Medicinal and Aromatic Plant Science and Biotechnology ©2009 Global Science Books

Chemical Composition and Biological Evaluation of *Vitex agnus-castus* L.

Nabawya Ibrahim[1] • Ahmed Shalaby[2] • Radwan Farag[3] • Gamal Elbaroty[3] • Emad Hassan[2*]

[1] Pharmacognosy Department, National Research Centre, 12311 Dokki, Cairo, Egypt
[2] Medicinal and Aromatic Plants Department, National Research Centre, 12311 Dokki, Cairo, Egypt
[3] Biochemistry Department, Faculty of Agriculture, Cairo University, Cairo, Egypt

Corresponding author: * **emadnrc@yahoo.com**

ABSTRACT

The chemical composition of the essential oil (EO) of chaste tree (*Vitex agnus-castus*) leaves, Verbenaceae, was identified by GC/MS analysis. The oil contained hydrocarbons (62.77%) and oxygenated compounds (36.38%) as the main chemical groups. Mono-, sesqui- and diterpenoid compounds represented 47.37, 49.12 and 2.69%, respectively. T-caryophyllene (18.76%) was the major constituent, followed by 1,8-cineole (17.38), sabinene (15.38%) and germacrene B (13.72%). The unsaponifiable matter (USM) content of chaste tree fruits was 65%. The most predominant hydrocarbons identified by GLC were: tricosane (9.99%), heptadecane (7.76%), dotriacontane (7.74%), eicosane (7.45%), hexadecane (7.28%), β-sitosterol (5.56%), campesterol (1.01), and stigmasterol (1.32%). GLC of fatty acid methyl esters revealed that oleic acid (26.11%) and linoleic acid (24.76%) were the major unsaturated fatty acids, while palmitic acid (21.01%) was the major saturated fatty acid. Crude proteins of chaste tree fruits amounted to 6.65%. The protein hydrolysate was analyzed with an amino acid analyzer. Seventeen amino acids were detected; the major essential ones were leucine (7.85%), phenylalanine (6.09%) and threonine (5.83%); the non-essential ones were glutamic acid (15.95%), aspartic acid (14.61%) serine (11.99%), glycine (7.38%) and alanine (6.62%). The mucilage hydrolysates, analyzed by HPLC, were characterized by the presence of a high concentration of galacturonic acid and rhamnose. The antimicrobial activity of the EO was performed and the minimum inhibitory concentration (MIC) determined. The growth of both Gram-positive and -negative bacteria yeast and fungi were inhibited at a 1:50 (v/v) dilution; the growth of *Candida albicans* (yeast) and *Aspergillus niger* (fungus) were completely inhibited at 19 mg/ml. Also, the ethanolic extract (70%) of fruits showed antioxidant and antidiabetic activities.

Keywords: antidiabetic, antimicrobial, antioxidant, chaste tree, chemical constituents

INTRODUCTION

Chaste tree (*Vitex agnus-castus* Linn.), Verbenaceae, is an ornamental shrub or small tree widely distributed in the Mediterranean costal region and central Asia (Brummitt 1992). Several authors reported that different parts of chaste tree are used for various medicinal areas. For instance, Zoghbi *et al.* (1999) reported that, chaste tree is used medicinally in Brazil as a carminative, an antispasmodic, an antiseptic, a diuretic, in the treatment of stomach and headache. It is also used against influenza and diarrhea. The essential oils (EOs) of this plant showed an antimicrobial activity, while the fruits of the plant are used for menstrual disorders (Sorensen and Katsiotis 1999; Ibrahim *et al.* 2008). Various flavonoids were isolated from the root bark of *V. agnus-castus* and *V. rotundifolia* (Ko *et al.* 2000). Labdan diterpene alkaloid was isolated from the fruits (Li *et al*. 2002). Ketosteroid hormones were found in the flowers and the leaves of *V. agnus-castus* (Saden-Krehula *et al.* 1990).

The available literature on *V. agnus-castus* leaves suggests the need for further details investigation of chemical constituents and biological evaluation as antidiabetic and antioxidant activities. So, this is study aimed to evaluate this plant chemically and biologically.

MATERIALS AND METHODS

Plant materials

Plant samples of chaste tree (*V. agnus-castus*) were collected from El-Sadat city, Menofyia Governorate, Egypt. The fruits were collected from the trees early in the morning, at the end of the flowering stage that took place in November. Samples of this plant were subjected to botanical identification in the Orman Botanical Garden, Giza. The collected samples (mainly the fruiting tops and leaves) were air-dried at 27 ± 3°C, powdered (30 µm for the grain diameter) and kept in paper bags in a desiccator over anhydrous calcium chloride until chemical analyses.

