December 2009 Volume 3 Issue 4

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 3(4), 2009 [231-235]

Antioxidant and anti-diabetic activities from leaf extracts of *Streblus asper* Lour

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Received: 23rd July, 2009 ; Accepted: 2nd August, 2009

ABSTRACT

The methanolic extract from leaves of *Streblus asper* Lour was screened for *in vitro* antioxidant activity, and for anti-diabetic activity. The *in vitro* antioxidant activity was evaluated using DPPH and H_2O_2 for their free radical scavenging property. The data were expressed as IC_{50} and compared with ascorbic acid and tocoferol that served as reference standard. The leaf extract showed an IC_{50} of 1.01mg/ml and 700 µg/ml as compared to the standards, ascorbic acid and tocoferol, that showed an IC_{50} of 4nm and 215 µg/ml respectively. Anti-diabetic activity was evaluated by the significant reduction in serum glucose levels in streptozotocin induced hyperglycemic rats treated with leaf extract (200 and 400 mg/kg body weight), and the standard drug glibenclamide (10mg/kg body weight]. The leaf extract produced significant anti-hyperglycemic effect comparable to the standard drug glibenclamide.

KEYWORDS

Streblus asper;
Methanolic extract;
Antioxidant activity;
Anti-diabetic activity;
IC_{50}.

INTRODUCTION

Free radicals are highly reactive molecules with unpaired electrons. They react with DNA and cause damage to it by breaking the phosphodiester bond or by altering the base composition. The damage caused to cells and tissues by free radicals leads to several diseases such as arteriosclerosis, hypertension, aging, cancer, diabetes mellitus, inflammation, renal failure, liver damage, AIDS etc[1]. Several studies have revealed that antioxidants that can scavenge free radicals are effective in treating such disorders. Butyl hydroxyl anisol (BHA) and butyl hydroxyl toluene (BHT) are active synthetic antioxidants that show adverse reactions[2,3]. Hence, there is an urgent need for the production of antioxidants from natural source and can be nontoxic, economical, showing high activity that can replace the synthetic compounds. A large number of plants are known to exhibit potent antioxidant activities[4,5]. Plant phenolic compounds such as flavonoids and non-flavonoids act as antioxidants through different mechanisms such as scavenging of free radicals, quenching of reactive oxygen species, inhibition of oxidative enzymes, chelating of transition metals, or through interaction with biomembranes[6].

Diabetes mellitus (DM) is a chronic disorder caused by deficiency in the production of insulin by pancreas, or by the ineffectiveness of the insulin produced. Such

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a deficiency results in increased concentrations of glucose in the blood which can cause damage to the body in particular to the blood vessels and nerves. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Many Indian medicinal plants are reported to be useful in the treatment of diabetes[7,8].

S. asper Lour (Family Moraceae) is a dioecious woody plant distributed in tropical countries such as India, China, Sri Lanka, Malaysia, the Philippines and Thailand. It is a valuable medicinal plant used in traditional medicine. The bark extract from this plant is been used as remedy for fever, dysentery, relief of toothache and antigingivitis[9]. The root extract is applied to unhealthy ulcers, sinuses and used as an antidote to snake bite. The milky juice prepared from tender nodal and leaf cuttings has been used as antiseptic and astringent applied to chapped hands and sore heels[10]. Anticancer, anti-microbial, anti-malarial and insecticidal activities were reported in this plant[11-14]. The medicinal properties attributed to this plant are mainly due to the presence of several cardiac glycosides in different parts of this plant[15,16]. The present investigation is to evaluate the antioxidant and anti-diabetic activities from leaf extracts of *S. asper*.

EXPERIMENTAL

Plant collection and identification

The leaves of *Streblus asper* Lour. were collected from mature plant (10-year-old) in the month of August-September, 2008 from Kakatiya University campus. Taxonomic identification of the specimen was performed and a voucher specimen is deposited in the herbarium of Department of Botany, Kakatiya University, Warangal.

Preparation of extract

The dried leaves were pulverized into fine powder in an electric blender. The powdered material (250 g) was extracted with methanol in soxhlet apparatus for 24 h at room temperature. The crude methanolic extract thus obtained was concentrated by evaporating at 40°C using a rotary vacuum evaporator. Approximately 2 g of the semisolid residue was used for further experiments.

Phytochemical screening

Phytochemical tests were carried out to know the nature of compounds present in the methanolic extracts from leaves[17]. Nnhydrin test for amino acids, Mayer's and Dragendorff's tests for alkaloids, $FeCl_3$ test for phenolic compounds, Shinoda's test for flavonoids, Libermann-Burchard test for terpenoid/steroids and froth formation test for saponins.

