

Analysis of phyto-constituents, antioxidant and antidiabetic activities of some medicinal plants of Nepal

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Abstract: The plant kingdom is a wide field to search for natural effective antioxidant and oral hypoglycaemic agents that have slight or no side effects. The present study was carried out to analyze the phytochemical, antioxidant and anti-diabetic activities of six ethno-medical plants of Nepal viz *Berberis aristata*, *Murraya koenigii*, *Xanthium strumarium*, *Cuscuta reflexa*, *Myrica esculenta* and *Syzygium cumini*. Phytochemical screening of methanolic extract of all selected plants showed the presence of different chemical constituents such as alkaloids, polyphenols, flavonoids, terpenoids, saponins, glycosides and tanins. The antioxidant activity was evaluated by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity. Ascorbic acid was used as standard during DPPH assay. *S. cumini* and *M. esculenta* were seen most active with IC₅₀ values 33.35 µg/mL, 35.19 µg/mL, respectively. The anti-diabetic activity was evaluated by using α -amylase inhibition method. Acarbose was used as positive control during the assay. *X. strumarium*, *S. cumini*, *M. esculenta*, *B. aristata* were found to be potent towards α -amylase inhibition with IC₅₀ values 197.21 µg/mL, 206.22 µg/mL, 246.07 µg/mL, 270.04 µg/mL.

Keywords: Phytochemical; Amylase inhibitor; Antioxidant; Diabetes; Medicinal plants.

Introduction

Diabetes is one of the most prevailing diseases around the world. Diabetes has been estimated to affect 177 million people worldwide, and this figure is projected to extend to 300 million by 2025¹. Diabetes is affected by decreased secretion or increased resistance of insulin leading to high postprandial glucose levels. Inhibition of enzymes such as alpha amylase and alpha glucosidase, involved in the digestion of carbohydrates can significantly decrease the postprandial hyperglycemia². Hence, in vitro inhibition of α -amylase, α -glucosidase are currently in trend as the attractive drug targets. Commercially available inhibitors of the digestive enzyme designed as drugs thus far are Acarbose, miglitol, and voglibose etc. But the use of these synthetic inhibitors are limited due to several side effects such as hyperglycemia at higher doses, liver problems, lactic

acidosis, and diarrhea, also like financial cost^{3,4}. Oxidative stress is also major problems in many diabetic patients. It causes oxidative damage to components of cell such as proteins, lipids, and nucleic acid, which is reported to be responsible for the long-term complication in diabetes⁵. It is proved that phytoconstituents of plants, with naturally occurring harmful alternative to synthetic antioxidant products⁶. Plants products with natural antioxidant properties will be antioxidant activity, can be used to control oxidative stress in diabetes patients, and possibly a less a good alternative source as natural enzyme inhibitors in the management of postprandial blood glucose levels.

Traditionally many plants extracts have been used in the management of diabetic all around the world without their scientific evidences. Literature review revealed that many articles had reported the mechanism and

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chemistry of the antidiabetic action of plant extract^{7,8,9}. Nepal is blessed with immense variety of plants with potential antidiabetic activities due to variable climate conditions, geographical variation. But not much researched have been done to seek more safe and efficient antioxidant, α -amylase inhibitors from its natural sources so far. Hence, this study was conducted to assessed α -amylase inhibition activity, phytochemical constituents, and their antioxidant properties of the selected plants of Nepal origin, that is, *Berberis aristata* (D.C.) Family: Berberidaceae, *Xanthium strumarium* (Linn.) Family: Asteraceae, *Myrica esculenta* (Buch. - Ham.) Family: Myricaceae, *Syzygium cumini* (Linn.), Family: Myritaceae, *Murraya koenigii* (Linn.) Family: Rutaceae, *Cuscuta reflexa* (Linn.) Family: Convolvulaceae.

Methods

Enzyme, chemicals and reagent

The enzyme porcine pancreatic α -amylase, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid were purchased from Sigma-Aldrich (Sigma-Aldrich, USA). The solvent used in this study was methanol (Merck, Germany). All other chemicals used in this study were of the commercially available analytical grade.

