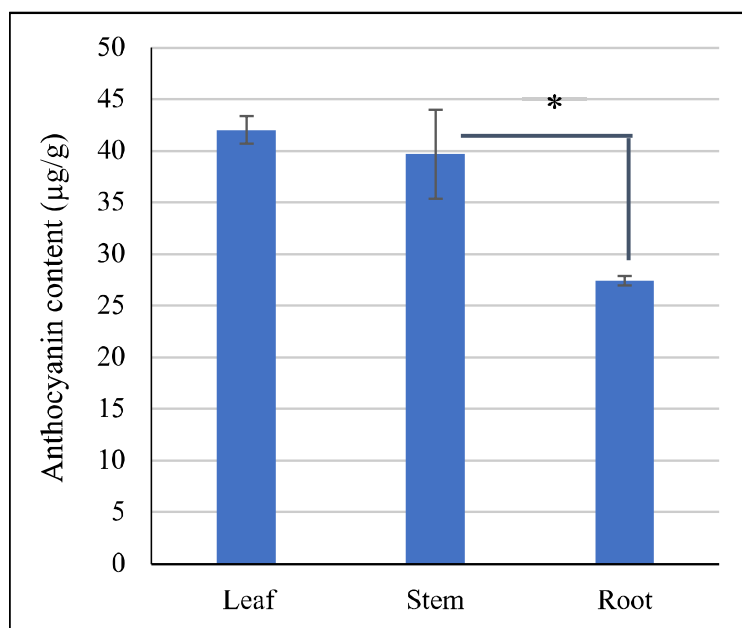


Fig. 3. Anthocyanin content of leaf, stem and root extracts of Skunk vine. Error bars indicate the standard deviation ($n = 3$). Asterisk indicates significant difference ($*P < 0.05$, t-test).

Anthocyanin content in Skunk vine

Anthocyanins have an antioxidant potential twice than other known antioxidants, such as catechin vitamin E, BHA (butylated hydroxyanisole) and BHT (He and Giusti, 2010). In the present study, it was found that leaf and stem contained more or less similar amount of anthocyanin content such as 42.04 $\mu\text{g/g}$ and 39.68 $\mu\text{g/g}$, respectively (Fig. 3). Among the plant parts used, significantly the lowest amount of anthocyanin content was recorded in root (27.44 $\mu\text{g/g}$). Although, anthocyanin content of the medicinal plants were reported in different medicinal plants (Mazandarani *et al.*, 2011; Asem *et al.*, 2015), the results of present study suggest that Skunk vine irrespective of leaf and stem are very good source of anthocyanins.

Carotenoid content in Skunk vine

Carotenoids play a great role in reducing risk for several human disorders, including various types of cancer, cardiovascular or ophthalmological diseases (Mayne, 1996). Along with phenols, flavonoids and anthocyanin, the carotenoids content was also analyzed in the fresh leaf and stem of Skunk vine and observed the plant as the rich source of carotenoids. Although both leaf and stem extracts contained carotenoids such as 41.96 mg/g and 25.58 mg/g, the leaf showed better performance than stem (Fig. 4). A lot of investigations reported that medicinal plant as the good source of carotenoids which has great value for scavenging increased ROS in the human body during health disorder. In previous investigation carotenoid content was reported in Skunk vine leaf as 2.805672 ± 0.13424 mg/g (Nayak *et al.*, 2015) which is much lower than that of present observation. The variation is due to the plant species, extraction materials and method of estimation.

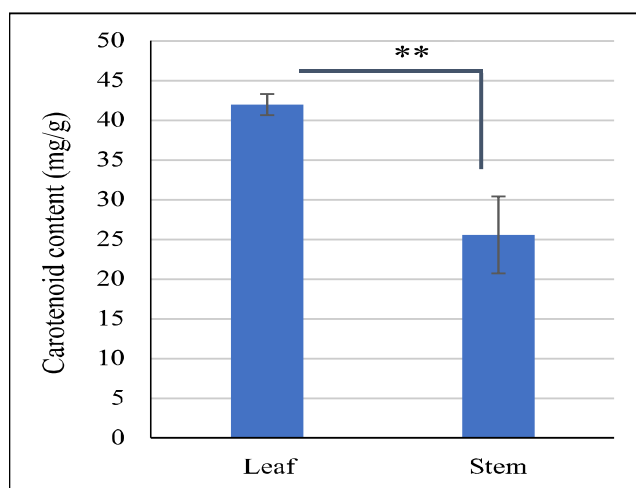


Fig. 4. Carotenoid content of leaf and stem extract of Skunk vine. Error bars indicate the standard deviation ($n = 3$). Asterisks indicate significant difference (** $P < 0.01$; t-test).