Determination of antioxidant activity

1,1-Diphenyl-2-picryl hydrazyl (DPPH) (Sigma Aldrich Co., St. Louis, USA), phosphate buffer saline (PBS) (Himedia, Mumbai, India) and ascorbic acid (SD fine chemicals Ltd., Mumbai, India) of analytical grade were used. Different concentrations (10.30, 50, 100 and 200 mg/L) of extract were subjected to antioxidant assay with DPPH, and radical scavenging activity with H_2O_2.

Diphenyl Picryl Hydrazyl (DPPH) free radical scavenging activity

In vitro DPPH free radical scavenging activity of leaf extract was determined as described by Blios (1958)[18]. To 0.1 ml of different concentrations (0.01 to 10 mg/ml) of each extract, 2.5 ml of methanol and 0.5 ml of 0.2mM DPPH solutions were added and thoroughly mixed. The absorbance was measured at 517 nm. Ascorbic acid was used as control. The experiment was performed in triplicates and the IC_{50} (inhibitory concentration) for each concentration was determined.

Hydrogen peroxide (H_2O_2) free radical scavenging activity

The ability of leaf extract to scavenge hydrogen peroxide was determined according to the method of Famey *et al.* (1998) and Sanchez, (2001)[19,20]. The solution of hydrogen peroxide (20 mM) was prepared in PBS (pH 7.4). Various concentrations (0.01 to 10 mg/ml) of 1 ml of leaf extracts and standard (tocoferol) were added to 2 ml of H_2O_2. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing PBS without H_2O_2. The percentage of H_2O_2 scavenging for the test and standard compounds was calculated as

$$\% \text{ scavenged } [H_2O_2] = [(A_{control} - A_{sample})/ A_{control}] * 100$$

Determination of anti-diabetic activity

Wister albino adult rats (200-220 g) of either sex (obtained from Mahaveer Enterprises, Hyderabad, India) were housed in polypropylene cages in a room where the congenial temperature was 27°C±1°C and 12 h light and dark cycles were maintained. Animals were maintained as per the guidelines of Institutional Animal Ethics Committee. They were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum*.

Collection of blood samples

The blood was collected from orbital plexus in heparinized tubes and plasma was separated by immediate centrifugation of blood samples at 3000 rpm for 5 min at room temperature and was directly used for estimating glucose levels in blood plasma.

Activity in streptozotocin induced-hyperglycemic (diabetic) animals

A group of rats starved for 18 h were made hyperglycemic by injecting streptozotocin (Sigma, USA) dissolved in citrate buffer (pH 4.3) at the rate of 65 mg/kg of body weight, 15 min after the intraperitoneal administration of 110 mg/kg of nicotinamide in normal saline[21]. After 5 days, their plasma glucose concentration was estimated and moderately streptozotocin diabetic rats having plasma glucose levels above 250 mg/ml were separated into four groups and each group contained 6 animals. Group 1 served as untreated diabetic control, group 2 was given a standard oral hypoglycemic agent, glibenclamide (10 mg/kg) while group 3 and 4 received 200 mg/kg and 400 mg/kg of methanolic extract of *S. asper.* Blood glucose level of each rat was estimated at 1, 2, 4 and 8 h after administration of these components.

Statistical analysis

The results were expressed as mean±S.D. Statistical analysis ($p<0.05$) were carried out using paired *t*-test and one-way ANOVA followed by Bonferroni test.

RESULTS

Phytochemical screening

Phytochemical screening for secondary metabolites in the methanolic extract of leaves revealed the presence of amino acids, phenolics and terpenoid/steroids.

Antioxidant activity of leaf extract: DPPH free radical scavenging activity

Figure 1 shows IC50 inhibition curve of DPPH radical with methanolic leaf extract of *S. asper*. The extracts showed variable antioxidant activity depending on the dose concentration of the extract. The amount of extract needed for 50% inhibition (IC50) of DPPH radical is 1.01 mg/ml whereas the IC50 for ascorbic acid was 4nm.

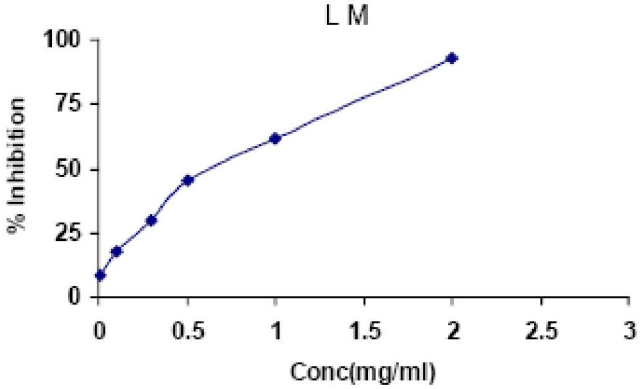

Figure 1 : Antioxidant activity of extract using DPPH method

H_2O_2 free radical scavenging activity

Antioxidant activity of *S. asper* leaf extract is represented in the Figure 2. Six different concentrations of methanolic extract of leaves were used for the free radical scavenging activity. It was evident that the extract showed a dose dependent inhibition of H_2O_2 free radical. The IC_{50} value of leaf extract was found to be 700 µg/ml and reference drug tocoferol showed an IC_{50} of 215 µg/ml.