Plant collection and preparation of their extract

The plants samples were collected from Arghakhanchi District and identified by an expert from the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu. All collected leaves materials for each test sample were washed with running tap water and dried in shade for few days. The dried samples were powdered by mechanical grinder and stored in cool and dry place in air tight container until further use. The methanol extract was prepared for each powdered sample by cold percolation method. All extracts of each plant were combined, filtered through a porous plug of absorbable cotton and concentrated using rotary evaporator. The concentrated product was then dried on a room

temperature until solvent is completely evaporated. Finally, it was stored 4°C in fridge in air tight container until future use.

Qualitative phytochemical analysis

The crude methanol extracts of each plant sample was analyzed for the presence of steroids, glycosides, phenols, tannins, terpenoids, alkaloids, flavonoids, saponins, carbohydrates, quinones as per the standard protocol of the qualitative phytochemical investigation¹⁰.

Free radical scavenging ability assay

DPPH assay was carryout as adopted methods in our laboratory by Subba et al. 2014^{11,12}. In brief, 1 mL each methanol extracts solution containing suitable concentration was mixed with 1 mL, 0.4 mM DPPH in methanol. The reaction mixtures were incubated in dark for 30 min. The absorbance of the reaction mixture was measured at 516 nm in a spectrophotometer immediately after incubation. The DPPH free radical scavenging ability in percent (1 %) was calculated using following formula thus

$$\text{Inhibition (\%)} = \left[\frac{A_0 + A_T}{A_0} \right] \times 100\%$$

Where A_T is the absorbance of samples extract and A_0 is the absorbance without samples extract.

Alpha-amylase inhibition assay

The determination of α -amylase inhibition was carried out by measuring the absorbance of the undigested starch remain under the assay conditions because of enzyme inhibition at 630 nm (blue, starch-iodine complex) following the standard method¹³. Substrate (Potato starch) was prepared by dissolving 200 mg of starch in 25 mL of NaOH (0.4 M) by heating at 100 °C for 5 minutes. After cooling, pH was adjusted to 7.0 and the final volume made up to 100 ml using distilled water. Enzyme solution of 50 μ g/mL was prepared by dissolving 5 mg of 3 U/mL α - amylase in 100 mL of 20 mM phosphate buffer of pH 6.9 containing 6.7 mM

sodium chloride. Acarbose was used as a standard inhibitor for alpha-amylase enzyme. The extracts and acarbose were dissolved in DMSO to give suitable concentrations for assay. To 200 µL of starch solution, 200 µL of acarbose or plant extract at varying concentrations (40, 80, 160, 320, 640, and 1000 µg/mL), was added and pre-incubated at 37°C for 5 min. After this 200 µL of α-amylase solution was added to each of them and then again incubated further for 15 minutes at 37 °C. Then the reactions were terminated by adding 800 µL of HCl (0.1 M). Finally, 1000 µL of iodine reagent (2.5 mM) was added, and absorbance was measured at 630 nm. Each assay was carried out in triplicates. Percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = (1 - [\text{Abs2} - \text{Abs1} / \text{Abs4} - \text{Abs3}]) \times 10$$

Where, Abs1 is the absorbance of the reaction mixture containing plant sample, α-amylase and starch, Abs2 is the absorbance of the reaction mixture of starch and sample Abs3 is the absorbance of the reaction mixture

of α-amylase and starch Abs4 is the absorbance of reaction mixture containing starch only.

Results and discussion

List of collected plants and their medicinal uses

On this research work the plants samples were collected on the basis of their traditional medicine value for the treatment a number of diseases including diabetic (Table 1).

Phytochemical screening

The result of phytochemical screening for each plant extract obtained here is shown as follows in Table 2. Preliminary phytochemical analysis of *B. aristata*, *X. strumarium*, *M. esculenta*, *S. cumini*, *M. koenigii* and *C. reflexa* showed positive results for alkaloids, terpenoids, seroids, and flavonoids. The result is well supported by previously reported literatures^{14, 15, 16}. Srinivas *et al* reported, high levels of phenolic acids, alkaloids, and diterpenes and significant concentrations of saponins, glycosides, fixed oils, and phytosterols in *X. strumarium*¹⁴.

