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ANTIHYPERLIPIDIMIC, ANTIHYPERGLYCEMIC AND CHEMICAL COMPOSITION OF *SENNA SURATTENSIS* (BURM.F.) LEAVES

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ABSTRACT

Senna surattensis was used in folk medicine as a hypoglycemic plant. GC/MS technique was used for the identification of volatiles, saponifiable and unsaponifiable matters. Fifty two compounds were identified in the volatiles. The volatiles were composed mainly of oxygenated compounds. Andro-encecalinol was the major component. Twenty three fatty acids were found in the sap. fraction. The unsaturated fatty acids represented (11.62%). The most abundant ones were oleic and linoleic acids. Thirty two compounds were identified in unsaponifiable matter, mostly hydrocarbons. The major compound was prehnitol. Limonene, azulene, phytol and squalene were present in considerable amounts. For the investigation of the biological activities, two groups of male albino rats were used. The first group was injected with alloxan to induce diabetes. The hyprglycemic rats were fed on the leaf extract for 8 weeks. The second group was fed for two months on high fat to induce hyperlipidemia. Four and eight weeks after administration of the extract, blood samples were collected. Blood glucose level was measured in the first group. Total serum cholesterol, triglycerides, HDL-c and LDL-c were determined in the second group. The significant reduction in glucose and lipid profiles of plasma indicates the efficacy of *S. surattensis* leaf extract as a feed supplement for controlling blood glucose and body lipid metabolism.

Keywords: *Senna surattensis*, Caesalpiniaceae, volatiles, antihyperglycemia, antihyperlipidemia, unsaponifiable matter, fatty acids.

INTRODUCTION

Diabetes is generally associated with hyperlipidemia, which has been found to be mainly due to overproduction of very low density lipoprotein– triglycerides in type 2 diabetes (Bourgeois *et al*., 1995) besides abnormalities in lipid metabolism, characterized by severe hypertriglyceridemia and hypercholesterolemia (Alaupovic and Fernandes, 1985).

Senna surattensis (Burm.f.) (Syn: *Cassia glauca*, Family Caesalpiniaceae) was used as a hypoglycemic plant in folk medicine (Chopra and Chopra, 1956). The present study shows that the leaf extract possesses definite antihyperlipedemic effects in hyperlipidemic rats and antihyperglycemic properties in diabetic rats after 8 weeks of treatment. The chemical composition of volatiles, fatty acids and unsaponifiable matter were also identified. Study of LD_{50} of 80% ethanol extract of *S. surattensis* proved that that this plant is non toxic.

MATERIALS AND METHODS

Plant material

The fresh leaves of *S. surattensis* were collected from the garden of National Research Centre, Egypt, and was identified by Mrs. Treeze Labib, specialist in plant taxonomy [A voucher specimen of the plant (voucher number S-195) was kept in Pharmacognosy Department, National Research Centre, Egypt].

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Preparation of volatiles

Fresh leaves were chopped and subjected to combine hydrodistillation/solvent extraction using a modified Likens and Nickerson apparatus with 2-methyl butane (15mL) as a solvent.

Investigation of lipid content

The residue obtained after evaporation of petroleum ether was subjected to saponification (Tsuda *et al*., 1960) then subjected to GC/MS for fatty acid methyl esters and unsaponifiable matter.

Conditions of GC/MS analysis

A Trace GC 2000 (produced by Thermo) was used. The Mass Spectrometer model was SSQ 7000 produced by Finnigan equipped with library software Wiley 138 and NBS 75 was used. Capillary DB5 (Methylpolysiloxane containing 5% phenyl groups) column 25m X 0.25 mm i.d., Temp. program for volatiles was; 2 min. at 60˚C, 60-100˚C (2˚C/min) and 100-250˚C (5˚C /min). Temp. program for fatty acids methyl esters and unsaponifiable matter was: oven temperature was programmed at 50°C isothermal for 5 min.; then heating to 280°C at a rate of 5°C/min. then isothermal at 280°C for 5 min. Carrier gas was helium at flow rate of 1.0 ml/min. Injection voltage 70 ev.; scan mass range 40-450.