Total antioxidant activity

Total antioxidant activity was also analyzed through % DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging ability of the extracts of leaf, root and stem of Skunk vine. A great contribution of the plant parts was found for scavenging DPPH although root showed little performance in this respect. The leaf extract showed great performance; 63.78% inhibition which is significantly higher than that of stem extract (45.75%) (Fig. 5). Root showed the lowest performance (24.19%) than leaf and stem. Another observation with Skunk vine leaves showed the great efficiency for scavenging DPPH and it was about 80% (Upadhya, 2013). In an experiment, shade dried leaves of Skunk vine exhibits a dose dependent DPPH free radical scavenging manner, where about 60% inhibition was recorded by 500 μ /ml Skunk vine extract (Uddin *et al.*, 2014). The antioxidant activity of fresh and dried plant extracts of Skunk vine and *Syzygium aqueum* were recorded using β -carotene bleaching and the 2,2-azinobis(3- ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical cation assay. The percentage of the antioxidant activity for the extract was between 58 and 80% (Osman *et al.*, 2009). The data of which is more or consistent with that of present observation. Along with these, it is also indicated that Skunk vine has very good potential for total antioxidant.

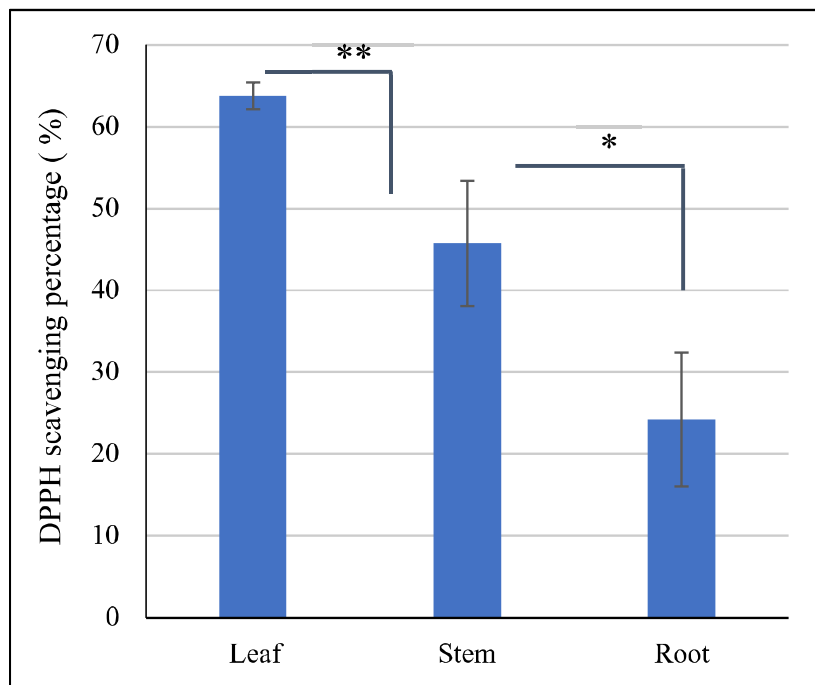


Fig. 5. % DPPH scavenging activity of extracts of different plant parts of Skunk vine. Error bars indicate the standard deviation ($n = 3$). Asterisks indicate significant difference ($*P < 0.05$, $**P < 0.01$; t-test).

Inhibition of α -glucosidase and α -amylase activity

Inhibition of digestive enzymes such as α -glucosidase and α -amylase reduce the amounts of monosaccharide, particularly glucose to be absorbed in the body. Medicinal plants have been considered as the potential source of inhibitor of those enzymes. Therefore, it was analyzed α -glucosidase and α -amylase inhibition efficiency of leaf and stem of Skunk vine and found positive responses of those plant parts to inhibit of those enzymes. Although, α -glucosidase activity was greatly inhibited by the extracts of both leaf and stem, leaf showed better performances than stem. At the concentration of 0.5 mg/mL and 1.5 mg/mL, the leaf extracts showed 39.64 and 59.13 % inhibition capacity of α -glucosidase activity which were higher than that of stem extracts which were 19.25 and 45.60% respectively (Fig. 6, a).

