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HYPOGLYCEMIC EFFECT OF METHANOLIC EXTRACT FROM FRUITS OF *Sonneratia caseolaris* - A MANGROVE PLANT FROM BAGERHAT REGION, THE SUNDARBANS, BANGLADESH

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ABSTRACT

Hasan MN, Sultana N, Akhter MS, Billah MM, Islam KK (2013) Hypoglycemic effect of methanolic extract from fruits of *Sonneratia caseolaris* - A mangrove plant from Bagerhat region, the Sundarbans, Bangladesh. *J. Innov. Dev. Strategy.* 7(1), 1-6.

The present study was designed to investigate the hypoglecemic activity for the methanolic (85%) extract of mature fruits from *Sonneratia caseolaris*. Hypoglycemic tests were done on Swiss albino mice which were administered with the extract at the dose of 50, 100, 200 and 400 mg/kg body weight respectively in different experimental groups. The mice were sacrificed followed by oral glucose treatment and their sera were collected for glucose level analysis. The results showed that the extract was able to decrease blood glucose levels. The methanolic extract of fruits of *S. caseolaris* exhibited statistically significant ($p<0.01$ & $p<0.1$) reduction of serum glucose at the dose of 50, 100, 200 and 400 mg/kg-bodyweight in white albino mice (Swiss-webstar strain) at oral glucose test whereas the controlled use of glibenclamide showed the significant result ($p<0.1$). Our findings are in good agreement with the usage of this plant in anti-diabetic activities in ethnomedicine and provide insight for further research.

Key words: *hypoglycemic effect, Sonneratia caseolaris, methanolic extract, mangrove plants, sunderbans*

INTRODUCTION

The diversity of medicinal plants is growing around the world includes more than a thousand species (Ghani 1998). More than 500 of these medicinal plants have been reported in Bangladesh. The number of the indigenous medicinal plants continues to grow with discovery and introduction of newer plants everyday (Ghani 2003). These indigenous medicinal plants have long been extensively used in preparation of unani, ayurvedic and herbal medicines in the country. However, the proper scientific evaluation of the species limits their uses in therapeutic applications. Mangrove plants have been used as sources of food, a variety of traditional products and artifacts. They have also been used in folk medicine but their biological activities and chemical constituents have not yet been studied in detail. *Sonneratia caseolaris* (L.) Engl. is a mangrove plant and belongs to the family Sonneratiaceae (Wan Jusoh and Hashim, 2009). It is found in the Sundarbans mangrove forest in Bangladesh and is also native of South and South-East Asia. The plant is also reported in Malay Peninsula, Timor, New Guinea, Solomon Islands, and Indonesia and northern Australia (Little 1983). In English, it is designated as Crabapple while locally it is known as Choilani or Choila.

S. caseolaris has been reported to be used in traditional medicinal systems in several countries. It is used as folk remedy for sprains, swellings, and helminthiasis (Duke and Wain, 1981). In Myammar, the fruit is used for poultices. In Indochina, poultices made of leaves and salt are applied to cuts and bruises. In Malay, old fruit walls are used for helminthiasis, half-ripe fruit for coughs, and pounded leaves are used for hematuria and small pox (Perry and Parsons, 1980). In Bangladesh, the plant extract are used as astringent and antiseptic agents in sprains and swellings, and in arresting hemorrhage (Ghani 2003). Young fruits taste sour and are used as flavoring agents. Mature fruits have a cheese-like taste and are eaten raw or cooked. Fermented fruit juice is known to be useful in arresting hemorrhage and the wall of an old fruit is given as a vermifuge. In addition, the juice of half-ripe fruit is used to treat coughs. The juice of the flowers enters into a compound for treating blood in the urine. Pneumatophores are used as corks and floats. The crude ethanol extract of leaves of *S. caseolaris* L. (Sonneratiaceae) have been reported for its antinociceptive and antidiarrhoeal activities. And also reported to be hemostat, sprains, swellings, and worms remedy by crabapple mangrove.

