

Full Length Research Paper

Evaluation of antidiabetic and antioxidant activity of aerial parts of *Hyptis suaveolens* Poit.

Praveen Singh Nayak^{1*}, D. M. Kar¹ and Shweta Purohit Nayak²

¹School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Kalinga Nagar, Ghatikia, Bhubaneswar, Odisha, India.

²GRY Institute of Pharmacy, Borawan, Khargone, M. P. India.

Accepted 3 October, 2012

Hyptis suaveolens Poit. (Lamiaceae) commonly called curry leaf and savanna plant which is abundantly found in farmlands. Traditionally, the plant is used in the treatment of diabetes mellitus, fever, eczema, cancers and headache. The present study was aimed to investigate the *in vitro* antioxidant and antidiabetic potential of aerial parts of *H. suaveolens* Poit at dose levels of 250 and 500 mg/kg, in acute normoglycemic and alloxan induced hyperglycemic rats including oral glucose tolerance and chronic study (11 days), keeping glibenclamide (5 mg/kg) as standard drug. The body weight measurement and selective serum biochemical estimation (blood glucose concentration, ALT, AST, ALP, urea, creatinine, triglyceride, and cholesterol) were undertaken in 11 days treated hyperglycemic rats. The test result revealed that in normoglycemic rats, the decrease in blood glucose level lies between 4 to 15% in extract treated groups, while the hyperglycaemic rats showed a significant ($p < 0.05$ to 0.001) fall of blood sugar level in a progressive manner and similar findings also observed in glucose tolerance test to an extent of $p < 0.05$. The results of body weight and tested biochemical parameters of blood serum of the 11-days treated animals showed in favor of the anti diabetic activity of the test extracts. In DPPH scavenging anti-oxidant assay, the IC₅₀ value of the test extracts were found as 30.06 and 28.76 µg/ml while the IC₅₀ value of ascorbic acid was 20.92 µg/ml. This study suggests that the aerial part of *H. suaveolens* is endowed with antidiabetic and antioxidant potential.

Key words: Antidiabetic activity, *in vitro* antioxidant, *Hyptis suaveolens*, alloxan.

INTRODUCTION

Diabetes mellitus (DM) is a syndrome which affects more and more people in all countries over the world. It is well known that diabetes mellitus is the commonest endocrine disorder that, according to the World Health Organization (WHO, 2004), affects more than 176 million people worldwide. From an ethnopharmacological perspective, it is important to understand that this disease is one at the interface of conventional biomedical and local (or traditional) treatment. Diabetes mellitus (DM) which not only lead to hyperglycemia also cause many complications, such as hyperlipidemia, hypertension and atherosclerosis

(Bakirel et al., 2008; Sepici et al., 2004; Luo et al., 2004; Abdel-Barry et al., 1997).

Many plant species are known in folk medicine of different cultures to be used for their hypoglycaemic properties and therefore used for treatment of DM (Abdel-Barry et al., 1997; Pushparaj et al., 2000). Despite this, few traditionally used antidiabetic plants have received proper scientific screening.

The plant, *H. suaveolens* (L) Poit commonly known as *W. tulsi* belongs to the family Lamiaceae and is an ethnobotanically important medicinal plant. The plant has been considered as an obnoxious weed, distributed throughout the tropics and subtropics. Almost all parts of this plant are being used in traditional medicine to treat various diseases. The leaves of *H. suaveolens* have been utilized as a stimulant, carminative, sudorific, galactagogue and

*Corresponding author. E-mail: praveen_nayak2000@yahoo.com, psnayak2000@gmail.com. Tel: +919926498776.

as a cure for parasitic cutaneous diseases (Anonymous, 1964).

Crude leaf extract is also used as a relief to colic and stomach ache. Leaves and twigs are considered to be antispasmodic and used in antirheumatic and antisudorific baths, anti-inflammatory, antifertility agents ((Kirtikar and Basu, 1991) and also applied as an antiseptic in burns, wounds, and various skin complaints. The decoction of the roots is highly valued as appetizer and is reported to contain urosolic acid, a natural HIV-integrase inhibitor (Chatterjee and Pakrashi, 1997).

