

ANTIDIABETIC EFFECT OF 50% HYDROETHANOLIC EXTRACT OF *ARISTALOCHIA BRACTEOLATA* LAM LEAVES IN STREPTOZOTOCIN- INDUCED DIABETIC RATS

K. Thangavelu*¹ and V A Doss²

¹Assistant Professor, Department of Biochemistry, PSG College of Arts & Science,
Coimbatore-641014, Tamil Nadu.

²Associate Professor, Department of Biochemistry, PSG College of Arts & Science,
Coimbatore-641014, Tamil Nadu.

Article Received on
16 Nov 2014,

Revised on 11 Dec 2014,
Accepted on 05 Jan 2015

***Correspondence for
Author**

Dr. K. Thangavelu

Assistant Professor,
Department of
Biochemistry, PSG
College of Arts &
Science, Coimbatore-
641014, Tamil Nadu.

ABSTRACT

The present work was aimed to evaluate the antidiabetic effect of 50% hydroethanolic extract of *Aristolochia bracteolata* Lam. leaves on 36 adult male albino Wistar rats weighing about 190-220 gms. Rats were divided into 6 groups of 6 rats in each. Group-I served as normal control while diabetes was induced in Group-II, III, IV, V and VI using Streptozotocin at a dose of 55 mg/kg bw by intraperitoneal injection. After induction of diabetes Group – II served as diabetic control. Group – VI rats were treated with 600 µg/kg bw/day of standard drug (Glibenclamide) while Group – III, IV and V rats were orally administered with 50% hydroethanolic leaf extract of *Aristolochia bracteolata* Lam. at the doses of 150, 300 and 450 mg/kg bw/day respectively for 28 days. To determine hypoglycemic effect of 50% hydroethanolic leaf extract of *A. bracteolata* Lam., blood and tissue biochemical parameters were assayed in normal, diabetic control and treated groups. Oral administration of 50% hydroethanolic extract of *A. bracteolata* Lam. leaves for 28 days exhibited a significant ($p < 0.05$) antidiabetic effect similar to those produced by Glibenclamide at a dose of 600 µg/kg bw/day for 28 days. This study also revealed potent antioxidant effect of 50% hydroethanolic leaf extract of *A. bracteolata* Lam. 9 major compounds from the leaf extract were identified through GC-MS analysis.

KEYWORDS: Leaf extract of *Aristolochia bracteolata* Lam., Streptozotocin (STZ), Glibenclamide (GB) and antidiabetic effect.

INTRODUCTION

Diabetic and related complications are the major health problem worldwide. Currently, there are over 150 million diabetic patients globally and this likely tends to increase to 300 million or more by the year 2025.^[1] Diabetes attributed by oxidative stress and this extended to neurological, cardiovascular, retinal and renal complications.^[2]

Diabetes are broadly classified into Type – 1, Type – 2 and Gestational diabetes. Type – 1, associated with 10% diabetic population including childhood, reduction of Insulin production. Type – 2, diabetes is the most common type and characterized by resistance to insulin activity found in 90% diabetic population of more than 30 years of age.^[3]

Diabetes treated by Insulin and chemical hypoglycemic agents, produce various side effects. Hence, many studies are required to investigate the herbal phytochemical principle to treat diabetes with efficient manner.^[4]

Aristolochia bracteolata Lam., Warm Killer is a perennial herb found in the upper Gangetic Plain, the Western Peninsula, Bengal, Gujarat and the South India. It is used in the treatment of syphilis, gonorrhoea, boils, bowel ulcers and other skin disease.^[5] Ethanolic leaf extract of *A. bracteolata* Lam. has antioxidant and wound healing effect in rats, using wound models, at two different dose levels of 400 and 800 mg/kg bw/day.^[6] Most of the plant extract exhibiting hypoglycemic, hypolipidemic and antioxidant effects in animals may be helpful to treat diabetes and associated complications in human.^[7]

MATERIALS AND METHODS

1. **Plant collection:** *Aristolochia bracteolata* Lam. was collected from near Marudhamalai, Coimbatore District, Tamil Nadu. Fresh leaves were washed and allowed to shade dry.
2. **Preparation of Leaf Extract:** Dried leaves were made to coarse powder and used for preparing extract using Benzene, Acetone, Ethanol, 50% ethanol and water. All the extracts are subjected to qualitative analysis. Among all, 50% hydroethanolic leaf extract was chosen to study the antidiabetic activity. 250 gm leaf powder was mixed in 1000 ml of 50% hydroethanol and allowed to stand for 72 hours with occasional shaking. The extract was filtered and the filtrate was evaporated. The residue was stored for further uses.