Identification of compounds
Compounds were identified by matching their mass spectra with those recorded in the MS library and further confirmed by comparing the mass spectra with those of reference compounds or with the published data (Adams, 1995).

Investigation of biological activity
Experimental animals
Adult male Albino rats of the Sprague Dawely strain weighing 130-150g. and Abino mice (25-30 g.) were obtained from the animal house of National Research Centre, Egypt. They were kept under the same hygienic conditions and well balanced diet and water.

Normal diet
Vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95% pure) 10.5% and starch 54.3%. Ethical issue was followed as reported (Ney *et al*., 1988).

Drugs, Biochemical Kits and Reagents
Alloxan, Sigma Co. Metformin (Cidophage)®, Chemical Industries Development (CID), Giza, ARE. Simvastatin (Zocor): Global Napi Pharmaceuticals, Egypt, under license from Merk & Co., NJ, USA. Biochemical kits used for assessment of blood glucose level. Biomerieux kits were used for the biochemical assessment of cholesterol, triglyceride, and high density lipoprotein levels.

Determination of LD_{50}
Total ethanol extract of *S. surattensis* leaves were prepared by percolation. Albino mice (25-30g) were divided into groups, each of 6 animals. Several doses at equal logarithmeic intervals were given orally to a group of six animals. The mice were then observed for 24h, after administration and symptoms of toxicity and mortality rates in each group were recorded and LD_{50} was calculated (Miller and Tainter, 1944).

Antihyperglycemic activity
The rats were injected intra-peritoneal with alloxan (150 mg/kg body weight) to induce diabetes mellitus (Eliason and Samet, 1969). Hyperglycemia was assessed after 72 hours by measuring blood glucose (Trinder 1969) and after 4 and 8 weeks intervals.

Animals were divided into 3 groups (each consisted of 10 rats):

1. First group: diabetic rats that serve as positive control.
2. Second group: diabetic rats that received 100 mg/kg b.wt. of plant extract.
3. Third group: diabetic rats that received 150 g/kg metformin.

At the end of each study period, blood samples were collected from the retro orbital venous plexus through the eye canthus of anesthetized rats after an overnight fast.

Serum was isolated by centrifugation and the blood glucose level was measured (Bucolo and David, 1973).

Antihyperlipidemic test
a- Induction of hyperlipidemia in rats: The rats were fed for two months on high fat for induction of hyperlipidemia (Ney *et al*., 1988). Hyperlipidemia was assessed by measuring serum triglycerides (Bucolo and David, 1973), total cholesterol (Allian *et al*., 1974), HDL-cholesterol. LDL- cholesterol fractions (Stein, 1986). Only hyperlipidemic rats were used for assessing the antihyperlipidemic activity of the different extract.

b- Three groups each consisted of ten induced hyperlipidemic rats were used in this test. The first group was kept as a control. The second group was treated with daily oral dose of 100 mg/kg body weight of the total ethanol extract for eight weeks. The third group was treated with daily oral dose of 2 mg/kg simvastatin. At zero time, 4 weeks and 8 weeks after administration of the extracts, blood samples were collected from the retro orbital venous plexus through the canthus of anaesthetized rats after an overnight fasting and serum was isolated by centrifugation. Total serum cholesterol, triglycerides, HDL cholesterol and LDL cholesterol were determined as mentioned above.

Statistical analysis
The data obtained were statistically analyzed using Student's "t" test (Snedecor and Cochran, 1971).

RESULTS

Volatile constituents
Data in table 1 showed that the volatiles consisted mainly of 52 compounds representing about 88% of the volatiles. The volatiles were composed mainly of oxygenated compounds (56.7%) of which 5.2% was monoterpenes and 8.9% was sesquiterpenes. Aldehydes was the major chemical class in the volatiles followed by ethers which was represented only by andro-encecalinol, the major compound. Both alcohols (9.5%) and esters (4.4%) constituted 7 compounds, while ketone was the least group in the volatiles (2.1%).

Hydrocarbons accounted for 31.6% of the volatiles of which 5.6% was monoterpenes, 7% sesquiterpenes and 15.7% straight chain hydrocarbons, mostly alkanes. The major hydrocarbon was tetracosane followed by mesitylene.