Fumes of the dried leaves are also used to repel mosquitoes and control insect pests of stored grains. The leaves of the plant have been shown to contain alkaloids, terpenes and volatile oils (Gills, 1992).

The present study has been designed to determine the role of extracts of aerial parts of *H. suaveolens* for *in vitro* antioxidant activity and potential antidiabetic activity if any, against normoglycemic and alloxan induced hyperglycemic rats.

MATERIALS AND METHODS

The plant material used in this study was aerial parts of *H. suaveolens*, collected from road side area from Khargone district Khargone M.P., India, during spring (mid-August to mid-October, 2010) and was authenticated by the Taxonomist Dr. S. K Mahajan, Botany Department, Government P G College Khargone M.P. The plant materials were initially rinsed with distilled water and dried on paper towel in laboratory at $(37 \pm 1^\circ\text{C})$ for 24 h and milled into coarse powder by a mechanical grinder.

Preparation of extract

The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with alcohol and water using Soxhlet apparatus. The yield of the plant extracts ethanol (95%) and aqueous measured about 20 g each after evaporating the solvent using water bath. The standard extracts obtained were then stored in a refrigerator at 4°C for further use (Akueshi et al., 2002).

Preparation of the test samples

Glibenclamide (5 mg/kg) was used as the reference control. The test extract was suspended in 25% Tween 20 in distilled water prior to oral administration to the experimental animals. Animals in the control group received only the 25% Tween 20 (2 ml/kg). All the test samples were administered through oral route.

Animals used

Male albino Wistar rats, weighing 150 to 200 g and Swiss albino mice, weighing 20 to 25 g were used. Prior to the experiments, the selected animals were housed in acrylic cages in standard environmental conditions (20 to 25°C), fed with standard rodent diet for 1 week in order to adapt to the laboratory conditions and water was given *ad libitum*. They were fasted overnight (12 h) before experiments, but were allowed free access to water. Six animals were used for each group of study. All the experiments on animals

were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee (IAEC No. 1171/C/08/CPCSEA).

Determination of blood glucose levels

Fasting blood glucose concentration was determined using a blood Glucometer (Optimum), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns (Aslan et al., 2007a; b).

Screening for antidiabetic activity

The Screening for antidiabetic activity was followed as per standard procedures (Dash et al., 2001). The test samples were suspended in 25% Tween 20 in distilled water. Glibenclamide (5 mg/kg) was used as reference control during the study. All the test samples were administered through oral route.

Single dose study

Normoglycaemic animals

The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. Plasma was separated following centrifugation and the glucose was estimated by GOD/POD method using Glucose estimation kit from M/s. Sigma Diagnostics (India) Pvt. Ltd., Baroda, India. The normal rats were then divided into six groups of six animals each. Group I served as solvent control and received only vehicle (2 ml/kg) through oral route, Group II received glibenclamide (5 mg/kg) and served as reference control. Groups III to VI received the alcohol and aqueous extract at a dose of 250 and 500 mg/kg, respectively, through oral route. Blood glucose levels were examined after 1, 2, 4, 6, 8 and 10 h of administration of single dose of test and control samples (Table 1).

Alloxan induced diabetic animals

The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. After 1 h, the animals were provided feed *ad libitum*. The blood glucose level was checked before alloxanisation and 24 h after alloxanization as aforementioned. Animals were considered diabetic when the blood glucose level was raised beyond 250 mg/100 ml of blood. This condition was observed at the end of 72 h after alloxanisation. The animals were segregated into six groups of six rats in each. Group I served as normal reference, Group II served as solvent control and received vehicle (2 ml/kg) through oral route. Group III received glibenclamide (5 mg/kg). Groups IV to VII received the test extract at doses of 250 and 500 mg/kg in a similar manner as per the aforementioned experiment. Blood glucose level of each rat was estimated at 1, 2, 4, 6, 8 and 10 h, respectively (Table 2).

Effect on oral glucose tolerance in rats

An oral glucose tolerance test (OGTT) was performed on diabetic

Table 1. Effect of ethanolic and aqueous extracts of *Hyptis suaveolens* on blood glucose level in normoglycemic rats.