Standard Drug: Glibenclamide (GB) is a sulphonylurea group used as oral hypoglycemic drug.

- Test Animals:** Albino Wister rats, weighing 190 – 220 gram were maintained in standard environmental condition (20 – 25⁰C) using acrylic cage. Fed them with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the IAEC and CPCSEA and our protocols were duly approved by the Institutional Ethical Committee (Reg No. 158/1999/CPCSEA).
- Acute Toxicity Studies:** Healthy albino rat fasted overnight and were fed with increasing doses (1, 2, 4 and 8 gm/kg bw) of the 50% ethanolic extract^[6a]. In doses of up to 8 g/ kg bw did not produce any evident sign of toxicity or mortality in rats up to 14 days after administration.
- Gas Chromatography – Mass Spectroscopy (GC – MS) Analysis of *Aristolochia bracteolata* Lam. Leaves:** GC-MS analysis was performed with THEROMO GC-TRACE ULTRA VER: 50, THERMO MS DSQ II equipment. DB 5-MS capillary standard non-polar column was used to analyze the compounds. Oven temperature was maintained at 80⁰C and then raised to 260⁰ C at 5⁰ C/min. The carrier gas used was helium (1ml/min) and the sample injected was 1µl. The total running time for GC was 45 minutes.

EXPERIMENTAL PROCEDURE

Induction of Diabetic: The animals were grouped and left for fasting overnight. Streptozotocin (STZ) solution of 10 mg/ml was prepared in ice-cold citrate buffer 0.2M, pH 4.5 kept in ice bath and was administered immediately at a dose of 55 mg/kg bw intraperitoneally. After 48 hrs of STZ injection, rats with blood glucose level (GBL) of 200-300 mg/dL were considered as diabetic rats.^[8] The treatment was started on 3rd day onwards and continued for 28 days.

Determination of Hypoglycemic Effect in Diabetic Rats: The overnight fasting rats were divided into six groups (Group – I, II, III, IV, V and VI) of six rats each. Group – I served as normal and Group – II served as diabetic control. Group – III, IV, V and VI received 150, 300 and 450 mg/kg bw/day 50% hydroethanolic leaf extract of *Aristolochia bracteolata* Lam. and GB 600 µg/kg bw/day respectively for 28 days. All the rats were sacrificed and their serum, tissue biochemical parameters and tissue architecture were examined.

Statistical Analysis: The data were expressed as mean \pm SD with CD values. Statistical comparisons were performed by One – way analysis of variance (ANOVA), followed by Duncun Multiple range test. The results were considered statistically significant if the p-values are 0.05 or less.

RESULT AND DISCUSSION

Phytochemical Constituents

Phytochemical constituent present in the various solvent extract of *A. bracteolata* Lam. leaves are given in the Table – 1. Primary phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, Phenolic compounds, glycosides and tannins.

Table 1: Phytochemical constituent of *Aristalochia bracteolata* Lam. leaves extract.

Sl.No	Plant Constituents	Solvent Extracts			
		Benzene	Chloroform	50% ethanol	Water
1	Alkaloid	+	+	++	+
2	Flavanoid	+	-	++	-
3	Saponin	+	-	++	++
4	Phenolics	-	-	+	++
5	Tannins	-	-	++	++
6	Carbohydrates	-	-	+	+
7	Glycosides	-	-	++	++
8	Proteins	-	-	+	+
9	Steroids	+	+	-	-

+ = Presence; - = Absence

The acute toxicity studies showed that the extract was safe up to a maximum dose of 8 g/ kg bw.