Extraction of the essential oils

The EOs of *V. agnus-castus* leaves and fruits were extracted by hydrodistillation using a Clevenger apparatus. The distilled EO was dried for 24 h over anhydrous sodium sulphate and the mean value was determined from triplicate hydrodistillation. The chemical composition of the EO was performed by a Hewlett Packard model (5890) series II plus, equipped with a carbowax 20 M capillary column (0.32 mm × 50 m, i.d.), flame ionization detector (FID), helium as carrier gas at a flow rate of 1 ml/min, initial column temperature was 60°C increased to 200°C at a rate of 3°C/min and held at 200°C for 40 min., injector and detector temperatures were 200 and 250°C, respectively. MS analysis was made using Hewlett Packard Mass Spectrometer (model 5970). A temperature ionization detector (TIC) detector was used, Carbowax 20 M capillary column (0.32 mm × 50 m, i.d.), temperature was increased from 60 to 200°C at 3°C/min and MS ionization voltage was 70 eV (Ibrahim *et al.* 2007). Qualitative and quantitative identification of the EO constituents were carried out by comparing the retention times and mass fragmentation patterns with those of available authentic EOs in the data base of Kato Aromatic Co. (El-Harrania, Giza) as well as from previously published data (Adams 1989; Jennings and Shimamoto 1980).

Received: 23 March, 200. Accepted: 9 July, 2009.

Original Research Paper

Identification of lipid matter of chaste tree fruits

Preparation of unsaponifiable (USM) and saponifiable (SM) matters, qualitative and quantitative identification were carried out adopting the method of Ibrahim and Bashandy (2006).

Qualitative and quantitative determination of chaste tree proteins

The defatted powdered fruit (10 g) of chaste tree was stirred in 10% NaCl for 1 h, then filtered. An equal volume of trichloroacetic acid was added. The white flocculent precipitate was collected. The precipitated proteins (25 mg) were hydrolyzed with 6 N HCl at 105°C for 24 h in a sealed tube. 20 µl was analyzed using an amino acid analyzer LC 3000, Eppendorf Co. (Harborne 1998).

Qualitative and quantitative determination of chaste tree mucilage

The mucilage of chaste tree fruits (10 g) was prepared by cold and hot extraction methods (Karawya *et al.* 1980). The prepared mucilage (100 mg) was hydrolyzed by heating with 2 ml of H_2SO_4 (0.5 M) in a sealed tube for 20 h and the components of mucilage hydrolysate were characterized by an HPLC apparatus under the following conditions:

Column, Hyper REZ XP carbohydrate Pb guard; H_2O as a mobile phase at a flow rate of 0.6 ml/min; column temperature was 80°C; injected volume was 20 µl; detector, Refractive index detector (RID-10A).

Peak identification was performed by comparing the retention time of each peak with those of standard sugars obtained from Sigma chemical company (St. Louis, USA).

The antimicrobial activity and the minimum inhibitory concentration (MIC) of the leaf EO (dissolved in paraffin oil) was evaluated by the inhibition zone method against different microorganisms (Simon 1959; Gabraith *et al.* 1971).

Antidiabetic and antioxidant activities of 70% ethanolic extract

The LD_{50} of 70% ethanolic extract of chaste tree fruits was found to be 12.5 g/kg body weight (bw) calculated according to the equation of Laurence and Bacharach (1974) as follows:

$$LD_{50} = Dm - \frac{\sum (Z \bullet d)}{N}$$

where Dm = the dose at which all mice died, Z = half the sum of dead mice from two successive doses, d = the difference between the two successive doses, N = number of mice in each group.