Treatment and dose	Blood glucose level (mg/dl)							% decrease at 10 h
	0 h	1 h	2 h	4 h	6 h	8 h	10 h	
Diabetic control (Tween + water)	94.6 ± 1.1	87.2 ± 4.62	91.43 ± 1.86	89.56 ± 0.81	91.58 ± 2.23	89.66 ± 0.46	92.67 ± 3.22	--
Glibenclamide (5 mg/kg)	91.43 ± 1.31	81.22 ± 2.63	67.53 ± 2.34*	58.12 ± 2.61**	54.72 ± 2.44***	73.83 ± 1.42***	71.63 ± 2.81***	21.65
Et. Ext. (250 mg/kg)	89.13 ± 1.2	87.6 ± 1.1	87.2 ± 2.65	86.73 ± 1.46	86.33 ± 1.43	86.12 ± 0.89*	85.32 ± 1.51	4.27
Et. Ext. (500 mg/kg)	88.4 ± 2.43	87.32 ± 2.16	86.49 ± 1.87	86.04 ± 1.67	85.6 ± 2.69	84.4 ± 1.43**	81.32 ± 2.49*	7.94
Aq. Ext. (250 mg/kg)	92.53 ± 1.27	91.46 ± 1.68	89.94 ± 1.09	87.11 ± 0.91	86.22 ± 2.13	84.11 ± 1.18**	82.11 ± 1.89**	11.22
Aq. Ext. (500 mg/kg)	91.18 ± 0.93	87.30 ± 0.78	85.49 ± 2.61	85.21 ± 1.37	84.33 ± 2.38*	82.66 ± 1.21***	77.42 ± 2.73***	15.10

Values are expressed in Mean ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t- test (t-value denotes statistical significance at *p<0.05, **p<0.01 and ***p<0.001 respectively, in comparison to group-I).

Table 2. Effect of ethanolic and aqueous extracts of *Hyptis suaveolens* on blood glucose level in single dose treated alloxan induced hyperglycemic rats.

Treatment	Blood glucose level (mg/dl)							% Age decrease at 10 h
	0 h	1 h	2 h	4 h	6 h	8 h	10 h	
Normal control	93.09 ± 1.38	89.50 ± 0.64	88.50 ± 0.64	90.50 ± 0.64	87.59 ± 0.64	88.67 ± 2.14	89.76 ± 2.33	-
Diabetic control (Tween + water)	307.5 ± 4.341	297.63 ± 4.93###	301.81 ± 2.89###	291.88 ± 3.51###	287.89 ± 3.67###	303.77 ± 4.29###	279.61 ± 2.71###	-
Glibenclamide (5 mg/kg)	315.7 ± 2.17	258.62 ± 3.21***	198.31 ± 2.89***	139.21 ± 2.63***	126.31 ± 3.28***	118.91 ± 2.96***	92.59 ± 2.87***	70.67
Et. Extract (250 mg/kg)	333.2 ± 3.98	328.38 ± 3.26	307.53 ± 3.24	242.79 ± 3.81***	203.44 ± 2.59***	163.53 ± 4.28***	134.36 ± 3.71***	59.67
Et. Ext. (500 mg/kg)	342.7 ± 4.15	369.68 ± 2.88	319.81 ± 2.36*	212.37 ± 3.77***	158.69 ± 3.91***	131.84 ± 3.28***	126.59 ± 2.93***	62.85
Aq. Ext. (250 mg/kg)	341.09 ± 5.22	322.23 ± 3.91	282.62 ± 3.11**	236.59 ± 4.12***	157.34 ± 3.86***	148.73 ± 3.57***	131.72 ± 2.98***	61.38
Aq. Ext. (500 mg/kg)	358.7 ± 7.25	312.33 ±	279.36 ± 2.47	186.34 ± 3.26***	153.76 ± 3.41***	137.34 ± 2.15***	119.34 ± 2.38***	66.72

Data represented as Mean ± SEM, (n=6). p< 0, 0.05 *significant, ###very significant, ****highly significant as compare to normal control group. p< 0.05 *significant, **very significant, ***highly significant as compare to diabetic control group. (One way analysis of variance (ANOVA) followed by Dunnett's t-test).

rats by feeding glucose (5 g/kg) per os. Animals were deprived of food 18 h before and during the experiment but were allowed free access to water. They were divided into 6 groups of 6 rats each. Four groups received the plant extract at the doses as per the aforementioned experiment by os. One group received 5 mg/kg of glibenclamide and the control group received the vehicle. The plant extract, glibenclamide and vehicle were orally administered 1 h before glucose administration. Blood glucose level was determined before drug and glucose administration (-1 and 0 h, respectively) and subsequently at 0.5, 1, 2 and 3 h after (Table 3).