Table 1. Names of the plants, family, parts used, and their medicinal uses

S. N	Scientific Names	Common Nepali Names	Family	Parts used	Medicinal uses
1.	<i>B. aristata</i>	Chautari	Berberidaceae	Leaves	Dysentery, diabetes, leprosy, piles.
2.	<i>M. koenigii</i>	Mitho neem	Rutaceae	Leaves	Ulcers, fractures
3.	<i>X. strumarium</i>	Kaade jhaar	Asteraceae	Shoot	diuretic, jaundice, heart diseases
4.	<i>C. reflexa</i>	Aakase beli	Convolvulaceae	Whole plant	Wounds, bruises, worms
5.	<i>M. esculenta</i>	Kaphal	Myricaceae	Leaves	Diarrhea, cold, arthritis
6.	<i>S. cumini</i>	Jamun	Myrtaceae	Whole plant	Jaundice, leprosy, tuberculosis

Table 2. Phytochemical screening of methanol plant extracts

Plants	Alkaloids	Flavonoids	Glycosides	Polyphenols	Terpenoids	Steroids	Carbohydrates	Saponins	Tannins	Quinones
<i>B. aristata</i>	+	+	+	+	+	+	-	-	+	+
<i>M. koenigii</i>	-	+	-	-	+	+	+	+	+	-
<i>X. strumarium</i>	+	+	-	+	+	-	-	+	+	-
<i>C. reflexa</i>	+	+	-	-	+	+	+	-	+	+
<i>M. esculenta</i>	+	+	-	+	+	-	+	+	-	+
<i>S. cumin</i>	+	+	+	+	+	+	-	+	+	+

Where, (+) =Present and (-) = Absent

DPPH as an antioxidant

The methanol extractives of *B. aristata*, *X. strumarium*, *M. esculenta*, *S. cumini*, *M. koenigii* and *C. reflexa* were subjected for free radical scavenging activity by DPPH assay. It was conducted for each plant extract by using ascorbic acid as standard. In this assay, different concentrations of different extract solution and ascorbic acid solution were incubated at room temperature and their absorbance was recorded by spectrophotometer. The IC₅₀ values of each extracts were calculated. The graph of concentration against the corresponding percentage radical scavenging activity of different samples was plotted (Figure 1) and concentration providing 50% inhibition was determined.

A half maximal inhibitory concentration (IC₅₀) value of *S. cumini*, *M. esculenta*, and *B. aristata* was found 33.35±0.1333 µg/mL, 35.19±0.2641 µg/mL, and 47.28±0.15 µg/mL, respectively (Table 3). IC₅₀ value of the standard, that is, ascorbic acid was found to be 22.17 µg/mL. Among the test plants, *S. cumini*, *M. esculenta*, and

B. aristata have lower IC₅₀ values followed by *M. koenigii*, *C. reflexa*, *X. strumarium*, respectively. The high antioxidant activity of these plants may be due to the presence of phytochemicals such as alkaloids, flavonoids, terpenoids, quinones, saponin and polyphenols, which are known for their antioxidant behavior¹⁵. The result is well supported by previously reported the literatures¹⁶. Antioxidant activity of the methanol extract of *X. strumarium* leaves was previously reported by Rad Sharifi *et al* and Kamboj *et al* and ascribed to the high levels of phenolics and the highest amount of flavonoids content in the leaves extract of *X. strumarium*. Antioxidant potential of the *M. esculenta* had been reported by Rawat *et al*^{17,18}. Moderate DPPH radical scavenging activity was reported for *B. aristata* from Sagarmatha National Park of Nepal by Bhatt *et al*¹⁹. *M. koenigii* curry tree berries are reported as a good natural source of antioxidative compounds to prevent oxidative damage in meat and meat products²⁰. Strong antioxidant activity had been reported for *S. cumini*¹⁵