The biochemical effects of 70% ethanolic extract of chaste tree fruits on diabetic rats were carried out. Induction of diabetes was performed by i.p. administration of alloxan monohydrate (150 mg/kg bw) to adult male albino rats weighing from 100-140 g were obtained from the animal house of National Research Centre, Dokki, Giza, Egypt. Rats were fed a standard diet and free access to tap water. They were kept for two weeks to acclimatize to environmental conditions (Rao *et al*. 1999). Rats were divided into three groups (10 rats each): Group I: Normal control rats; Group II: Control non-treated diabetic rats and Group III: Diabetic rats treated with 70% ethanolic extract over a period of 21 successive days. The blood samples were withdrawn from the retro-orbital venous plexus at 0, 10 and 21 days. The experimental diabetic rats were orally administered a dose of 1.25 mg/kg bw (0.10 LD_{50}) from the 70% ethanolic fraction daily for 2 weeks. The blood glucose levels were estimated (Howanitz and Howanitz 1984) as well as other clinical parameters such as alanine aminotransferase (ALT) (Henry *et al*. 1974), aspartate aminotransferase (AST) (Bergmeyer 1978) as liver function tests and blood urea (Henry *et al*. 1974) and creatinine (Di Gorgio 1974) as kidney function test, were measured. Moreover, changes in the antioxidant status were also measured mainly by glutathione (Moron *et al*. 1979), glutathione reductase GR (Beutler 1969) and peroxidase GPX (Kokatnur and Jelling 1941), lipid peroxidation (Jagota and Dani 1982), vitamins E (Baker and Frank 1968) and C (Ohkawa *et al*. 1979), which altered due to the application of alloxan-monohydrate as a free radical inducer. These parameters were estimated in diabetic rats before and after treatment with 70% ethanolic extract of chaste tree fruits and all the experiments were conducted according to animal ethic rules but without Animal Ethics Committee approval.

RESULTS AND DISCUSSION

GC/MS analysis (**Table 1**) of the chaste tree leaves EO (0.12%) revealed that it contained hydrocarbons (62.77%) and oxygenated compounds (36.38%) as the main chemical groups of which the mono-, sesqui- and diterpenoid compounds represented 47.37, 49.12 and 2.69%, respectively. T-caryophyllene (18.76%), 1,8-cineole (17.38%), sabinene (15.38%) and germacrene B (13.72%) were the major constituents followed by α-humulene (5.40%) and α-terpineol (4.37%). The comparison between the reported constituents of leaves chaste tree EO and those obtained in this study indicate that there were qualitative and quantitative differences. Zoghbi *et al.* (1999) stated that the main constituents by GC/MS of the EO of fresh leaves were 1,8-cineole (33.5%), sabinene (18.5%), α-pinene (8.9%), α-terpinylacetate (6.4%) and (*E*)-β-farnesene (5.2%). While, in fresh flowers were 1,8-cineole (13.5%), (*E*)-β-farnesene (15.3%), β-caryophyllene (8.2%), bicyclogermacrene (6.7%), and in fresh fruits oil were (*E*)-β-farnesene (23.1%), 1,8-cineole (18.2%), α-terpinylacetate (8.5%), α-pinene (8.1%), sabinene (7.7%), β-caryophyllene (5.3%) and bicyclogermacrene (5.2%). Elgengaihi *et al.* (1992) mentioned that the EO percentages in the leaves, flowers and fruits of *V. agnus–castus* were 2.0, 0.24 and 0.16, respectively. Terpineol was the main component in leaves (11.2%) and fruits (11.1%) oil, while alloaromadendrene was the main constituent of flowers (19.0%) by GLC. In this study, however, the flowers of chaste tree contained the highest amount of EO (0.14%) compared with that from leaves (0.12%) or fruits (0.11%).

The USM content of chaste tree fruits was 65%. GLC analysis of USM revealed the presence of hydrocarbons ranging from C_{14} to C_{32} of which tricosane (9.99%), heptadecane (7.76%), dotriacontane (7.74%), eicosane (7.45%) and hexadecane (7.28%), campesterol (1.01), stigmasterol (1.32%) and β-sitosterol (5.56%) were the most predominant components (**Table 2**). The total fatty acid fraction of chaste tree fruits represented 5% of the petroleum ether extract and it contained a high percentage of total unsaturated fatty acids (65.28%). Oleic acid (26.11%) and linoleic acid (24.76%) were the major unsaturated fatty acids. On the other hand, the saturated fatty acids represented 34.29%, of which palmitic acid (21.01%) was identified as the major saturated fatty acid (**Table 2**).

Crude proteins of chaste tree fruits amounted to 6.65%. Seventeen amino acids were detected as shown in **Table 3**.

The yield (%) of mucilage extracted by the two cold and hot methods were 1.45 and 0.43%, respectively. Mucilage hydrolysates were analyzed by HPLC. The results revealed that a high concentration of galacturonic acid (95.24% of the total hydrolyzed) and rhamnose as the minor monosaccharide (4.76%). The USM, SM, crude protein and mucilage of chaste tree fruits were identified for the first time in this study.