Study of blood glucose level on alloxan induced 11-days treated diabetic animals

The animals were kept fasting for 24 h with water *ad libitum* and injected alloxan monohydrate intraperitoneally at a dose of 150 mg/kg in normal saline. After 1 h, the animals were provided rodent- feed *ad libitum*. The blood glucose level was measured 72 h after administration of alloxan. The animals showing blood glucose level beyond 250 mg/dl were considered for the study. The diabetic animals were segregated into six groups of six rats each. Group II served as solvent control and received only

vehicle (2 ml/kg) through oral route. Group III received glibenclamide (5 mg/kg). Groups IV and V received ethanol extract at doses of 250 and 500 mg/kg. Similarly, Groups VI and VII received aqueous extract at same dose level respectively in a similar manner for 11 days. The Group I served as a normal reference. The blood glucose level was measured on 0, 3, 7 and 11th day of treatment (Table 4).

Determination of body weight and serum biochemical of 11- days treated alloxan induced diabetic rats

The body weight of the 11-days treated animals comprised

Table 3. Effect of ethanolic and aqueous extracts of *Hyptis suaveolens* on oral glucose tolerance in normal rats.

Group	Treatments and dose	Blood glucose concentration (mg/dl)					% Reduction at 3 h, w.r.t 5h
		0 min	0.5 h	1 h	2 h	3 h	
I	Normal control	83.75 ± 0.47	86.50 ± 0.64	88.50 ± 0.64	83.50 ± 0.64	86.50 ± 0.64	
II	Solvent control (2 ml/kg)	90.50±0.64	135.52±0.64 ^{###}	118.83±0.85 ^{###}	98.50±0.64 ^{###}	91.50±0.64 ^{###}	32.48
III	Glibenclamide (5 mg/kg)	89.43 ± 0.40	95.50±1.04 ^{***}	81.53±0.91 ^{***}	72.50±0.64 ^{***}	62.51±0.64 ^{***}	34.54
IV	Et. Ext. (250 mg/kg)	83.62±0.40	98.25±0.85 ^{***}	97.61±0.91 ^{***}	93.50±0.64 ^{***}	89.13 ± 0.64	9.28
V	Et. Ext. (500 mg/kg)	87.50 ± 0.64	107.31±1.37 ^{***}	102.32±1.10 ^{***}	94.50±0.64 ^{**}	91.50 ± 0.64	14.73
VI	Aq. Ext. (250 mg/kg)	91.50 ± 0.64	116.84±1.10 ^{***}	109.83±0.85 ^{***}	102.65 ± 0.91 [*]	92.50 ± 0.64 ^{**}	20.83
VII	Aq. Ext. (500 mg/kg)	84.87 ± 0.91	128.36±0.85 ^{***}	117.36 ± 1.10	103.51±0.64 ^{***}	96.53±0.64 ^{***}	24.79

Data represented as Mean ± SEM, (n=6). p<0.05 ^{*}significant, ^{##}very significant, ^{###}highly significant as compared to normal control group. p<0.05 ^{*}significant, ^{**}very significant, ^{***}highly significant as compare to solvent control group. (One way analysis of variance (ANOVA) followed by Dunnett's t-test).

Table 4. Effect of ethanolic and aqueous extract of *Hyptis suaveolens* on blood glucose level in alloxan induced diabetic rats.