Table 1. Results of GC/MS analysis of volatile constituents of *Senna surattensis* Leaves.

KI	Compound	Area %
	Acetogenin	
	a-alkans	
1700	Heptadecane	0.4
1800	n-Octadecane	0.7
1900	Nonadecane	0.7
2000	Eicosane	1.0
2100	Henicosane	1.2
2200	Docosane	1.6
2300	Tricosane	1.3
2400	Tetracosane	8.0
2500	Pentacosane	0.5
	b-alkenes	
1292	Tridecene	
	c-aliphatic alcohols	
2082	octadecanol	0.1
	d-Aliphatic Aldehydes	
854	2-Hexenal	7.7
899	Heptanal	8.0
1102	Nonanal	2.0
	e-Aliphatic Acids	
1129	2-Ethyl hexanoic acid	0.9
	Terpenoids	
	a-Monoterpen hydrocarbons	
908	Santolinatriene	0.3
939	α-Pinene	1.5
1040	Cis-ocimine	2.0
1072	p-Mentha-3,8-diene	0.7
1082	m-Cymene	0.5
1111	p-Mentha-1,3,8-triene	0.6
	b-Sesquiterpene hydrocarbos	
1368	Cyclosativene	0.3
1390	β-Cubebene	0.4
1418	Trans-caryophyllene	0.5
1420	α-Santalene	4.1
1433	γ-Elemene	0.5
1508	α-Farnesene(E,E)	1.2
	c-Oxygenated monoterpenes	
1033	1,8-Cineole	1.6
1098	Linalool	1.0
1144	Cis-β-terpineol	0.1
1177	Terpin-4-ol	0.3
1235	Myrtenyl acetate	0.1
1241	Cyclocitral	0.8
1282	α-Terpinen-7-al	0.3
1306	Iso-verbanol acetate	0.1
1325	Limonene aldehyde	0.4
1350	α- Terpinyl acetate	0.2
1420	P-Menth-1-en-9-ol acetate	0.2
	d-*Oxygenated* sesquiterpens	
1426	α-Ionone	0.8
1566	Longipinanol	0.1
1574	Dendrolasin	0.2
1652	α-Eudesmol	4.9
2314	Palustrol	2.98
	Miscellaneous	
994	Mesitylene	3.28
1079	p-Methylbenzaldehyde	0.53
1190	Methyl salysilate	2.79
1246	Carvotanacetone	0.91
1324	Z-3-Hexenyl tiglate	0.67
1380	Damascenone	0.26
1576	Hexyl benzoate	0.26
1675	Andro-encecalinol	18.34
1922	Carissone	0.19

KI: Kovat index

Table 2. Results of GC/MS analysis of unsaponifiable matter of *Senna surattensis* leaves.

Peak no.	RRT	Compound	Area %
1	0.79	Mesitylene	1.3
2	0.81	Pseudocumol	1.1
3	0.85	Limonene	7.9
4	0.93	Diethyl benzene	6.0
5	1.00	Prehnitol	11.0
6	1.05	Benzene, 1-ethyl-2,3 dimethyl	1.1
7	1.095	1,2,3,5 Tertramethyl benzene	6.4
8	1.14	Benzene, 1,3-diethyl-5- methyl	1.3
9	1.17	Cymol	6.8
10	1.21	Benzene, 1- methyl-4-(1-methylpropyl)	0.6
11	1.24	1-Isopropyl-3,5-dimethyl benzene	0.5
12	1.26	3-Methyl-(3H)-isobenzofuran-1-one	1.2
13	1.28	Azulene	7.1
14	1.34	Benzene, 1,3-dimethyl-5-(1-methylethyl)	0.4
15	1.40	2-Methyl-5-isopropenyl-2-cyclohexenone	8.1
16	1.54	Naphthalene-2-methyl	0.5
17	1.57	Naphthalene-1-methyl	0.3
18	2.43	Neophytadiene	0.2
19	2.73	Ethyl tetradecanoate	1.3
20	2.86	Phytol	6.1
21	3.00	Phytol isomer	2.2
22	3.35	Pentacosane	0.3
23	3.46	Hexacosane	0.3
24	3.58	Heptacosane	0.6
25	3.71	Squalene	7.1
26	3.81	Nonacosane	1.6
27	3.94	Triacontane	0.5
28	4.11	Henitriacontane	1.4
29	4.22	Pentatriacontane	0.4
30	4.27	Campesterol	1.1
31	4.38	Stigmasterol	3.4
32	4.47	β-Sitosterol	8.4