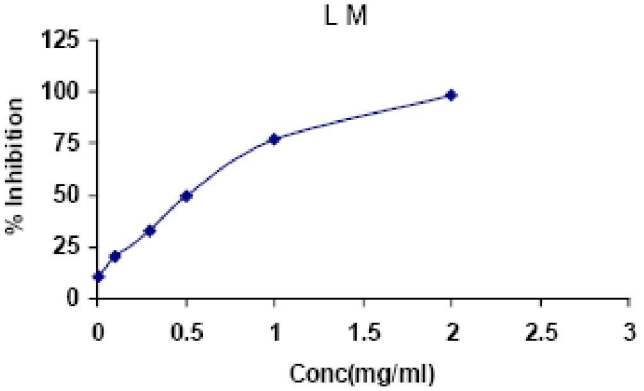

Figure 2 : H_2O_2 radical scavenging activity of *S. asper*

Activity in streptozotocin induced-hyperglycemic animals

The studies on the methanolic leaf extract of *S. asper*

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revealed that the extract produced significant reduction in glucose levels in the serum in hyperglycemic rats at both dose levels (200 and 400 mg/kg body weight) as compared to diabetic control. The reduction in the mean value of glucose level in the serum was dose dependent. The glucose levels in the blood serum of the normal and streptozotocin rats on 7th, 14th and 21st days are depicted in TABLE 1. Similar to the glibenclamide, the extract produced significant ($p<0.01$) antihyperglycemic effects after 1 h of administration of extract in hyperglycemic rats.

TABLE 1 : Effect of methanolic extracts of S. asper (MESA) on plasma glucose levels of hyperglycemic rats

Treatment	0hr	1hr	2hr	4 hr	8 hr
Normal rats-	83.9 ± 3.3	83.5± 3.6	83.1± 4.1	82.6± 2.4	81.5± 3.2
Diabetic Control	276.6 ± 4.5	274.4 ±5.2	272.3±4.9	270.6 ± 3.8	269.6 ± 4.5
Glibenclamide	283.3 ± 4.7	267.4 ±4.9**	253.6 ±5.1**	239.5 ±4.5**	230.2 ±4.6**
MESA(200mg/kg)	281.6 ± 4.9	273.8 ±5.2*	259.7±4.7**	246.2 ±4.2**	239.2 ±4.2**
MESA(400 mg/kg)	282.8 ± 5.1	271.2 ±3.9**	255.4 ±4.9**	243.4 ±4.7**	235.2 ±4.2**

Values are in mean ± SD; n =6; *p<0.01; **p<0.001

DISCUSSION

In this investigation we demonstrate that the leaf extracts of *S. asper*, a woody medicinal plant has antioxidant and anti-diabetic activities. Phytochemical studies of leaf extract revealed the presence of phenolics and terpenoid/steroids, which are believed to be responsible for antioxidant and antidiabetic activities. The antioxidant activity of methanolic leaf extract was assessed by standard DPPH assay performed for the inhibition of superoxide anion formation. The free radical scavenging activity of methanolic leaf extract was evaluated through its ability to quench the synthetic free radicals. DPPH scavenging activity was earlier used as a quick and reliable parameter to assess the *in vitro* antioxidant activity from crude extracts of several medicinal plants[22-25].

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly with Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects[26]. From our results, the H_2O_2 scavenging activity of the plant extract is comparable to that of the standard tocopherol. In the present investigation *S. asper* leaf extract at different doses demonstrated significant DPPH and H_2O_2 scavenging activity indicating its ability to act as free radical scavengers.

Diabetes mellitus is possibly the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy has increased[27]. Oral antihyperglycemic agents and insulin are widely used in diabetes treatment, but they also have prominent side effects. Effective control of the blood glucose level is a key step to prevent or reverse the diabetes complications[28].

Fasting blood glucose level in diabetic rats is an important parameter for monitoring diabetes[29]. Our data suggests that the methanolic extract of *S. asper* (MESA) causes the antidiabetic effect by reducing the fasting blood glucose level.

The present study concludes the free radical scavenging and anti-diabetic activities from leaf extracts of *S. asper*.

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