Groups	Treatment	Blood glucose concentration (mg/dl)				% Decrease at 11 day
		0 day	3 day	7 day	11 day	
I	Normal control	88.33 ± 2.155	89.33 ± 1.745	89.33 ± 1.820	90.33 ± 1.687	-
II	Diabetic control	407.5 ± 4.341	407.7 ± 6.458 ^{###}	419.5 ± 5.708 ^{###}	452.7 ± 10.53 ^{###}	-
III	Glibenclamide (5 mg/kg)	395.7 ± 27.17	212.3 ± 3.603 ^{***}	173.5 ± 3.630 ^{***}	101.8 ± 4.90 ^{***}	74.27
IV	Et. Ext. (250 mg/kg)	345.2 ± 7.998	297.8 ± 8.080 ^{***}	143.2 ± 11.57 ^{***}	164.5 ± 5.97 ^{***}	52.34
V	Et. Ext.(500 mg/kg)	367.7 ± 15.15	306.7 ± 7.360 ^{***}	214.8 ± 5.081 ^{***}	136.3 ± 4.787 ^{***}	62.93
VI	Aq. Ext.(250 mg/kg)	327.0 ± 21.22	283.8 ± 12.16 ^{***}	241.3 ± 12.44 ^{***}	146.3 ± 3.921 ^{***}	55.25
VII	Aq. Ext.(500 mg/kg)	321.7 ± 18.25	289.3 ± 9.330 ^{***}	216.2 ± 2.770 ^{***}	118.7 ± 5.207 ^{***}	63.10

Data represented as Mean ± SEM, (n=6). p<0, 0.05 ^{*}significant, ^{##}very significant, ^{###}highly significant as compared to normal control group. p<0.05 ^{*}significant, ^{**}very significant, ^{***}highly significant as compared to diabetic control group. (One way analysis of variance (ANOVA) followed by Dunnett's t-test).

of similar group distribution as earlier mentioned and was determined by simple weighing, using standard balance on 0, 3, 7 and 11 day of the study. The animals were sacrificed at the end of the study and blood samples were collected by standard method for estimation of serum urea, creatinine, triglycerides, AST, ALT, ALP and cholesterol (Tables 5 and 6).

In vitro antioxidant activity

DPPH radical scavenging activity

Quantitative analysis

The free radical scavenging capacity of the both extract

was determined using DPPH. An ethanolic DPPH solution (0.004%) was mixed with serial dilutions (10 to 100 µg/ml) of crude extracts and after 30 min, the absorbance was read at 515 nm using a spectrophotometer (Schimadzu 1700 UV – visible spectrophotometer). Ascorbic acid was used as reference. The inhibition curve was plotted and IC₅₀ values obtained by Probit analysis (Viturro et al.,

Table 5. Effect of ethanolic and aqueous extract of *Hyptis suaveolens* on body weight on treated alloxan induced diabetic rats.

Groups	Treatment	Body weight in (g)			
		0 day	3 day	7 day	11 day
I	Normal Control	182.3 ± 4.2	188.34±3.93	193.22±4.38	199.8 2± 6.8
II	Diabetic control	190.8 ± 3.8	182.56±4.61	173.36±3.28 ^{##}	164.74 ± 7.2 ^{###}
III	Glibenclamide (5 mg/kg)	176.5 ± 4.9	182.31±3.72	187.74±2.89 ^{**}	198.56 ± 6.1 ^{**}
IV	Et. Extract (250 mg/kg)	182.6 ± 4.6	186.53±3.77	190.87±3.44 ^{**}	193.53 ± 6.1 ^{**}
V	Et. Extract (500 mg/kg)	176.2 ± 5.1	187.59±3.46	191.82±4.68 ^{**}	194. 3 3± 5.8 ^{**}
VI	Aq. Extract (250 mg/kg)	184.4 ± 4.4	187.37±2.86	191.91±3.73 ^{**}	192.54 ± 6.0 ^{**}
VII	Aq. Extract (500 mg/kg)	182.3 ± 4.2	184.73±3.41	188.39±3.66 ^{**}	196.62 ± 5.1 ^{**}

Data represented as mean ± SEM, (n=6). p<0.05 [#] significant, ^{##} very significant, ^{###} highly significant as compare to normal control group. p<0.05 *significant, **very significant, ***highly significant as compare to diabetic control group. (One way analysis of variance (ANOVA) followed by Dunnett's t-test).

1999).

Phytochemical Screening

Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. To perform the tests, the following chemicals and reagents were used: steroids with chloroform and sulphuric acid, tannins with ferric chloride solution, gum with Molish reagents and concentrated sulfuric acid, flavonoids with Mg and HCl and saponins with the capability of producing suds. Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagendorff's reagent. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

Statistical analysis

The data obtained in the animal experiments like body weight, fasting blood sugar and biochemical estimations were subjected to statistical analysis. All values are expressed as Mean ± SEM (Standard Error of Mean). The data were assessed by the analysis of variance (ANOVA). Mean values were considered significantly different if P< 0.05 and 0.001.