RRt: retention time relative to prehnitol

GC/MS analysis of the unsaponfiable matter

Thirty two compounds were identified in unsaponifiable matter, accounting for 96.5% of this fraction (Table 2). The unsaponifiable fraction was composed mainly of hydrocarbons (64.5%). The major compound was prehnitol. Limonene, azulene, phytol and squalene were

also present in considerable amounts. The previous four hydrocarbons were proved to posses many biological activities. Eight oxygenated compounds were identified in the unsaponifiable matter of which three sterols were detected. The most abundant sterol was β-sitosterol, followed by stigmasterol. The total concentration of sterols reached 12.8%.

GC/MS analysis of the fatty acid methyl esters

Twenty three fatty acids were identified constituting 90.7% of the total fraction (Table 3). Methyl palmitate, methyl myristate, methyl stearate and methyl laurate were the major ones. Six unsaturated fatty acids constituting 11.6% were detected. The most important mono-unsaturated fatty acid esters were methyl oleate (6.5%) and methyl linoleate (3.2%).

***In vivo* antihyperlipidemic activity**

The repeated administration of *S. surattensis* leaf extract for a period of 8 weeks resulted in a significant decrease in lipid parameter levels of blood when compared to the control (Table 4). Serum cholesterol level were reduced by 28.3% and 43.2% after 4 and 8 weeks, respectively, while there were decreases in cholesterol level in control equal 3.3% after 4 weeks and significant increase (11.1%) after 8 weeks. Also triglycerides level significantly decreased by 18.8% and 49.2% after 4 and 8 weeks, respectively, while there were increases in the triglyceride level of the control group by 6.5% and 3.4%, respectively. Leaf extract treatment significantly lowers low density lipoprotein-cholesterol fraction (LDL-c) and raised high density lipoprotein-cholesterol fraction (HDL-c) levels. As concerning the cholesterol fractions, LDL-c level was decreased by 44.6% and 69.4% after 4 and 8 weeks, respectively, while the control group showed significant increase after 8 weeks (21.9%). The HDL-c was significantly increased after 4 weeks by 21% and after 8 weeks by 44.2%, while the control showed decreases in HDL-c levels by 7.6% and 12.2%, respectively. The risk factor (LDL/HDL) decreased from 2.8 in control to 1.6 in treated animals after 4 weeks and from 3.8 to 0.7 after 8 weeks. The relative potency of the leaf extract in reducing serum cholesterol, triglycerides and LDL-c reached 81%, 99.8% and 86.6%, respectively, as compared to those of simvastatin, while the extract induced an increase in HDL-c reached 105.5% of that of simvastatin.

***In vivo* antihyperglycemic activity**

The effect of 8 weeks oral administration of alcoholic extract of *S. surattensis* leaves in alloxan induced diabetic rats, at the dose of 100 mg/kg, on fasting blood glucose

Table 3. Results of GC/MS analysis of the fatty acid methyl esters of *Senna surattensis* leaves.