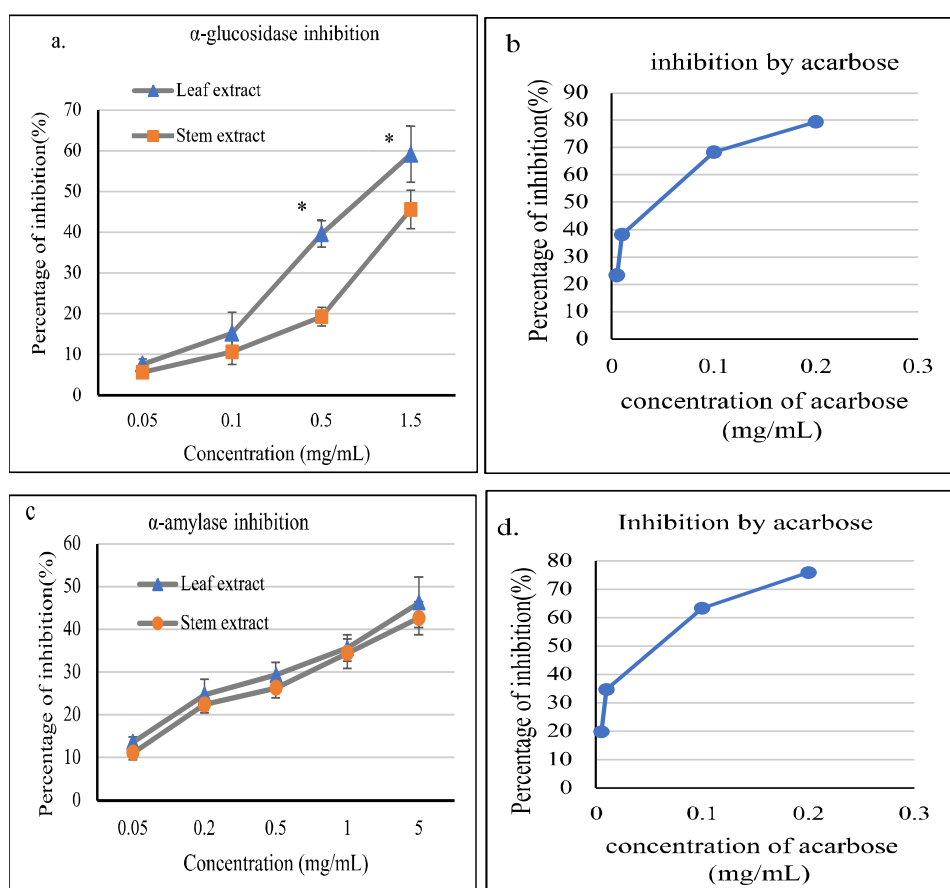


Fig. 6. α -glucosidase and α -amylase enzyme inhibition efficiency of Skunk vine. % inhibition of α -glucosidase activity by leaf and stem extracts (a) and acarbose (b). % inhibition of α -amylase activity by leaf and stem extracts (c) and acarbose (d). Error bars indicate the standard deviation ($n = 3$). Asterisk indicates significant difference ($*P < 0.05$); t-test).

The inhibition percentage was consistent to the positive control acarbose which showed up to 80% inhibition at the concentration level of 0.2 mg/mL (Fig. 6, b). In case of α -amylase activity, it was found more or less similar inhibition efficiency of leaf and stem extracts which were 46.23 and 42.56%, respectively at the concentration level of 5 mg/mL (Fig. 6, c). The positive control acarbose showed about 76% inhibition which was consistent to the inhibition made by plant extracts (Fig. 6, d). Similar trend of results were reported in the leaf extract of Skunk vine by Bhatnagar and Sahoo (2016). Several studies reported the presence of antidiabetic role regarding inhibition of α -glucosidase and α -amylase enzymes in different medicinal plants (Tamil *et al.*, 2010; Okoli *et al.*, 2011; Mohammed and Atiku, 2012). Along with these, present results suggest that the medicinal plant Skunk vine has the potentials to inhibit α -glucosidase and α -amylase enzyme activity.

Conclusion

The plant parts of Skunk vine such as leaf, stem and root showed very good potentials of antioxidants. Leaf showed the best performance in the content of total phenolic, flavonoids, anthocyanins, carotenoids and exhibited highest antioxidant activity (DPPH scavenging ability). The leaf extract also showed higher inhibition of α -glucosidase and α -amylase activity. So, it can be concluded that, Skunk vine has both antioxidative and antidiabetic properties which may be considered as the valuable source of natural safe drug. However, further analysis by changing the extraction methods, assay techniques and application strategies towards animal model might clarify the future implication of this natural asset.

Acknowledgement

The authors are very grateful to the Ministry of Science and Technology (MOST), Bangladesh for providing financial support for successful implementation of the research.

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