The antimicrobial properties of chaste tree EO were evaluated by the inhibition zone method. From **Table 4** it can be concluded that *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* possessed high degrees of sensitivity. The growth of *C. albicans* and *A. niger* were completely inhibited at 19 mg/ml medium while *B. subtilis* and *E. coli* were more resistant to the EO, in which the MIC values for both strains was 94 mg/ml. There is little information in the literature about the antimicrobial activity of chaste tree EO. For example, Hossain *et al*. (2001) mentioned that the petroleum ether and ethanolic extracts of *Vitex trifolia* leaves exhibited moderate antibacterial activity against $Gram^{+ve}$

Table 1 GC/MS analysis of essential oil of chaste tree leaves.

Peak No.	Identified constituent	Relative conc. (%)	MS (m/e)		
			M^+	Base peak	Main significant fragments
1	α-Pinene	2.02	136	93	121, 105, 91, 77, 55, 39
2	β-Pinene	0.94	136	93	121, 105, 91, 79, 69, 41
3	Sabinene	15.83	136	93	121, 108, 91, 79, 61, 41
4	β-Myrcene	0.98	136	41	119, 107, 79, 69, 67, 39
5	α-Terpinene	0.52	136	93	121, 105, 91, 79, 69, 39
6	l-Limonene	0.10	136	93	121, 107, 91, 79, 69, 41
7	1,8-Cineole	17.38	154	43	139, 125, 108, 81, 79, 41
8	γ-Terpinene	1.58	136	93	121, 105, 91, 77, 69, 43
9	*Trans*-Ocimene	0.54	136	93	121, 105, 91, 77, 43, 41
10	ρ-Cymene	0.29	136	119	93, 91, 77, 65, 43, 41
11	α-Terpinolene	0.32	136	93	121, 119, 77, 68, 43, 41
12	1-Octen-3-ol	0.79	128	57	99, 81, 72, 55, 43, 41
13	*Trans*-Sabinene hydrate	0.28	154	43	136, 120, 111, 93, 79, 71
14	Selin-4,7 (11)-diene	0.75	204	41	189, 161, 133, 119, 105
15	Linalool	0.46	154	43	139, 125, 111, 93, 71, 41
16	Linalyl acetate	0.22	204	43	158, 139, 121, 93, 69, 58
17	*Trans*-Caryophyllene	18.76	204	41	189, 161, 133, 93, 78, 69
18	Isocaryophyllene	1.60	204	41	189, 161, 133, 93, 78, 69
19	α-Humulene	5.40	204	93	147, 121, 103, 79, 59, 41
20	α-Terpineol	4.37	154	59	139, 121, 93, 77, 43, 41
21	Germacrene B	13.72	204	41	136, 121, 103, 93, 77, 45
22	δ-Cadinene	0.26	204	41	161, 134, 120, 105, 69, 51
23	Ledol	0.34	222	41	180, 161, 111, 95, 69, 55
24	Caryophyllene oxide	0.33	121	43	107, 95, 79, 55, 41, 40
25	Globulol	1.15	222	43	189, 161, 133, 109, 93, 69
26	α-Eudesmol	1.03	224	43	187, 151, 136, 119, 91, 69
27	Veridiflorol	0.28	222	43	204, 189, 161, 109, 93, 69
28	Spathulenol	1.34	206	43	205, 159, 135, 119, 91, 69
29	Caryophyllenol-II	0.34	220	43	187, 159, 185, 119, 91, 69
30	δ-Cadinol	2.60	222	43	204, 161, 134, 105, 81, 65
31	α-Cadinol	0.66	222	43	161, 137, 121, 95, 69, 41
32	Longipinocarvone	0.69	218	41	203, 175, 134, 107, 79, 58
33	*Epi*-α-Bisablol	0.62	204	43	187, 147, 131, 91, 69, 41
34	Unknown	0.24	279	191	219, 161, 135, 95, 69, 43
35	*Epi*-13-Manool	0.78	272	41	219, 189, 161, 107, 91, 69
36	Manool	1.68	272	43	257, 191, 175, 136, 119, 80
37	Unknown	0.61	286	187	204, 145, 119, 91, 71, 43
38	Sclareol	0.23	290	43	273, 257, 177, 137, 119

Table 2 GLC analysis of USM and SM of chaste tree fruits.