Effect of *Aristalochia bracteolata* Lam. leaves extract on diabetic rats

To chose optimum dose for the diabetic animal, different doses of 50% hydroethanolic leaf extracts (150, 300 and 450 mg./kg bw/day) were used. Blood Glucose Levels evaluated in experimental rats along with the normal rats. Table - 2 exhibits the effect of different doses of *A. bracteolata* Lam. leaves extracts and GB on BGLs, HbA_{1c} and serum Insulin in experimental rats. The hypoglycemic effect of 50% hydroethanolic leaf extract of *A. bracteolata* Lam. and GB (Group -III, IV, V and VI) were compared with untreated diabetic rat (Group – II). A reduction of BGL to an extent of 94% was observed in GB treated diabetic rats at dose of 600 μ g/kg bw/day and 81.63%, 82.33% and 85.16% reduction in BGLs seen doses of 150, 300 and 450 mg/kg bw/day respectively for 28 days of 50%

hydroethanolic leaf extract of *A. bracteolata* Lam. There is a significant reduction of HbA_{1c} and increased level of Insulin and liver glycogen observed in treated groups (Table - 2).

Increased HbA_{1c} level in diabetic rats were due to increased blood glucose level. Excess load of blood sugar glycosylates some proteins including haemoglobin, albumin, collagen and crystalline protein non-enzymatically in diabetes.^[9]

On oral administration of leaf extracts of *A. bracteolata* Lam. with doses of 150, 300 and 450 mg/kg bw/day for 28 days, HbA_{1c} level in blood decreased significantly ($p < 0.05$) compared to Glibenclamide treated diabetic rats. This result is similar to the effect of ethanolic extract of *Annonu squamosa* on diabetic rats (100mg/kg bw/day) treated for 30 days.^[10]

Streptozotocin-induced diabetic rats (Group-II) showed significant decrease in plasma insulin when compared to other groups. Streptozotocin up-regulates Lipid Peroxidation (LPO), SOD and Glutathione peroxidase (GPx) of pancreas and causes oxidative stress by increasing TBARS in the diabetic pancreas to destroy β -cells.^[11]

Table 2: Levels of Blood Glucose, HbA1c and serum Insulin of experimental animals.

GROUPS	Blood Glucose Mg/dL	HbA1c Mg/dL	Serum Insulin μ IU/L	Liver Glycogen Mg/gm tissue
Group I (NC) Normal control	109.50 \pm 07.60	5.08 \pm 0.24	14.50 \pm 0.64	59.50 \pm 3.73
Group II (DC) Diabet control	392.17 \pm 18.80	9.73 \pm 0.45	8.96 \pm 1.00	28.17 \pm 5.19
Group III DC – 150mg Ab	161.00 \pm 14.04	8.48 \pm 0.52	9.31 \pm 0.53	46.67 \pm 3.33
Group IV DC – 300mg Ab	159.00 \pm 12.08	7.65 \pm 0.56	11.73 \pm 0.37	50.83 \pm 2.14
Group V DC – 450mg Ab	151.50 \pm 22.21	7.12 \pm 0.55	10.17 \pm 0.47	50.50 \pm 1.87
Group VI DC - 600 μ g GB	128.50 \pm 17.22	6.68 \pm 0.52	11.88 \pm 0.58	41.83 \pm 6.71
CD ($P < 0.05$)	1.61	0.499	0.678	3.977

Values are mean \pm SD of six samples in each group

Gh = *Gymnema hirsutum*; GB = Glibenclamide; NC = Normal Control;

DC = Diabetic Control.

There were significant ($p < 0.05$) changes in the plasma Insulin level of *A. bracteolata* Lam leaf extracts and Glibenclamide treated diabetic rats (Group-III to Group-VI) (Table-3). These results indicate that the standard drug and phytochemical principles of plant significantly

($p < 0.05$) increase the pancreatic secretion of Insulin from the existing but damaged β -cells by regenerating them. Similar reports are available that show that treatment with *Cassia glaucain* diabetic rats for 21 days increase in serum insulin level.^[12] The increased serum Insulin levels of treated animals (Group -III to Group-VI) may due to the stimulatory effect on cellular permeability, regeneration or reutilization of residual β -cells or by enhancement of endogenous insulin in diabetic rats. The results of the present study was similar to the results obtained by Kohli^[13] after treatment with *G. sylvestre*.