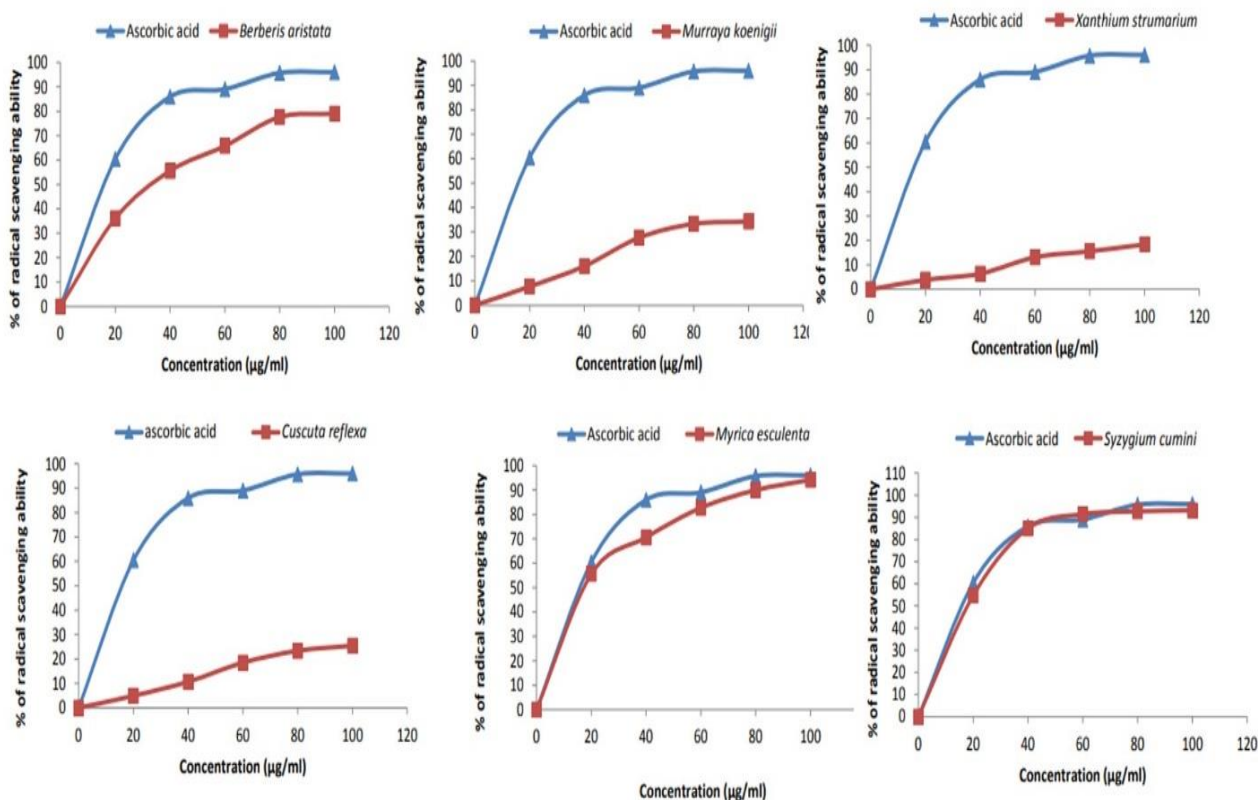


Figure 1: Results of 2, 2-diphenyl-1-picrylhydrazyl scavenging activities of the methanolic extracts of the plants

Table 3. IC₅₀ values of the plant extracts along with the standard ascorbic acid

Sample name	IC ₅₀ (µg/mL) (mean)
Ascorbic acid	22.17
<i>S. cumini</i>	33.35
<i>M. esculenta</i>	35.19
<i>B. aristata</i>	47.28
<i>M. koenigii</i>	127.86
<i>C. reflexa</i>	180.66
<i>X. strumarium</i>	258.10

In vitro α-amylase inhibition study

The mode of α-amylase inhibition by the tested plant's leaf extract was determined by the lowest IC₅₀ according to the method given by Bernfeld¹³. The comparisons of percentage α-amylase inhibition between different plant extracts and ascorbic acid as standard are presented in Figure 2. In the present study, among the medicinal plants tested, *X. strumarium* and leaves showed good inhibition of α-amylase activity. Moderate inhibition activity by *M. esculenta* and *B. aristata* and weak activity by *C. reflexa* and *M. koenigii* on comparison with standard acarbose although literature talks a lot about their antidiabetic activity on alloxan induced diabetic rats (Table 3)²¹⁻²⁴.

Almost all plant extract showed an effective α-amylase inhibition. *X. strumarium* was seen most active with an IC₅₀ value 197.21 µg/mL (Table 4). These results are pretty exciting because the literature talks a lot about anti-diabetic

plants but *X. strumarium* is not so much celebrated for its anti-diabetic properties in most of the studies. This work here by, showed the same plant as most active to inhibit α-amylase enzyme. The present study also shows; plants that are active in antioxidant activity are also active in anti-diabetic activity but not necessarily to similar extent. For instance, *S. cumini* was found most active in antioxidant assay but in anti-diabetic assay *X. strumarium* was found to be the most active instead. According to the past studies the phytochemicals like alkaloids, terpenoids, and polyphenols are the major antioxidants. Similarly, the phytochemicals like flavonoids, tannins, polyphenols and terpenoids are good anti-diabetics. Hence, this study here suggests *S. cumini* bark as good anti-oxidant as well as good anti-diabetic and *X. strumarium* leaves as a good anti-diabetic. *M. koenigii* was reported for the α-glucosidase inhibitory property and was found to inhibit α glycosidase²⁵.