Fig. 1. (A. Pneumatophore, B. Flower and C. Fruit of *Sonneratia caseolaris*)

In the effort for identifying anti-diabetic properties from different mangrove flora, it was noticed that methanolic extract of the fresh fruits of *S. caseolaris* possessed intestinal α-glucosidase inhibitory property (Mangala Gowri *et al.* 2007). In folk medicine, fruit is used as remedy to stop bleeding and in the treatment of piles and cough,

removal of intestinal worms and as sprain poultices (Bandaranayake 2002; Jayanta and Hrudaya, 2011). It has also been reported to be toxic against mosquito larvae (Devi *et al.* 1997) and possess hepatoprotective activity (Charoenteeraboon *et al.* 2007). Fatty acids, sterols, hydrocarbons (Hogg and Gillan, 1984), flavonoid, luteolin and its glycosides (Sadhu *et al.* 2006) was found in its leaves but chemical and biochemical properties of the fruits yet to be reported.

Therefore, the present study was undertaken to identify the pharmacological parameter of extracts from mature fruits of *S. caseolaris* because there is a possibility that it may contains substances with potential therapeutic uses and these could serve a basis of precursors for synthesis of useful drugs. To fullfil the objective, considering the ethnomedicinal and traditional uses of this plant an evaluation was conducted to find the hypoglycemic effect of oral administration of the methanolic extracts from fruit, on the level of serum glucose in mice was tested.

MATERIALS AND METHODS

The study was carried out at Pharmaceutical Biotechnology Laboratory, Department of Genetic Engineering and, Biotechnology, Jessore Science and Technology University (JSTU), Jessore and Biotechnology and Genetic Engineering Discipline of Khulna University.

Animals

A total number of 48 young Swiss-albino female and male mice were used for hypoglycemic test with the extract of fruit of *S. caseolaris.* These mice were collected from Animal Resource Branch, ICDDR'B, Mohakhali, Dhaka. They were 4-5 weeks old weighing 20-30 gm on an average. The animals were provided standard laboratory food and tap water and this was maintained at natural day night cycles. The experiments were conducted in an isolated place and noiseless condition was ensured. The mice were housed in standard size metallic cages (8 mice /cage) in properly ventilated room. For hypoglycemic experiments, 48 mice was divided them into 6 groups**.** Each group included 8 mice in which 8 mice in control, 8 mice to be treated with standard (Glibenclamide) and 8 per groups for four others for 4 different doses against body weight (50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg respectively).

Materials for extraction

The extraction of *S. caseolaris* fruits was carried out with methanol. The materials required for this extraction was distilled water**,** beakers**,** hot water bath, oven**,** weighing balance**,** glass rods**,** tissue paper**,** spatula**,** titrating pipettes**,** foil papers**,** vials**,** Soxhlet apparatus and thimble.

Chemicals

The chemicals used for the spectrometric analysis in determining the serum glucose level in response to oral administration of different plant extracts included reagent of glucose (GOD-PAP Cronolab AG; Swizerland). This reagent was supplied with buffer (Cat no: 101- 0014**,** GOD-PAP reagents; Lot no: 12679), glucose standard, alcohol, distilled water, acetone, detergent and methanol.