Peak no.	RRt	Compound	Area%
1	0.68	Methyl 2-methoxycarbonyl-3-methyl-3-butenoate	0.3
2	0.70	Methyl dodecanoate (methyl laurate)	7.4
3	0.74	Nonadecanoic acid dimethyl ester (dimethyl azelate)	0.4
4	0.78	Methyl tridecanoate	0.3
5	0.85	Methyl tetradecanoate (methyl myristate)	14.4
6	0.89	Tridecanoic acid,4, 8, 12-trimethyl-, methyl ester	0.2
7	0.92	Methyl pentadecanoate	0.9
8	0.97	Pentadecanoic acid, 14-methyl- methyl ester	0.2
9	0.99	9-Hexadecanoic acid, methyl ester, (Z)- (methyl palmitoleate)	0.3
10	1.00	Methyl hexadecanoate (methyl palmitate)	36.4
11	1.06	Methyl heptadecanoate	0.9
12	1.10	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (methyl linoleate)	3.2
13	1.11	9-Octadecenoic acid (Z)-, methyl ester (methyl oleate)	6.5
14	1.12	Methyl octadecanoate (methyl stearate)	7.7
15	1.16	Methyl 6,9- octadecadienoate	0.3
16	1.18	Methyl nonadecanoate	0.3
17	1.22	Methyl ester of ricinoleic acid	0.3
18	1.24	Methyl eicosanoate (arachidic acid methyl ester)	3.1
19	1.25	14-Oxo-nonadec-10-enoic acid methyl ester	1.0
20	1.33	Octadecanoic acid, 9,10-dichloro-, methyl ester	0.4
21	1.34	Methyl docosanoate (methyl behenate)	4.4
22	1.39	Methyl tricosanoate	0.4
23	1.44	Methyl tetracosanoate (methyl lignocerate)	1.5

RRt: retention time relative to methyl palmitate.

Table 4. Effect of alcoholic extract of leaves of *Senna surattensis* on total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) in hyperlipidemic rats.

Group	I ml saline					Leaf extract (100mg/kg body weight)					Simvastatin (2 mg/kg body weight)				
Time	Chol	TG	HDL	LDL	RF	Chol	TG	HDL	LDL	RF	Chol	TG	HDL	LDL	RF
	M±SE					M±SE					M±SE				
Zero	168.7 ± 5.1	142.4 ± 6.8	37.8 ± 0.7	102.4 ± 4.2	2.76	172.3 ± 6.9	143.8 ± 8.1	31.9 ± 1.1	111.6 ± 7.3	3.5	182.4 ± 9.6	162.3 ± 7.9	31.9 ± 1.2	118.0 ± 5.9	3.7
4 weeks	163.2 ± 8.9	151.6 ± 7.2	34.9 ± 0.6	98.0 ± 3.9	2.81	123.6* ± 5.2	116.2* ± 5.4	38.6* ± 1.5	61.8* ± 3.2	1.6	102.3* ± 4.7	95.2* ± 4.3	37.5* ± 1.6	45.8* ± 1.4	1.22
% of change	3.3	6.5	7.6	4.3	-	28.3	18.8	21.0	44.6	-	43.9	41.3	17.6	61.1	-
8 weeks	187.4 ± 9.1	147.2 ± 6.3	33.2 ± 0.9	124.8 ± 5.7	3.76	97.9* ± 4.1	72.5* ± 3.6	49.2* ± 1.3	34.2* ± 1.4	0.7	85.1* ± 2.5	81.7* ± 3.2	45.3* ± 2.7	23.5* ± 0.8	0.52
% of change	11.1	3.4	12.2	21.9	-	43.2	49.6	44.3	69.4	-	53.3	49.7	42.0	80.1	-

Chol: cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein, RF: risk factor= LDL/HDL, M±SE: mean± standard error.
*Significantly different at P< 0.01

Table 5. Effect of alcoholic extract of leaves of *Senna surattensis* on blood glucose level in diabetic rats.

Time	Group						
	Control	Diabetic non treated		Diabetic treated with 100 mg/kg alc. ext.		Diabetic treated with 150 mg/kg metformin	
	M± S.E.	M± S.E.	% of change	M± S.E.	% of change	M± S.E.	% of change
Zero	89.3 ± 2.7	267.5 ± 11.4	-	268.9 ± 2.1	-	269.1 ± 11.7	-
4 weeks	85.4 ± 3.1	265.3 ± 10.7	0.82	187.2* ± 9.8	30.38	142.6* ± 8.3	42.72
8 weeks	87.9 ± 2.6	271.2 ± 3.4	0.14	126.7* ± 6.3	49.56	86.4* ± 3.8	67.89

*Significantly different from control at P< 0.01

(FBG) were examined (Table 5). FBG levels of treated animals reduced by 30.4% and 49.6% after 4 weeks and 8 weeks treatment, respectively, with the aqueous leaves extract. The relative potency of leaf extract reached 71% of that of metformin after 4 weeks and 73% after 8 weeks.