Liver glycogen levels of diabetic control rats (Group-II) were significantly low ($p < 0.05$) when compared with normal rats. This may be due to failure of utilization of glucose by hepatocytes in Streptozotocin-induced diabetic rats as claimed by Mitra.^[14] Glycogen content of all treated groups (Group-III to Group-IV) showed significant ($p < 0.05$) increase which is similar to the effect of *Aegle marmelose* leaf extract on diabetic rats.^[15]

Levels Serum and Tissue Biochemicals

The effect of oral administration of 50% hydroethanolic extract of *Aristolochia bracteolata* Lam. leaves at the doses of 150, 300 and 450 mg/kg bw/day for 28 days on STZ – induced diabetic rats were shown in the following Table – 3 and figure – 1, 2 and 3. Total cholesterol, Triglycerides, VLDL-Cholesterol and LDL-Cholesterol levels were significantly ($p < 0.05$) increased in Steptozotocin induced diabetic rats. Similar result had been reported in diabetic rats.^[16] This increased lipid profile level in diabetes is believed to be mainly due to the increase in the mobilization of free fatty acid from Extra Cellular Fluids (ECF) into the cell and increased activity of lipolytic hormones on the fat deposit.^[17]

Kaushal^[18] reported that administration of Bitter Gourd fruit juice on Streptozotocin-induced diabetic rats showed significant return of serum TC, TAG, VLDL, LDL, PL and HDL to the normal levels. Similar result found in treatment with 150, 300 and 450 mg/kg bw/day doses of 50% hydroethanolic extract of *A. bracteolata* Lam. leaves. HDL-Cholesterol level which was found to be reduced in diabetic rats showed good increase after treatment with all 3 doses of *A. bracteolata* Lam. leaves.

Table 3: Levels of Serum Total Cholesterol, TAG, HDL, VLDL and LDL-Cholesterol.

Experimental Animal Groups	Serum Total Cholesterol Mg/dL	Serum Triglyceride Mg/dL	Serum HDL Mg/dL	Serum VLDL Mg/dL	Serum LDL Mg/dL
Group I (NC) Normal control	88.83 ± 2.32	95.50 ± 12.73	38.50 ± 2.43	19.00 ± 1.16	31.33 ± 1.37
Group II (DC) Diabet control	150.00 ± 11.87	159.83 ± 7.76	30.17 ± 5.04	32.00 ± 1.41	88.83 ± 6.44
Group III DC – 150mg Ab	124.00 ± 6.23	113.82 ± 9.89	39.83 ± 2.64	23.50 ± 2.17	66.50 ± 11.4
Group IV DC – 300mg Ab	115.00 ± 10.53	106.33 ± 8.02	40.83 ± 2.32	21.33 ± 1.51	52.83 ± 12.2
Group V DC – 450mg Ab	119.33 ± 5.89	112.50 ± 5.09	40.17 ± 2.32	22.00 ± 1.41	57.17 ± 3.71
Group VI DC - 600µg GB	108.83 ± 6.21	109.50 ± 6.16	31.33 ± 3.14	22.00 ± 1.26	77.17 ± 5.2
CD (p<0.05)	10.121	12.06	2.817	2.223	6.71

Values are mean ± SD of six samples in each group; CD= Critical Difference;

Ab= *Aristolochia bracteolata* Lam.

Activities of Stress Marker Enzymes

Normalization of serum ALP, ALT, AST and LDH (diabetic markers) activities were found in 50% hydroethanolic leaf extract of *A. bracteolata* Lam. treated groups (figure – 1, 2, and 3).