RESULTS AND DISCUSSION

Alloxan-induced type 2 diabetes is a chemical model of experimental diabetes mellitus developing a severe hyperglycaemia and widely used in the diabetic studies. This model was used in our investigation to evaluate the effects of aerial parts of *H. suaveolens* ethanolic and aqueous extracts on hyperglycaemia and some metabolic disorders related to diabetic mellitus. The effects of ethanol and aqueous extracts of aerial parts of *H. suaveolens* on fasting blood glucose levels of normal and diabetic rats are presented in Tables 1 and 2 respectively.

The plant extracts induced 4 to 15% fall of fasting blood glucose which is not significant enough to interpret hypoglycemic effect on normal rats (Table 1). Treatment

of normal rats with glibenclamide produced a significant (p<0.01) hypoglycaemic effect from first to 6 h, reaching a 41.15% maximum fall (p<0.01) in the blood glucose, as compared with the normal control group or with time 0. In alloxan induced diabetic rats as shown in Table 2, a dose dependent effect of the plant extract was observed. The test extracts showed a persistent decrease in blood glucose level till the end of 10 h, with maximal decrease noted in aqueous extract at 500 mg/kg dose, reaching 66.72% (p<0.01), while the standard drug glibenclamide showed 70.67% decrease. The test result presented in Table 3, indicates that the test extracts induce reduction in hyperglycaemia during the glucose tolerance test in diabetic rats. The alloxan-induced hyper glycaemia was significantly (p<0.01) corrected by the plant extract at the end of the treatment (11 days) in a sustained dose dependent manner, the result of which is presented in table 4. The maximal reduction 63.10% was observed with aqueous extract at high dose of 500 mg/kg. The potency of the extract in the light of fall of blood sugar level is dose dependent and in the order of aqueous extract followed by ethanol extract. *H. suaveolens* recover the body weight of treated diabetic group in a significant extent (p<0.01) when compared with diabetic control group and approach towards the untreated control normal animal group (Table 5).

The biochemical parameters of plasma urea, creatinine, triglyceride, cholesterol, AST, ALT, ALP values of treated diabetic groups at the end of the treatment (11 days) decreases in a significant extent (p<0.001) when compared with diabetic control group (Table 6). The extent of decrease is in a dose dependent order and the potency rest first with aqueous extract followed by ethanolic extract.

The preliminary phytochemical investigation report indicates that the aqueous extract of *Hyptis suaveolens* found to contain carbohydrates, saponins, tannins, alkaloids, flavonoids, terpenoids, steroids and sterols as

Table 6. Effect of ethanolic and aqueous extract of *Hyptis suaveolens* on some serum biochemical parameters on treated alloxan induced diabetic rats.

Group	Treatment	Urea (mg/dl)	Creatinine (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
I	Control	42.25±0.47	0.83±0.004	84.25±0.47	63.25±0.75	36.42 ± 0.8	136.14 ± 2.6	118.20 ± 4.8
II	Diabetic control	183±0.64 ^{###}	1.53±0.008 ^{###}	145.5±0.64 ^{###}	172.8±1.10 ^{###}	64.26 ± 1.2*	170.20 ± 4.8*	134.28 ± 2.2*
III	Glibenclamide (5 mg/kg)	89±0.40 ^{***}	1.13±0.006 ^{***}	104.8±0.85 ^{***}	85±0.91 ^{***}	42.46 ± 1.4**	141.80 ± 2.6**	128.12 ± 1.9**
IV	Et. Ext.(250 mg/kg)	143.8±0.47 ^{***}	1.29±0.006 ^{***}	138.3±0.85 ^{***}	128.5±1.04 ^{***}	47.15 ± 1.1 ^{***}	144.00 ± 4.4 ^{***}	126.6 ± 3.8 ^{***}
V	Et. Ext. (500 mg/kg)	109±0.40 ^{***}	1.27±0.004 ^{***}	132.5±1.19 ^{***}	114.5±1.32 ^{***}	44.26±2.2 ^{***}	142.00±4.2 ^{***}	120.26±2.4 ^{***}
VI	Aq. Ext. (250 mg/kg)	92.50±0.64 ^{***}	1.17±0.006 ^{***}	115.0±1.08 ^{***}	96.75±1.31 ^{***}	44.26±2.2 ^{***}	144.22±2.4 ^{***}	124.24±4.6 ^{***}
VII	Aq. Ext.(500 mg/kg)	89±0.40 ^{***}	1.15±0.006 ^{***}	108.3±0.85 ^{***}	94.25±1.10 ^{***}	43.62±2.8 ^{***}	143.20±4.6 ^{***}	121.42±1.2 ^{***}

