Research Article

EVALUATION OF ANTI-DIABETIC POTENTIAL OF ALKALOIDS AND FLAVONOIDS EXTRACTS FROM *MIMUSOPS ELENGI* L.

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ABSTRACT

Objective: The anti-diabetic activity was evaluated using alkaloids and flavonoids extract of leaf, stem, bark and fruit of *Mimusops elengi* L. **Materials and methods:** The alkaloids and flavonoids were extracted using cold extraction method. *In vitro* salivary amylase activity was done to evaluate the antidiabetic potential of plant part extracts. **Results:** The maximum yield was found in alkaloids of leaf extract (7.85mg/g) and in free flavonoids of stem extract (8.72mg/g). The lowest IC₅₀ value was found in alkaloids extract of leaf *i.e.*, 24.05 µg/ml and free flavonoids extract of leaf *i.e.*, 27.55 µg/ml. **Conclusion:** Plant parts especially leaf shows considerable inhibitory activity at low concentrations of extracts. This revealed that plant have high anti-diabetic potential and medicinal use.

Keywords: *Mimusops elengi* L., Alkaloids, Flavonoids, Salivary alpha amylase assay etc.

INTRODUCTION

Type 2 Diabetes is a chronic metabolic disease characterized by hyperglycemia, impaired carbohydrate metabolism and relative insulin deficiency secretion and action. It is a serious issue as this disease is increasing alarmingly and causing pre-mature death in developing countries.¹

Medicinal plants contain various bioactive compounds which can be used as a source of alpha amylase inhibitors. Alpha amylase inhibitors lower the level of postprandial hyperglycemia by controlling starch break down. Hence, retardation of starch digestion by inhibition of enzyme such as α -amylase would play a key role in the control of diabetes^{2,3}. Many synthetic antidiabetic drugs acarbose, sulfonylureas and biguanide are available but have many side effects⁴. Therefore safe, economic and reliable drugs are needed. Plant extracts with high anti- hyperglycemic potential are offering the new drug discovery.

Mimusops elengi is tall evergreen tree belongs to family Sapotaceae. It is native to Western Ghat region of the peninsular India and well known for its medicinal properties. The plant parts are reported to have promising therapeutic value in prevention of Diabetes type-2. The methanolic extract of leaves and flowers of the plant have been reported for their hypoglycemic activity⁵. The bark extract of *Mimusops elengi* L. was also evaluated for antidiabetic activity in alloxan induced diabetic rat⁶.

MATERIAL AND METHODS

Plant material

Plant parts (Leaf, Stem, Bark and Fruit) of *Mimusops elengi* L. were collected from Jaipur district, Rajasthan, India. It was

authenticated as (RUBL 211587) by Herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.

Extraction of Alkaloids

Leaf, Stem, Bark and Fruit of plant were subjected to the alkaloid extraction by method of Djilani. Take 5 gram of dried powdered material and then wetted with 15ml of NH₄OH and cold extracted with 150ml of ethyl acetate for 72 hours. The extract was filtered and air dried. Dissolve the residue in distilled water and acidified with H₂SO₄ to adjust pH around 3-4 to make the extract acidic. The acidic plant extract was extracted with petroleum ether and diethyl ether using separating funnel to remove all lipophilic, acidic and other material. Then plant extract was basified with the help of NH₄OH solution and adjust the pH around 9-10. The basic plant material is extracted with chloroform. The extract was washed with distilled water to neutral pH and dried to obtain alkaloids⁷.

Extraction of Flavonoids

Leaf, Stem, Bark and Fruit of plant were subjected to the flavonoids extraction by method of Subramanian and Nagarajan. 5 gram of dry material of plant material was cold extracted in 50ml of 80% methanol. The extract was dried and dissolve in distilled water. The extraction is done using petroleum ether to remove lipophilic, acidic and other material. Further extract it with diethyl ether and separate the upper layer and label it as F₂ (free flavonoids). Extract the lower layer with ethyl acetate and again separate the upper layer and discard the lower layer. Dry the upper layer in petriplate and reflux it with 7% Sulphuric acid for two hours. Now extract with ethyl acetate using separating funnel. Use the upper layer and label it as F₃ (Bound flavonoids) and wash it with distilled water to neutralize the pH at 7 to obtain crude flavonoids^{8,9}.

In vitro Salivary α amylase Inhibitory Assay

The inhibition assay was performed using the chromogenic DNSA method^{10,11}. The different concentrations (20-100 $\mu\text{g/ml}$) of plant extracts were prepared. 100 μl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 1 ml of salivary alpha amylase enzyme was added and incubated with 100 μl of extracts at 37°C for 10 min. After pre-incubation, 100 μl of 1% starch solution prepared in the phosphate buffer was added to each tube and incubated at 37°C for 10 min. The reaction was terminated with 200 μl DNSA (3,5-dinitrosalicylic Acid) reagent, placed in boiling water bath at temperature 85-90°C for 5 min, cooled to room temperature, diluted adding 1 ml of distilled water and the absorbance measured at 540 nm.