The present study showed an increased AST level in serum, liver and kidney in experimental rats (Fig. 1 and 2). These results indicate hepatic damage and increased AST transport across the membrane. The levels of AST in serum, liver and kidney of treated group (Group-III to V) showed significant return of the values to normal level. This is similar to the effect of hydromethanolic extract of sepals of *Salmalia malabarica* in diabetes that restored the serum and tissue AST levels.^[19]

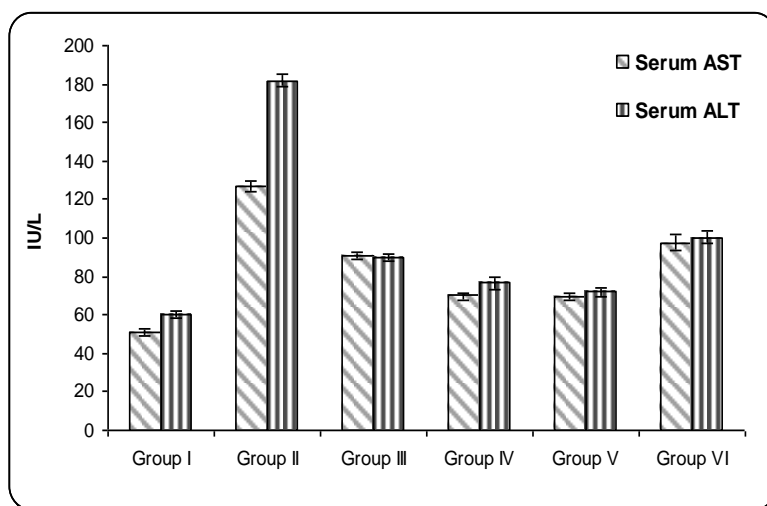


Fig. 1: Activities of serum AST and ALT of experimental animals.

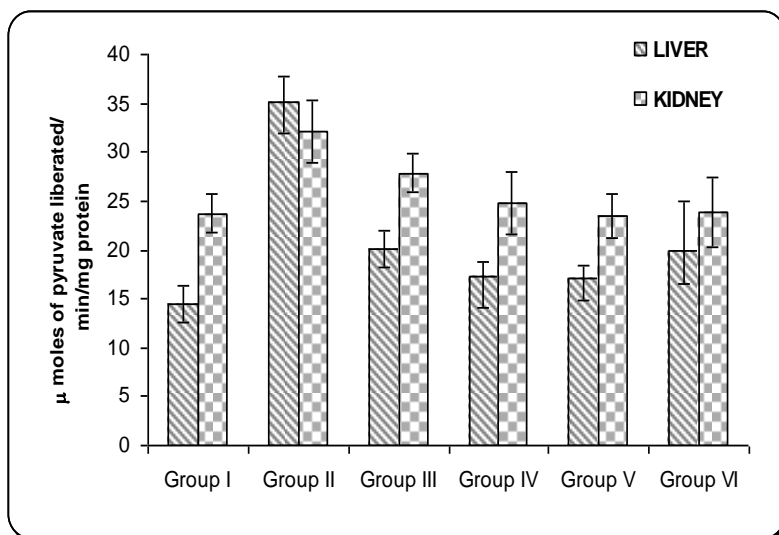


Fig. 2: Activities of AST in Liver and Kidney of experimental animals.

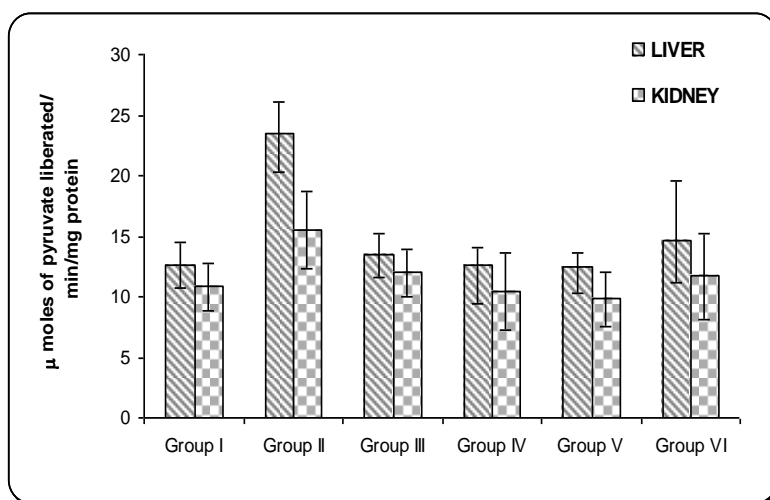


Fig. 3: Activities of ALT in Liver and Kidney of experimental animals.