Data represented as Mean ± SEM, (n=6). p<0.05 # significant, ^{##}very significant, ^{###}highly significant as compare to control group. p<0.05 *significant, **very significant, ***highly significant as compare to negative control group. (Analysis of variance (ANOVA) followed by Dunnett's test).

Table 7. Effect of ethanolic and aqueous extract of *Hyptis suaveolens* on DPPH scavenging activity.

Test substances	Concentration (µg/ml)				IC ₅₀ (µg/ml)
	10	20	50	100	
Ascorbic acid	28.8±0.22	62.8 ± 0.70	80.8 ± 1.14	95.17 ± 1.45	20.92
Ethanolic ext.	21.6 ± 0.19 ^{***}	54.2 ± 0.71 ^{***}	73.1 ± 1.01 ^{**}	90.5 ± 1.36*	30.06
Aqueous ext.	22.4 ± 0.23 ^{***}	57.5±0.33 ^{***}	72.2.4 ±0.02	93.2 ±1.23	28.76

Data are expressed as Mean ± S.E.M., n = 6; *p<0.01 vs. control, ** p< 0.001 vs control and ***p< 0.0001 vs. control by student's 't' test.

phytoconstituents. DPPH is relatively stable nitrogen and centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515 nm and also for a visible deep purple color. When DPPH

accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC₅₀ value of the test extracts was found to be 30.06 and 28.76 µg/ml for ethanolic extracts and aqueous extracts while the IC₅₀ value of ascorbic acid was 20.92 µg/ml (Table 7). From the results, it may be postulated that both the plant extracts have hydrogen donors thus, scavenging the free radical DPPH.

It is generally accepted that alloxan treatment causes permanent destruction of β-cells and

impairment of renal function and sulfonyl ureas are known to lower the blood glucose level by stimulating β-cells to release insulin (Pari and Maheswari, 1999). The hypoglycemic effect comparable to glibenclamide suggested that the extract may act by regenerating the β-cells in alloxan-induced diabetes (Ghosh and Suryawanshi, 2001) and the decreased activity in glucose level in OGTT might be due to a decrease in the rate of initial glucose absorption when plant fiber is given orally with glucose (Day et al., 1990). Diabetes mellitus results from the failure to use

glucose for energy, which leads to increased utilization and decrease storage of protein responsible for reduction of body weight essentially by depletion of body proteins (Guyton and Hall, 2000). It has been reported that the increase in glycaemia in alloxan or streptozotocin-induced diabetic rats was associated with dislipidoemia characterized by elevated serum triglycerides total cholesterol levels (Dhanbal et al., 2006). The improvement of blood glucose level induced by most hypoglycaemic treatment is associated with a reduction of serum triglycerides and total cholesterol. The presence of alkaloids, carbohy-drates, flavonoids, tannins, steroids and/or terpenes in the whole plant of *H. suaveolens* either as single constituents or in combination may be responsible for the observed anti-diabetic activity.

Conclusion

In this study, several animal models and *in vitro* assay were applied to evaluate the antidiabetic activity of ethanolic and aqueous extract of aerial parts of *H. suaveolens* Poit. As a conclusion, it could be speculated that the observed antidiabetic activity of *H. suaveolens* Poit might be related to the presence of tannins, terpenoids, steroids and flavonoid contents and having the potential to impart beneficial therapeutic effect in diabetes.