The percentage inhibition is calculated as follows:

Percentage inhibition = $\frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$ / A control
Where, A control = Absorbance of control reaction and A test = Absorbance of the samples

STATISTICAL ANALYSIS

All experiments were performed in 3 different sets with each set in triplicates. The data are expressed as mean $\pm$ SEM (standard error of the mean). The IC_{50} value was calculated by linear regression equation method by using Graph Pad Statistical Software.

RESULTS

The alkaloids, free flavonoids and bound flavonoids extracts of plant parts are used for illustrating yield of extracts (mg/g) as shown in Table-1. The highest yield of alkaloids was obtained in leaf 7.85 mg/g. The highest yield of flavonoids was found in stem

i.e., 8.72 mg/g (free flavonoids) and 7.56 mg/g (bound flavonoids).

In studies it was observed that the alkaloids extract shows higher percent inhibition with low IC_{50} values in comparison to flavonoids extracts of plant parts. The IC_{50} value of leaf and bark were found lower in comparison to stem and fruit in case of alkaloids extracts as shown in Table 2. The alkaloids extract of leaf and bark shows maximum inhibitory effects of alpha amylase activity with lowest IC_{50} value of 24.05 $\mu\text{g/ml}$ and 24.72 $\mu\text{g/ml}$ respectively. The good inhibitory effects were also shown by stem and fruit extracts with IC_{50} value of 46.18 $\mu\text{g/ml}$ and 47.88 $\mu\text{g/ml}$ respectively.

The free flavonoids extract of leaf shows maximum inhibitory activity with lowest IC_{50} value of 27.55 $\mu\text{g/ml}$ in comparison to other extract as shown in Table 3. The bound flavonoids extract of leaf exhibits IC_{50} value of 55.75 $\mu\text{g/ml}$. The free flavonoids and bound flavonoids extract of stem shows IC_{50} value of 64.94 $\mu\text{g/ml}$ and 92.89 $\mu\text{g/ml}$ respectively. The free and bound flavonoids extract of bark are found to have IC_{50} value of 77.56 $\mu\text{g/ml}$ and 107.90 $\mu\text{g/ml}$ respectively. The free and bound flavonoids of fruit extracts found to have IC_{50} value of 80.34 $\mu\text{g/ml}$ and 59.35 $\mu\text{g/ml}$ respectively. The p value < 0.01 was found significant in all plant extract activity.

The comparative study of alkaloid, free and bound flavonoids extract of leaf, stem, bark and fruit was studied and shown in Figure 1, 2, 3 and 4. In Figure 1 and Figure 2 the free flavonoids extract of leaf and stem shows strongest inhibitory activity. In Figure 3 and Figure 4 alkaloid extract of bark and fruit shows highest inhibitory activity in comparison other extract.

Table 1: Dry weight and yield of flavonoids and alkaloids of plant parts

Plant parts	Yield of alkaloids (mg/g)	Yield of free flavonoids (mg/g)	Yield of bound flavonoids (mg/g)
Leaf	7.85	6.75	7.10
Stem	4.14	8.72	7.56
Bark	2.01	3.29	7.55
Fruit	4.98	5.06	5.69

Mean $\pm$ Standard Deviation

Table 2: Salivary alpha amylase Inhibitory activity of Alkaloids of *Mimusops elengi* L.

Plant parts	Concentration ($\mu\text{g/ml}$)	Mean% Inhibition	Regression Equation	IC_{50} value ($\mu\text{g/ml}$)
Leaf	20	47.76 $\pm$ 1.63	Y = 0.4475x + 39.235	24.05
	40	56.69 $\pm$ 1.36		
	60	67.45 $\pm$ 1.20		
	80	75.32 $\pm$ 1.98		
	100	83.19 $\pm$ 3.18		
Stem	20	24.38 $\pm$ 5.46	Y = 0.65x + 19.977	46.18
	40	54.86 $\pm$ 1.52		
	60	65.20 $\pm$ 2.82		
	80	67.25 $\pm$ 1.52		
	100	83.18 $\pm$ 0.88		
Bark	20	44.08 $\pm$ 1.86	Y = 0.4418x + 39.078	24.72
	40	62.18 $\pm$ 1.52		
	60	66.48 $\pm$ 3.14		
	80	71.68 $\pm$ 0.31		
	100	83.51 $\pm$ 0.62		
Fruit	20	25.80 $\pm$ 2.42	Y = 1.0462x - 0.095	47.88
	40	37.94 $\pm$ 0.88		
	60	55.38 $\pm$ 3.08		
	80	89.73 $\pm$ 6.21		
	100	-		

Mean $\pm$ Standard Deviation

Table 3: Salivary alpha amylase Inhibitory activity of Flavonoids of *Mimusops elengi* L.