DISCUSSION

Elevated levels of plasma cholesterol and triglycerides have been implicated as causative factors in the development of atherosclerosis and coronary heart diseases (Ross, 1999). Various plant-based formulations are known till date, which in one way or the other are known to have an effect on cholesterol level.

Diabetes is associated with profound alterations in the plasma lipid, triglycerides and lipoprotein profile and with an increased risk of coronary heart disease (Maghrani *et al*., 2004). High level of total cholesterol is one of the major factors for coronary heart diseases and it is well known that hyperlipidemia and the incidence of atherosclerosis is increased in diabetes (Tan *et al*., 2005). Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications (Betteridge, 1997).

The effect of *S. surattensis* leaf extract on plasma glucose and lipid profiles may be due to its content of unsaturated fatty acids, sterols, phytol and squalene. Dietary fatty acids play an important role in the regulation of plasma HDL levels, and the atheroprotective effect of HDL is related to cellular cholesterol efflux, which in turn depends on the function of cholesterol transporters. While saturated fatty acids had no effect, mono and polyunsaturated fatty acids markedly inhibited cholesterol efflux (Wang and Oram, 2002).

There is ample evidence that structural modifications in cholesterol molecule suppress its intestinal absorption. Thus, plant sterols with 1 or 2 extra carbons in the side chain incorporate in intestinal micelle to displace cholesterol and are poorly absorbed and interfere with cholesterol absorption from the intestine. It has been reported that stigmasterol (24-ethyl-cholesta-5, 22-dien-3β-ol; Δ^{22} derivative of sitosterol [24-ethyl-cholest-5-en-3β-ol]) is poorly taken up by the brush border membrane and inhibits cholesterol synthesis via competitive

inhibition of sterol Δ^{24}-reductase (Kitareewan *et al*., 1996; Schluter *et al*., 2002).

Phytol, a branched-fatty alcohol, is a carbon side-chain of chlorophylls. The peroxisome proliferator-activated receptor (PPAR) is one of the indispensable transcription factors for regulating lipid metabolism in various tissues. It is indicated that phytol is functional as a PPARα ligand and that it stimulates the expression of PPARα-target genes in intact cells. Because PPARα activation enhances circulating lipid clearance, It is thought that such effects of phytol are valuable for control of lipid abnormalities in common diseases including obesity, diabetes, and hyperlipidemia through PPARα activation in the liver (Kitareewan *et al*., 1996; Schluter *et al*., 2002).

Phytanic acid, which is a metabolic derivative of phytol, has been reported to activate PPARγ and the retinoid-X-receptor (RXR). Therefore, the intake of phytol as a precursor of phytanic acid is valuable for the management of lipid metabolism through the activation of PPARs (Baxter, 1968).

Squalene is an isoprenoid intermediate of cholesterol biosynthesis which can be obtained from the diet where it is abundant, for example, in olive oil. Serum squalene originates partly from endogenous cholesterol synthesis and partly from dietary sources. Dietary squalene is found in postprandial lipoprotein fractions (Gylling and Miettinen, 1994) generally in association with very low density lipoproteins, from which it is distributed to various tissues. Several studies have also demonstrated that dietary squalene decreases serum triglyceride and cholesterol levels in humans (Miettinen and Vanhanen, 1994; Strandberg *et al*., 1990; Simonen *et al*., 2007; Chan *et al*., 1996) and animals (Richter and Schafer, 1982). These results have been attributed to increased elimination of cholesterol as fecal bile acids and to inhibition of HMG Co A reductase by dietary squalene due to negative feed-back regulation (Strandberg *et al*., 1990).

From this study we can conclusively state that *S. surattensis* leaf extract has shown remarkable effects on blood glucose level and marked improvement on hyperlipidemia. Its specific effect on HDL has additional advantage in checking coronary risks. The extract seems to have no toxicity since LD_{50} (8.6 gm/kg) was found up to 80 times of effective dose.

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