The levels of ALT, after treatment with Glibenclamide and *A. bracteolata* Lam leaf extracts at various doses returned to values near to the control group, while that of treated normal control rats remained unchanged.

Any remarkable change in liver function shows increase in ALT level. Oral administration of aqueous extract of the aerial part of *Achyrocline satureioides* at a dose of 300mg/kgbw/ day reduced the ALT level effectively in diabetic rats.^[20]

Felig^[21] also reported that the activity of ALT had been increased in the absence of insulin because of availability of amino acids in blood of diabetes and are responsible for the increased gluconeogenesis.

Gas Chromatography – Mass Spectroscopy (GC – MS) Analysis of Leaf Extract of *Aristolochia bracteolata* Lam.

GC-MS analysis of leaf extract of *A. bracteolata* Lam. revealed that the presence of 9 major compounds including Bismethoxy Carbonyl derivative, Phenyl derivatives, Saturated and Unsaturated fatty acids, Methyl Esters, Octanoic acid, Phytol, Benzamide and 6-Methyl-1-Phenanthrenol (Table-4).

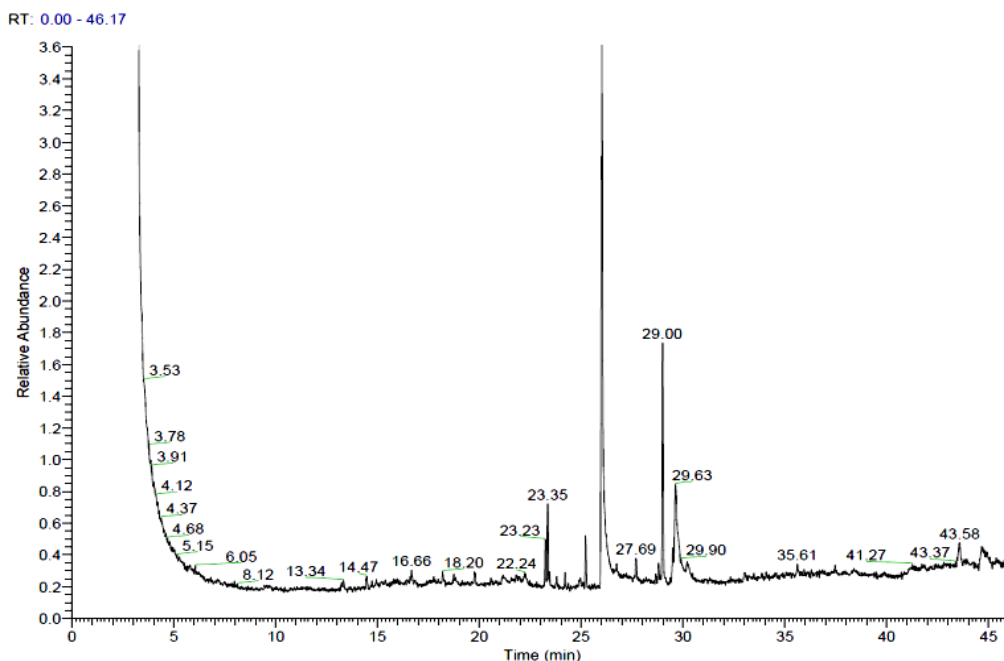


Fig. 4: GC spectrum of leaf extract of *A. bracteolata* Lam.

Table-4: Major compounds present in leaf extract of *A. bracteolata* Lam.

Sl.No	Chemical Name of the Compound	Molecular formula	M. Wt.	Retenti on time	Peak area %
1.	3-Isopropylidene-2,2-bismethoxy carbonyl - 5,5-dimethyl-4-phenylbicyclo[2.1.0]pentane	C ₂₀ H ₂₄ O ₄	328	9.57	0.46
2.	(4S,5S,Rs)-4-Tosyloxymethyl-5-(4-methylsulfinylphenyl)-2-phenyl-4,5-dihydro-1,3-oxazole	C ₂₄ H ₂₃ NO ₅ S ₂	469	13.31	0.57
3.	(2R,3R)-1,2-Epoxy-3-hexanol	C ₆ H ₁₂ O ₂	116	22.24	0.61
4.	2-Octyldodecan-1-ol	C ₂₀ H ₄₂ O	298	23.35	4.29
5.	Tridecanoic acid	C ₁₃ H ₂₆ O ₂	214	26.01	29.42
6.	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	26.01	29.42
7.	(S)-N-(1-Benzyl-2-chloroethyl) benzamide	C ₁₆ H ₁₆ ClNO	273	27.69	1.07
8.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]] (Phytol)	C ₂₀ H ₄₀ O	296	29.00	7.43
9.	6-Methyl-1-phenanthrenol	C ₁₅ H ₁₂ O	208	43.58	1.75