Procedure for S. caseolaris extraction with methanol

Green mature fruits were collected from Bagerhat region, the Sundarban's. The collected samples were separated from undesirable materials or plants or plant parts. Then drying of the fruits was carried out for 15 days under shaded sunlight to prevent the decomposition of the active constituents and any their photochemical degradation. The samples were ground into a coarse powder with the help of a suitable grinder. The powder was then stored in an airtight container and kept in a cool, dark and dry place until analysis was conducted. A porous cellulose thimble was filled with 45 gm dried and shredded sample of *S. caseolaris*. The thimble was then placed in an extraction chamber, which was suspended above a flask containing 400 ml. methanol solvent and below a condenser was placed. The flask was heated at 45^0C and the solvent evaporated and moved up into the condenser where it was converted inside the extraction chamber containing the sample. The extraction chamber was designed in a manner so that when the solvent surrounding the sample exceeds a certain level, it would overflow and trickle back down into the boiling flask. This created a siphon and the process was repeated 7 times over 6 hours. The thimble was subsequently filled with 32 gm and then 30 gm *S. caseolaris* powder and the whole procedure was repeated. At the end of the extraction process, the flask containing the solvent and lipid was removed from the thimble after squeezing to leave the soaked thimble. The extract residues were dried on tray and covered by aluminum foil paper and the filtered solution (extract) was dried by hot water bath for 3 days at 40^0C. After 3 days a sticky oily precipitate was found in the beaker and the extracts were collected by spatula in the designated glass vials. The amount of the extract was 9 gm. The extracted residues were kept in plastic jars and the extract containing vials were kept in the refrigerator at temperature of 7-10^0C.

Determination of hypoglycemic effects of methanolic extracts of S. caseolaris in mice

As mentioned earlier, 48 mice were used for this experiment and they were divided in to 6 groups. First group which was control group contained 8 mice. The second group with 8 mice was treated with standard

(Glibenclamide 10mg/kg body weight). The remaining groups were treated with different doses of plant extracts depending on the body weight in which group-3 of 8 mice was administered with 50mg/kg body weight while group-4, 5 and 6, each with 8 mice received the doses of 100mg/kg body weight and 200mg/kg body weight 400mg/kg body weight, respectively. The dose of glucose and Glibenclamide administered in oral glucose test was 2g/kg and 10mg/kg body weight. Before the day of gavaging and serum glucose analysis, the mice were fasted overnight and on the day of gavaging, the methanolic extract weighed in 0.4 gm and then suspended in corn oil. The net volume was prepared as 3ml and the mixture was shaken till the extract was suspended thoroughly. Then 10gm glucose was dissolved in distilled water and net volume was made 10ml. Simultaneously, 5mg/ml Glibenclamide was dissolved in distilled water and net volume was prepared 1ml. The extract or Glibenclamide was administered by gavaging and the mice were kept for 60 minutes before any treatment was carried out. Then glucose solution was administered by gavaging and again the mice were kept for 60 minutes without any treatment. After 120 minutes of gavaging with glucose, the sera from the mice were collected. Then, glucose in serum was measure by spectrophotometric analysis.

Spectrophotometric analysis

The spectrophotometric analysis was carried out according to the instruction for glucose measurement (Cromatest, Linear Chemicals, S.L., Barcelona, Spain). This measurement was based on the Trinder reaction (Trinder 1969; Barharn and Trinder, 1972). In principle, glucose was oxidized to D-gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of phenol and 4-aminoantipyrine (4-AA) was oxidized by hydrogen peroxide, to from a red quinoneimine dye proportional to the concentration of glucose in the sample.

B-D-Glucose + H_2O + O_2 →GOD→D-Gluconate + H_2O_2----------(i)
4-AA + Phenol →→→ H_2O_2 →→POD →→Quinoneimine + H_2O-------(ii)

The reagent for serum glucose measurement contained **R1(**monoreagent) which containedphosphate buffer (100 mmol/ L , pH 7.5), glucose oxidase (10 KU/L), peroxidase (2 KU/L), 4-aminoantipyrine (0.5 mmol/L), phenol (5 mmol/L). The other reagent was **CAL** which contained Glucose Standard: Glucose (100 mg/dl ≈5.55mmol/L). This standard was organic matrix based.

The Monoreagent and the Standard were supplied as ready –to-use.

The procedure for determining the serum glucose level was as follows:
1. The reagents and the sample were brought to room temperature.
2. Pipetting of samples and reagents were preformed into labeled tubes according to Table below (Table 1).