Plant parts	Concentration (µg/ml)	Mean% Inhibition	Regression Equation	IC ₅₀ value (µg/ml)
Free flavonoids of Leaf	20	34.25 ± 2.12	Y = 1.4005x + 11.41	27.55
	40	77.77 ± 2.77		
	60	90.27 ± 1.39		
	80	-		
	100	-		
Bound flavonoids of Leaf	20	16.57 ± 8.88	Y = 1.0792 x -10.174	55.75
	40	33.14 ± 7.65		
	60	37.74 ± 4.07		
	80	88.76 ± 2.49		
	100	96.68 ± 1.99		
Free flavonoids of Stem	20	13.18 ± 2.52	Y = 0.977x - 13.455	64.94
	40	19.62 ± 2.16		
	60	42.11 ± 0.84		
	80	60.43 ± 3.05		
	100	90.47 ± 4.92		
Bound flavonoids of Stem	20	33.17 ± 4.53	Y = 0.2066x + 30.807	92.89
	40	41.62 ± 7.67		
	60	42.33 ± 0.59		
	80	48.49 ± 2.80		
	100	50.39 ± 1.07		
Free flavonoids of Bark	20	17.64 ± 8.39	Y = 0.5196x + 9.695	77.56
	40	35.06 ± 0.72		
	60	41.05 ± 0.52		
	80	46.93 ± 0.19		
	100	63.66 ± 0.91		
Bound flavonoids of Bark	20	12.22 ± 0.39	Y = 0.3789x + 9.116	107.90
	40	28.25 ± 1.59		
	60	34.71 ± 0.52		
	80	39.67 ± 0.87		
	100	44.40 ± 0.87		
Free flavonoids of Fruit	20	20.75 ± 0.91	Y = 0.4003x + 17.836	80.34
	40	46.36 ± 19.17		
	60	33.29 ± 2.19		
	80	49.82 ± 0.69		
	100	59.05 ± 0.87		
Bound flavonoids of Fruit	20	9.33 ± 1.20	Y = 0.8293x + 0.776	59.35
	40	35.75 ± 0.39		
	60	63.78 ± 0.39		
	80	67.35 ± 0.20		
	100	76.46 ± 0.34		

Mean ± Standard Deviation

Figure 1: Salivary alpha amylase Inhibitory activity of Leaf extract of *Mimusops elengi* L. Figure 2: Salivary alpha amylase Inhibitory activity of Stem extract of *Mimusops elengi* L.

Figure 3: Salivary alpha amylase Inhibitory activity of Bark extract of *Mimosa elengi* L.

Figure 4: Salivary alpha amylase Inhibitory activity of Fruit extract of *Mimosa elengi* L.

DISCUSSION

Alpha Amylase enzyme plays important role in hydrolysis of the α -1, 4 glycosidic linkages between starch, glycogen and some other carbohydrates. The starch converts into glucose, and uptake by cells. The insulin will come into action and metabolize the excess sugar moieties and store into glycogen. But in disease cases, due to excess activity of amylase enzyme level, the glucose rises in blood which might results in hyperglycaemia ¹². In *Mimosa elengi* L. various secondary metabolites especially alkaloids, flavonoids, steroids, terpenoids are present ¹³. These metabolites have medicinal properties and are used in preparation of various herbal dug.

Research studies revealed that phytochemicals present in plants could to helpful in controlling excess starch breakdown and thus helps in management of high blood glucose in human beings. The plant extract exhibit the property of high amount of amylase inhibitory activity. Flavonoids maintains glucose metabolism, enhance glucose uptake and insulin secretion and modulating immune function to prevent Diabetics mellitus type 2 ^{14, 15}. Hyperglycemia could be controlled by management of blood glucose which has been considered essential in treatment of diabetes and for decreasing the risk of microvascular and macrovascular complications. In above study it was found that alkaloids and flavonoids extract of *Mimosa elengi* L. helps in management of controlling diabetics.

CONCLUSION

Plant based drugs need to be used in treatment of diabetes to avoid the complications of artificial drug substances. The healthcare cost is a need to an alternative or complementary therapies. The active bioactive compounds are identified and extracted to study its inhibitory activity and higher antidiabetic potential. The lowest IC₅₀ in both alkaloids and flavonoids extract of leaf extract in comparison to all other plant parts. This study justifies the plant parts *Mimosa elengi* L. especially leaves can be used in the controlling diabetes.

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