No. of peaks	Unsaponifiable matter (USM)			Saponifiable matter (SM)		
	$^{*}RR_t$	Conc. (%)	Identified compound	$^{**}RR_t$	Conc. (%)	Identified compound
1	0.67	3.20	Tetradecane (C_{14})	0.35	0.03	Caprylic (8:0)
2	0.72	4.44	Pentadecane (C_{15})	0.38	0.06	Unknown
3	0.78	7.28	Hexadecane (C_{16})	0.55	0.05	Capric (10:0)
4	0.83	7.76	Heptadecane (C_{17})	0.63	0.03	Undecanoic (11:0)
5	0.87	6.11	Octadecane (C_{18})	0.71	0.43	Lauric (12:0)
6	0.89	5.27	Nonadecane (C_{19})	0.78	1.05	Tridecanoic (13:0)
7	0.92	7.45	Eicosane (C_{20})	0.81	0.38	Unknown
8	0.96	6.20	Heneicosane (C_{21})	0.84	2.64	Myristic (14:0)
9	0.97	2.89	Docosane (C_{22})	0.93	5.35	Myristoleic (14:1)
10	1.00	9.99	Tricosane (C_{23})	0.96	2.24	Pentadecanoic (15:0)
11	1.04	5.35	Tetracosane (C_{24})	1.00	21.01	Palmitic (16:0)
12	1.14	5.30	Hexacosane (C_{26})	1.11	3.14	Palmitoleic (16:1)
13	1.19	4.19	Heptacosane (C_{27})	1.16	3.08	Heptadecanoic (17:0)
14	1.24	2.34	Octacosane (C_{28})	1.30	26.11	Oleic (18:1)
15	1.30	4.87	Squalene	1.40	24.76	Linoleic (18:2)
16	1.37	1.75	Triacontane (C_{30})	1.55	5.92	Linolenic (18:3)
17	1.45	7.74	Dotriacontane (C_{32})	1.76	1.53	Arachidic (20:0)
18	1.60	1.01	Campesterol	1.99	2.20	Behenic (22:0)
19	1.64	1.32	Stigmasterol			
20	1.69	5.56	β-Sitosterol			

$^{*}RR_t$ of tricosane was 21.04 min., $^{**}RR_t$ of palmitic acid was 17.58 min.

and Gram^{-ve} bacteria. The previous results showed that the oxygenated compounds in chaste tree EO represented 36.38% of the total components. Also, T-caryophyllene (18.76%), 1,8-cineole (17.38%), sabinene (15.38%) and germacrene B (13.72%) were the main components in the oil. Kim *et al.* (1995) stated that the antimicrobial activities of EOs against bacteria was attributed to their interference with the phospholipid bilayer of the cell membrane causing an increase in the permeability and loss of cellular constituents.

Table 3 Amino acids of chaste tree fruits.

Amino acids	RR_t*	Relative amino acid percent
***Essential**:		
Threonine	0.82	5.83
Valine	1.81	4.79
Methionine	2.02	0.07
Isoleucine	2.10	4.61
Leucine	2.16	7.85
Phenylalanine	2.46	6.09
Lysine	3.17	2.77
Total		32.01
***Non essential**:		
Aspartic acid	0.63	14.61
Serine	0.90	11.99
Glutamic acid	1.00	15.98
Proline	1.14	0.36
Glycine	1.44	7.38
Alanine	1.50	6.62
Cystine	1.59	0.04
Tyrosine	2.35	2.77
Histidine	2.99	4.21
Arginine	3.68	4.03
Total		67.99

RR_t* to glutamic acid Rt= 16.25 min

Table 4 Antimicrobial activity of leaves of chaste tree essential oil.

Microorganism	Diameter of inhibition zone (mm)	MIC (mg/ml medium)
Bacillus substilis (Gram+ve bacteria)	16	94
Escherichia coli (Gram-ve bacteria)	15	94
Candida albicans (yeast)	17	19
Aspergillus niger (fungus)	17	19

Biological evaluation

Fasting blood glucose levels of untreated diabetic rats (147 ± 27.55 mg/dl) were significantly higher than that in normal rats (83 ± 11.10 mg/dl). Significant decreases in blood glucose levels were observed in treated diabetic group from an initial level of 158 ± 31.01 to 115 ± 15.08 and 100 ± 9.48 after 1 and 2 weeks (**Table 5**). The serum creatinine levels of the treated diabetic was significantly reduced (-17.24%) after two weeks. Also, blood urea nitrogen level was also decreased (-7.14%). ALT and AST indicate the liver function. Serum ALT level was significantly decreased by 65.52% while there was no significant effect on AST after activity 2 weeks (**Table 6**).

Lipid peroxide levels increased in diabetic rats (20.24%) compared to non-diabetic rats (normal control). Treatment with the chaste tree ethanolic extract (70%) significantly decreased the lipid peroxide levels (17.33%). On the other hand, GSH level in normal rats was about 1.5 times higher than that of diabetic rats. The treatment with ethanolic extract (70%) of chaste tree fruits induced an elevation of free radical formation and oxidative stress may act as common pathways to diabetes (Wolff 1993). Hyperglycaemia in diabetes is thought to be associated with increased oxidative stress via glucose auto-oxidation which produces superoxide radicals and free radicals generated from glycolated proteins (Hamden *et al.* 2008). Accordingly, the 70% ethanolic extract of chaste tree fruits was evaluated as antidiabetic and as free radical scavenger.