Table 1. Mixtures of samples and reagents for serum glucose measurement

Tubes	Blank	Sample	Standard
Monoreagent	1.0 ml	1.0 ml	1.0 ml
Sample	-	10 µl	-
Standard	-	-	10 µl

3. The mixtures were mixed and the tubes were incubated for 10 minutes at room temperature or 5 minutes at 37ºC.
4. The absorbance (A) of the samples and the standard were performed at 500 nm against the reagent blank. The color was stable for about 2 hours if protected from light. The amount of glucose was determined according to formula given below:

$(A_{Sample} / A_{Standard}) \times C_{Standard} =$ mg/dl. Glucose----------(iii)

Here, A_{Sample} = Sample absorbance, $A_{Standard}$ = Standard absorbance, $C_{Standard}$ = Standard concentration

Statistical Analysis

The data for animal experiment were expressed as mean ± SEM and were evaluated by Student's t-test to determine the significant difference between control groups and the experimental groups. The results obtained were compared with the control groups. The $p<0.05$ and 0.001 were considered to be statistically significant.

Study design

Experimental animals were randomly selected and divided into six groups denoted as group-I, group-II, group-III, group-IV, group-V and group-VI, consisting of 8 mice in each group. Each group received a particular treatment i.e. control, positive control and the four doses (50, 100, 200, 400mg/Kg body weight) of the extracts. Each mouse was weighed properly and the doses of the extracts and control reagents were adjusted accordingly.

RESULTS AND DISCUSSION

The present study was conducted to determine the hypoglycemic potential of methanolic extract of fruits of *S. caseolaris* L. in Swiss albino mice. Serum glucose level of the mice was determined spectrophotometrically at

500 nm. in presence of different doses of methanolic extracts of *S. caseolaris* fruits, standard drug Glibenclamide and controls. Significant hypoglycemic activity of methanolic extract of fruits was observed in doses determined based on the body weight of the mice (Table 2). In this case, methanolic extract of fruits showed strong hypoglycemic activity.

Table 2. Serum glucose levels of mice after 120 minutes glucose gavaging followed by oral administration of methanolic extracts of fruits

Sample no.	Control	Standard (Glibenclamide) (mg/dl)	Dose 50mg/kg (mg/dl)	Dose 100mg/kg (mg/dl)	Dose 200mg/kg (mg/dl)	Dose 400mg/kg (mg/dl)
1	100	30	115	87.5	62.5	87.5
2	120	100	120	120	85	72.5
3	160	65	105	117.5	75	87.5
4	150	75	115	77.5	127.5	105
5	145	85	112.5	105	100	112.5
6	112.5	97.5	95	85	115	67.5
7	150	92.5	110	117.5		107.5
8	160	97.5	112.5	85	120	100
Sum	1097.5	642.5	885	795	685	740
Mean ± SD	137.18 ± 23.044	80.3125 ± 23.7335	110.625 ± 7.647	99.375 ± 17.5127	97.857 ± 24.513	92.5 ± 16.5219
SE		9.265	5.843	2.703	6.1916	8.148
T cal		4.862666	3.094	3.694	3.187	4.4566
Significance level%		0.1	1	1	1	0.1
5% Significance level		Significant	Significant	Significant	Significant	Significant

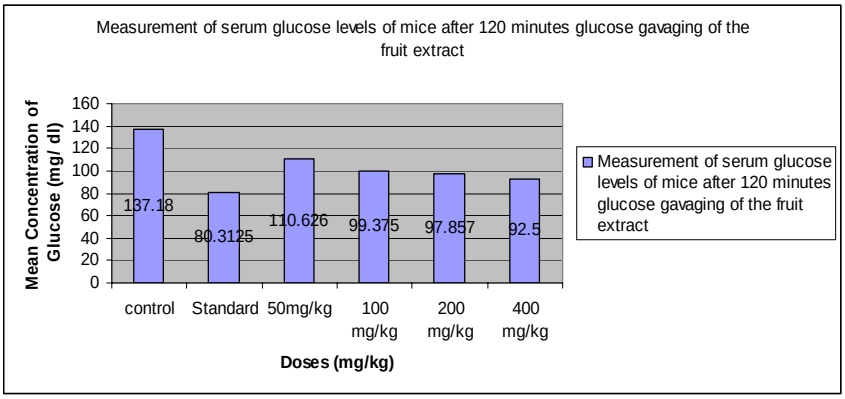

Fig. 2. Serum glucose levels of mice after 120 minutes glucose gavaging followed by oral administration of methanolic extract of fruits