2 - Hexadecen - 1 - ol, 3,7,11,15 - tetramethyl - tetramethyl -, [R-(R*,R*-(E))], naturally called as phytol and containing antimicrobial, anticancer, antiinflammatory and diuretic effects.^[22]

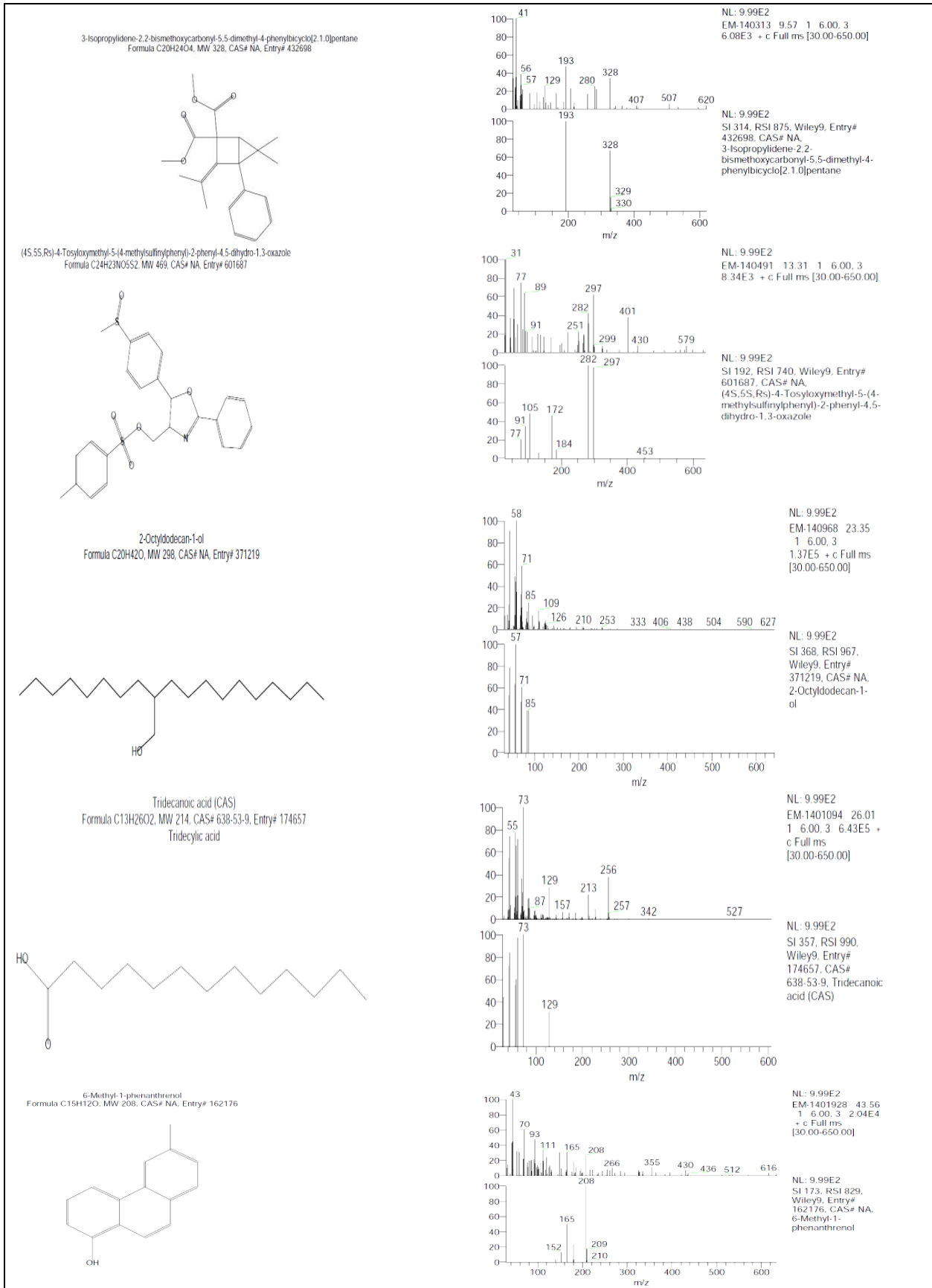


Fig.5: MS molecular spectrum fragment and structure of Major compounds present in leaf extract of and *A. bracteolata* Lam.

Certain flavonoids and phytol exhibit hypoglycemic activity.^[23] Carnitine Palmitoyl Transferase-1 (CPT-1) is associated with Type – 2 diabetes and Insulin resistance, causing increased mobilization and mitochondrial oxidation of fatty acids. This in turn produces more acetyl-CoA and results with increased gluconeogenesis and hyperglycemia.^[24]

Inhibition of fatty acid oxidation (FAO) is useful in reducing hypoglycemia by reducing gluconeogenesis. Inhibition of FAO and gluconeogenesis were attributed by reducing the release of fatty acid from adipose tissue (Nicotinic acid); by inhibition of acyl dehydrogenase (hypoglycin); by inactivation of mitochondrial CoA (p-tert butyl benzoic acid) and inhibition of CPT-1 activity (Tetra adenyl glycidate).^[25]

Phytol is a precursor of the natural retinoid phytanic acid that triggering Retinoid-X-Receptor (RXR), activates the full spectrum of peroxisome proliferator - activated receptors (PPARs).^[26] Heterodimerized Retinoid-X-Receptor (RXR) which coupled with CPT-1 lowers hyperglycemia in Type – 2 diabetes and obesity.^[27]

Analysis of ethanolic extract of *C. dactylon* in GC-MS revealed the presence of 2-Hexadecen-1-ol, 3,7,11,15 Tetramethyl, Hexadecanoic acid, Ethyl Ester, γ -sitosterol, Octadecanoic acid, Methyl ester, Tetracontane and N-Nonacosane. Since, they are potent antioxidants this plant extract used as a drug for various diseases like epilepsy, diabetes, cancer etc., and can serve as secondary metabolites targeting of many receptors.^[28] These study results were as similar as that of leaf extract of *A. bracteolata* Lam.