As shown in **Table 7**, GR activity was significantly decreased in diabetic rats compared to normal rats (-32.93%). On the other hand, diabetic-treated rats treated with chaste tree fruits extract had higher GR (37.96%) activity than that in diabetic rats. Concerning the levels of GPX, diabetic rats had higher activities than that normal control and diabetic-treated rats. Diabetic-treated rats had GPX activity nearly similar to that of control normal rats. This means that chaste tree fruits extract possessed an obvious reduction in GPX activity in the diabetic-treated rats group (-24.50%) after two weeks and nearly restored its normal activity. Diabetic rats treated with chaste tree fruits ethanolic extract (70%) induced a significant increase in both vitamin levels (74.01 and 55.29%, respectively) after two weeks (**Table 7**).

Generally speaking, the ethanol extract (70%) of chaste tree fruits was found to be relatively non toxic to mice (LD_{50} = 12.5g/kg bw). The antihyperglycaemic effect of the extract may be attributed to the presence of polysaccharides (mucilage) and proteins. Some mucilages and protein have been reported to have a hypoglycaemic effect (Tomada *et al.* 1987; Raghuram *et al.* 1994) by delaying glucose absorption and enhancing its utilization. Moreover, the 70% ethanolic extract of chaste tree fruits possessed remarkable antioxidant activity. The presence of some phenolic compounds is generally responsible for the antioxidant effect (17.33%). From previous studies, some flavonoids are inhibitors of lipid peroxidation by scavenging the CCl_4–derived radicals

Table 5 Effect of 70% ethanolic extract of chaste tree fruits on blood glucose level (mg/dl) of diabetic rats*.

Animal group	Treated period (week)					
	Zero time	First week		Second week		L.S.D
		Glucose level	Change (%)	Glucose level	Change (%)	(period)
Normal control	83 ± 11.10 Aa	88 ± 8.14 Aa	+6.02	93 ± 3.01 Aa	+12.05	10.01
Diabetic control	147 ± 27.55 Ba	145 ± 20.14 Ba	-1.36	143 ± 13.68 Ba	-2.72	26.12
Diabetic-treated rats	158 ± 31.01 Ba	115 ± 15.08 Cb	-27.22	100 ± 9.48 Ab	-36.71	25.41
L.S.D (treatment)	30.51	18.79	-	12.02	-	-

Values in a row followed by the same lower-case letter and in a column followed by the same capital letter are not significantly different at $p = 0.05$.
* Each value refers to the average of ten rats (± S.D).

Table 6 Effect of 70% ethanolic extract of chaste tree fruits on blood urea (mg/dl), creatinine (mg/dl), alanine aminotransferase (ALT) activity (U/l) and aspartate aminotransferase "AST" activity (U/l) of diabetic rats*.

Animal group	Urea (mg/dl)			Creatinine (mg/dl)		
	Zero time	Second week	L.S.D (period)	Zero time	Second week	L.S.D (period)
Normal control	18 ± 2.95 Aa	18 ± 3.03 Aa	3.84	0.65 ± 0.15 Aa	0.68 ± 0.20 Aa	0.22
Diabetic control	28 ± 2.88 Ba	29 ± 2.25 Ba	3.32	0.86 ± 0.16 Ba	0.83 ± 0.11 Aa	0.18
Diabetic-treated rats	28 ± 3.02 Ba	26 ± 2.23 Ba	5.51	0.87 ± 0.13 Ba	0.72 ± 0.11 Ab	0.15
L.S.D (treatment)	3.63	3.12		0.18	0.18	
Animal group	**ALT (U/l)**			**AST (U/l)**		
	Zero time	**Second week**	**L.S.D (period)**	**Zero time**	**Second week**	**L.S.D (period)**
Normal control	21 ± 5.32 Aa	20 ± 8.07 Aa	8.79	16 ± 3.94 Aa	16 ± 3.19 Aa	4.61
Diabetic control	26 ± 2.80 ABa	23 ± 5.54 Aa	5.65	34 ± 9.01 Ba	70 ± 15.93 Bb	17.69
Diabetic-treated rats	29 ± 7.01 Ba	10 ± 5.71 Bb	8.22	34 ± 8.90 Ba	32 ± 8.02 Ca	16.65
L.S.D (treatment)	6.65	8.05	-	13.36	14.52	-

Values in a row followed by the same lower-case letter and in a column followed by the same capital letter are not significantly different at $p = 0.05$.
*Each value refers to the average of ten rats (± S.D).