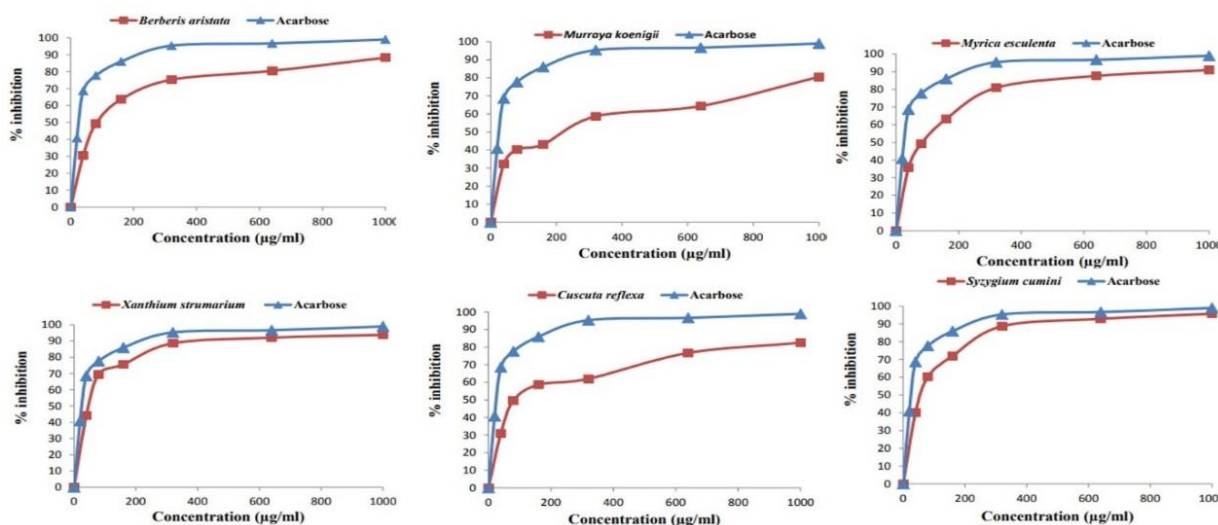


Figure 2: Inhibition of α-amylase activities by plant extracts

Table 4. IC₅₀ value of extracts on inhibition of α -amylase

Sample name	IC ₅₀ (μ g/mL) (mean)
Acarbose	144.56
<i>X. strumarium</i>	197.21
<i>S. cumini</i>	206.22
<i>M. esculenta</i>	246.07
<i>B. aristata</i>	270.04
<i>C. reflexa</i>	304.40
<i>M. koenigii</i>	374.22

(Each value is a mean of triplicate data)

Conclusion

This study provides some scientific support for their traditional use for diabetes management and other ailments. Phytochemical screening of methanolic extract of all six selected plants revealed the presence of different chemical constituents such as alkaloids, polyphenols, flavonoids, terpenoids, saponins, glycosides and tanins. From antioxidant activity, *S. cumini*, *M. esculenta*, were seen most active with IC₅₀ values 33.35 μ g/mL, 33.41 μ g/mL, 35.19 μ g/mL, 47.28 μ g/mL, respectively. *S. cumini* found to be most active amongst the samples. Anti-diabetic activity of methanolic plant extracts *X. strumarium*, *S. cumini*, *M. esculenta*, *B. aristata* were found to be potent towards α -amylase inhibition with IC₅₀ values 197.21 μ g/mL, 206.22 μ g/mL, 229.94 μ g/mL, 270.04 μ g/mL, respectively. *X. strumarium* being most active with an IC₅₀ value 197.21 μ g/mL. The data of our studies suggests that among the studied plants *S. cumini* and *X. strumarium* has beneficial effects in antioxidant activity and alpha amylase inhibition assay respectively holding the hope of a new generation for antihyperglycaemic drugs.

The present study may endow with the valuable idea for a complete study on the bioactive compound that contributes to these biological properties and also their possible mechanism of action is suggested.

Conflict of interest

The authors report no conflict of interest. The authors are responsible for the content and writing of the paper.

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