SUMMARY AND CONCLUSION

Diabetes Mellitus is a chronic metabolic disorder of carbohydrates, lipids and protein due to deficiency of Insulin secretion or resistance in its activity. It is characterized with high blood glucose and mortality. Diabetes increases the risk of long-term complications *viz.*, damage to blood vessels, cardiovascular risk, etc.^[29]

The present study showed that oral administration of *A. bracteolata* Lam. leaves extract reduced the blood glucose level in diabetic rats. The results suggest that the 50%hydroethanolic leaf extract produces an antidiabetic effect mediated by an increase in Insulin secretion, liver glycogen and peripheral glucose uptake like in GB treated diabetic rats.

The levels of serum Triglycerides, total cholesterol and LDL – cholesterol were significantly reduced in 50% hydroethanolic leaf extract of *A. bracteolata* Lam. treated STZ-induced

diabetic rats. Phytochemicals like flavanoid, alkaloids, phenolics and tannins are potent antioxidants and are used as antidiabetic agents for the management of diabetes.^[30] Activities of oxidative stress marker enzymes like AST, ALT and LDH in serum and key tissues of experimental animal become normal level. Thus, the significant effect of 50% hydroethanolic leaf extract of *A. bracteolata* Lam. may be due to the presence of more than one antihyperglycemic principles and their synergetic properties. From this study we may conclude that 50% hydroethanolic extract of *A. bracteolata* Lam. leaves have significant effect on blood glucose, serum lipids and marker enzyme like AST and ALT of STZ-induced diabetic rats.

ACKNOWLEDGEMENT

The authors are thankful to the management of PSG College of Arts & Science, Coimbatore, Tamil Nadu for providing laboratory facilities to carry out the research work.

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