This study also establishes a correlation between antidiabetic and antioxidant potential and may be of considerable interest in preventing the ill effects of diabetes and oxidative stress *in vivo*. However, the plant extracts should be investigated to find out the chemical compounds responsible for antidiabetic action. Furthermore, *in vitro* antioxidant activity should be evaluated by other antioxidant methods, for example, nitric oxide scavenging activity, reducing power, lipid peroxidation assay.

ACKNOWLEDGEMENTS

The authors are grateful to SPS, SOA University, Bhubaneswar for providing necessary facilities to carry out the research work in the faculty of pharmacy, SOA University.

REFERENCES

Abdel-Barry JA, Abdel-Hassan IA, Al-Hakim MHH (1997). Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J. Ethnopharmacol. 58: 149-155.

Akueshi CO, Kadiri CO, Akueshi EU, Agina SE, Ngurukwem B (2002). Antimicrobial potentials of *hyptis suaveolens* Poit (Lamiaceae), Nigeria. J. Bot., 15: 37-41.

Aslan M, Deliorman OD, Orhan N, Sezik E, Yesilada E (2007a). In vivo antidiabetic and antioxidant potential of *Helichrysum plicatum* ssp. *Plicatum capitulum* in streptozotocin-induced diabetic rats. J. Ethnopharmacol., 109: 54-59.

Aslan M, Deliorman Orhan D, Orhan N, Sezik E, Yesilada E (2007b). A study of antidiabetic and antioxidant effects of *Helichrysum graveolens capitulum* in streptozotocin-induced diabetic rats. J. Med. Foods, 10: 396-400.

Bakirel T, Bakirel U, Keleş OU, Ülgen SG, Yardibi H (2008). In vivo assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. J. Ethnopharmacol. 116:64-73.

Chatterjee A, Pakrashi SC (1997). The Treatise on Indian Medicinal Plants, PID, New Delhi. 5:15.

Dash GK, Suresh P, Ganapaty S (2001). Studies on hypoglycaemic and wound healing activities of *Lantana camara* Linn. J. Nat. Remed. 1:105-110.

Day C, Catwright T, Provost J, Bailey CJ (1990). Hypoglycaemic effect of *Momordica charantia* extracts. Planta Med. 56:426-429.

Dhanbal SP, Kokate CK, Ramnathan M, Kumar EP, Suresh B (2006). Hypoglycaemic activity of *Pterocarpus marsupium* Roxb. Phytother. Res. 20:4-8.

Ghani A (2003). Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh, Dhaka, Bangladesh. pp. 500-504.

Ghosh S, Suryawanshi SA (2001). Effect of *Vinca rosea* extracts in treatment of alloxan rats, Indian J. Exp. Biol. 39:748-759.

Gills LS (1992). Ethnomedicinal uses of plants in Nigeria. University of Benin Press, Benin City, Nigeria. p 276.

Guyton AC, Hall JE (2000). Guyton and Hall Text Book of Medical Physiology. 10th ed. Elsevier, Reed Elsevier India Pvt. Ltd., New Delhi, India. pp. 894-897.

Kirtikar KR, Basu BD (1991). Indian medicinal plants. Singh B & Singh, M.P. Publishers, India. Vol. 3, p 2032.

Luo Q, Cai Y, Yan J, Sun M, Corke H (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. Life Sci. 76:137-149.

Pari L, Maheswari J (1999). Hypoglycemic effect of *Musa sapientum* L. in alloxan-induced diabetic rats. J. Ethnopharmacol. 68:321-325.

Pushparaj P, Tan CH, Tan BK (2000). Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J. Ethnopharmacol. 72:69-76.

Sepici A, Gurbuz I, Çevik C, Yesilada E (2004). Hypoglycaemic effects of myrtle oil in normal and alloxan-diabetic rabbits. J. Ethnopharmacol., 93: 311-318.

Anonymous (1964). The Wealth of India: Raw, Materials. Council for Scientific and Industrial Research, New Delhi. Vol. 5, p 159.

Vituro C, Molina A, Schmeda-Hirschmann G (1999). Free radical scavengers from *Mutisia friesiana* (Asteraceae) and *Sanicula graveolens* (Apiaceae). Phytother. Res. 13:422-424.

World Health Organization (2004). Diabetes action now. Web page <http://www.who.org> (accessed January 2004).