Table 7 Effect of 70% ethanolic extract of chaste tree fruits on lipid peroxides, glutathione, glutathione reductase (GR), glutathione peroxidase (GPX) and vitamins E and C of diabetic rats.

Animal group	Lipid peroxides (µM/g tissue)	Glutathione (mg/g tissue)	GR (µM/g proteins)	GPX (µM/g proteins)	Vit. E (mg/g tissue)	Vit. C (mg/g tissue)
Normal control	1.68 ± 0.21 a	31.70 ± 0.56 a	14.06 ± 1.57 a	2.40 ± 0.18 a	7.27 ± 0.20 a	6.33 ± 0.15 a
Diabetic control	2.02 ± 0.12 b	20.63 ± 0.97 b	9.43 ± 0.73 b	2.98 ± 0.12 b	4.81 ± 0.11 b	4.63 ± 0.05 b
Change (%)	+20.24	-34.92	-32.93	+46.08	-33.84	-26.86
Diabetic-treated	1.67 ± 0.07 a	27.87 ± 0.65 c	13.01 ± 0.21 a	2.25 ± 0.28 a	8.37 ± 0.32 c	7.19 ± 0.16 c
Change (%)	-17.33	+35.04	+37.96	-24.50	+74.01	+55.29
L.S.D	0.18	0.92	1.24	0.25	0.28	0.16

Values in a row followed by the same lower-case letter and in a column followed by the same capital letter are not significantly different at $p = 0.05$.
*Each value refers to the average of ten rats (± S.D).

(Cholbi *et al.* 1991; Ibrahim *et al.* 2008). The alteration in the antioxidant level in the blood of diabetic animals occurs as a result of a disruption in free radical metabolism. The changes in antioxidant levels in blood and tissues of diabetic rats were observed by Wohaieb and Godin (1987). Glutathione is an important element in circumvention of cellular oxidative stress and biotransformation of electrophiles. GSH is reported to be essential for regeneration of other antioxidants such as vitamins E and C (Constantinescu *et al.* 1993). Reduction in blood GSH and vitamins E and C levels may be due to excessive oxidation and lack of regeneration from their oxidative radical form to reduced form. The findings of the present study show that increased lipid peroxidation associated with diabetic induction could be reduced by treatment with 70% ethanolic extract of chaste tree fruits. This extract induced a powerful effect on lipid peroxidation, antioxidant status and other clinical parameters, including liver and kidney functions of rats.

REFERENCES

Adams RP (1989) *Identification of Essential Oils by Ion Trap Mass Spectroscopy*, Academic Press, Inc., London

Baker H, Frank C (1968) *Determination in Serum Tocopherol*, In: Gowenlock AH (Ed) *Practical Clinical Biochemistry* (6th Edn), Heinemann Medical Books, London, UK, pp 222-223

Bergmeyer HU (1978) *Principles of Enzymatic Analysis*, Verlag Chemic, London, 42 pp

Beutler E (1969) Effect of flavin compounds on glutathione reductase activity: *in vivo* and *in vitro* studies. *Journal of Clinical Investigation* **48**, 1957-1966

Brummitt RK (1992) *Vascular Plants: Families and Genera*, Royal Botanic Gardens, Kew, 690 pp

Cholbi MR, Paya M, Acaraz MJ (1991) Inhibitory effects of phenolic compounds on CCl_4-induced microsomal lipid peroxidation. *Experientia* **42**, 195-199

Constantinescu A, Han D, Packer R (1993) Vitamin E recycling in human erythrocyte membrane. *Journal of Biological Chemistry* **288**, 10906-10913

Di Gorgio J (1974) *Clinical Chemistry – Principles and Techniques*, Harper and Row, Hagerstown, MD, pp 549-550

Elgengaihi SE, Motawe HM, Omar EA, El-Bazaza ZE (1992) Chemical and biological studies on *Vitex agnus-castus* L. volatile oils. *Indian Perfumer* **36**, 293-296

Gabraith H, Miller T, Paton A, Thompson J (1971) Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferole and cholesterol. *Journal of Applied Bacteriology* **4**, 803-813

Hamden K, Boujbiha MA, Masmoudi H, Ayadi FM, Jamoussi K, Elfeki A (2008) Combined vitamins (C and E) and insulin improve oxidative stress and pancreatic and hepatic injury in alloxan diabetic rats. *Biomedicine and Pharmacotherapy* **20**, 1-5