The extract of fruits showed significant results in a dose-dependent manner and the level of serum glucose decreased if compared to control. The extracts from fruits showed significant result ($p<0.01$) at the 400mg/kg-bw which were 92.50 ± 16.5219 and 80.3125 ± 23.7335 in case of standard (glibenclamide) showed significant results at 1% level. The results showed that the methanolic extract of *Sonneratia caseolaris* decreased blood glucose levels in glucose fed compared with their respective control groups. Glibenclamide caused significantly more hypoglycaemia in comparison with the plant extract. It has been reported that biguanide compounds produce hypoglycaemia in normal animals by reducing hepatic gluconeogenesis and by decreasing glucose absorption from gastrointestinal tract and also by increasing insulin sensitivity by increasing peripheral utilization of glucose. Therefore, it is conceivable that hypoglycemic principles in the extract exert effect probably by a mechanism similar to Glibenclamide.

In glucose tolerance test, the oral administration of *Sonneratia caseolaris* suppressed the increase in glucose level induced by glucose loading. Such an effect might be due to decrease in the rate of intestinal glucose absorption or by potentiating of pancreatic secretions or increasing the glucose uptake. It is known that the factors influencing the glucose metabolism under various physiological conditions do influence lipid metabolism as well. It has also been revealed that triglyceride accumulation increase considerably in diabetes mellitus (Iams and Wexler, 1977). Hypercholesterolemia and hypertriglyceridemia have been reported to occur

in diabetic (Riyad *et al.* 1988) and a significant increase in cholesterol and triglyceride observed in our experiment in accordance to this studies. Reduction in the body weight in diabetic animals as well as in humans is well known. In case of diabetes, the body weight will increase when normal glycaemic levels is achieved which is particularly seen in sulfonyl ureas or insulin. *Sonneratia caseolaris* leaf extract increases body weight in glucose loaded mice, which might be due to increased insulin secretion and better glyceamic control (Rasheda *et al.* 2010).

The present study on pharmacognostical characteristics and preliminary phytochemical screening of *S. caseolaris* provide valuable information which may help in authenticating the genuine mangrove plant along with the nature of phytoconstituents present in it. The above studies provide information regarding their identification and chemical constituents which may be useful for the standardization and preparation of monograph of *Sonneratia caseolaris*. The constituents of *S. caseolaris* may have several medicinal properties and can be utilized for the treatment of various diseases. Sherine *et al.* (2010) stated all of the results of bark powder coupled with the effect of chloform extract (bark) reveal that the bark of *Alstonia scholaris* possesses different significant pharmacological activities (Hypoglycemic, hyperlipidemic, anxiolytic & analgesic) at different doses.

CONCLUSION

In conclusion, the result suggests that the fruit extract of this mangrove plant (*S. caseolaris*) showed the hypoglycemic and/or antidiabetic activity in normal mice. Improved glucose tolerance suggests that the extract may stimulate the peripheral utilization of glucose by increasing insulin sensitivity to cells or by decreasing glucose absorption from gastrointestinal tract or by reducing hepatic gluconeogenesis and glycolysis, which is similar to that of glibenclamide. The present study gives some preliminary ideas; however, further study is necessary for the identification of the possible mechanism of action, isolation, purification and characterization of active components that may reveal new agents for diabetic therapy. It is important to note that although the research with pure natural compounds delineates mechanism of their biological actions and help in development of new therapeutics, in traditional medicines they are present in mixtures and that mixture of compounds in traditional medicinal preparations are thought to be more effective than a single compound. The constituents of the fruit of *S. caseolaris* may have several medicinal properties and can be utilized for the treatment of various diseases. Future research on this particular mangrove species is recommended to identify therapeutically potent compounds with pharmacological activities and could open new avenues in the use of natural products for therapeutic purpose.

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