Harborne JB (1998) *Phytochemical Methods* (3rd Edn), Chapman and Hall, London, pp 187-189

Henry JB, Sanford T, Davidsohn N (1974) *Clinical Diagnosis and Measurements by Laboratory Methods* (16th Edn), W.B. Saunders and Co., Philadelphia, PA, pp 260, 332-335

Hossain MM, Paul N, Sohrab MH, Rahamn E, Rashid M (2001) Antibacterial activity of *Vitex trifolia*. *Fitoterapia* **72**, 695-697

Howanitz PJ, Howanitz JH (1984) *Clinical Diagnosis and Management by Laboratory Methods* (17th Edn), W.B. Saunders, Philadelphia, PA, USA, 168 pp

Ibrahim NA, Bashandy S (2006) Phytochemical and biological investigations of *Cucurbita foetidissim* H. B. K. cultivated in Egypt. *Bulletin of Faculty of Pharmacy, Cairo University* **44**, 111-119

Ibrahim NA, Mohamed SM, Farid MA, Hassan EM (2006) Chemical composition. antiviral and antimicrobial activities of the essential oils of *Aster novi-belgii*, *Solidago anadensis* and *Myoporum laetum* growing in Egypt. *Bulletin of Faculty of Pharmacy, Cairo University* **44**, 103-109

Ibrahim NA, Shalaby AS, Farag RS, Elbaroty GS, Nofal SM, Hassan EM (2008) Gynecological efficacy and chemical investigation of *Vitex agnus-castus* L. fruits growing in Egypt. *Journal of Natural Product Research* **22**, 537-546

Jagota SK, Dani HM (1982) A new colorimeteric technique for the estimation of vitamine C using Folin phenol reagent. *Analytical Biochemistry* **127**, 178-182

Jennings W, Shibamoto T (1980) *Quantitative Analysis of Flavour and Fragrance Volatile by Glass Capillary Gas Chromatography*, Academic Press, Inc., New York

Karawya MS, Wassel GM, Baghdadi HH, Ammar NM (1980) Mucilages and pectins of *Opuntia*, *Tamarindus* and *Cydonia*. *Planta Medica* **45**, 68-75

Kim JM, Marshall MR, Cornell JA, Preston JF, Wei CI (1995) Antibacterial activity of carvacrol, citral, and geraniol against *Salmonella typhimurium* in culture medium and on fish cubes. *Journal of Food Science* **60**, 1364-1369

Ko WG, Kang TH, Lee SJ, Kim NY, Sohn DH, Lee BH (2000) Polymethoxyflavonoids from *Vitex rotundifolia* inhibit proliferation by inducing apoptosis in human myeloid leukemia cells. *Food and Chemical Toxicology* **38**, 861-865

Kokatnur VR, Jelling M (1941) Iodometeric determination of peroxygen in organic compounds. *Journal of American Chemical Society* **63**, 1432-1433

Laurence DR, Bacharach AL (1974) *Peget and Barnes; Evaluation of Drug Activities Pharmacometrics* (Vol 1), Academic Press, London, 135 pp

Li S, Zhang H, Qui S, Niu X, Santarsiero BO, Mesecar AD, Fong HH, Farnsworth N, Sun H (2002) Vitexlactam A, a novel labdane diterpene lactam from the fruits of *Vitex agnus-castus*. *Tetrahedron Letters* **43**, 5131-5134

Mora A, Paya M, Rios M, Alcaraz M (1990) Structure-activity relationships of polymethoxy flavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. *Biochemical Pharmacol*ogy **4**, 793-797

Moron M S, Depierre JW, Mannervik B (1979) Levels of glutathione, glutathione reductase and glutathione-*S*-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta* **582**, 67-76

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* **95**, 351-358

Raghuram TC, Sharma RD, Sivakumar B, Sahay BK (1994) Effect of fenugreek seeds on intravenous glucose dispostion in non-insulin dependant diabetic patients. *Phytotherapy Research* **8**, 83-86

Rao BK, Kesavulu MM, Giri KR, Rao CA (1999) Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook. Fruits powder in alloxan diabetic rats. *Journal of Ethnopharmacology* **67**, 103-109

Saden-Krehula M, Kustrak D, Blazevic N (1990) Δ^4-3-ketosteroids in flowers and leaves of *Vitex agnus-castus*. *Planta Medica* **56**, 547

Simon A (1959) The theory of agar diffusion method. *Antibiotiki* **4**, 112-117

Sorensen JM, Katsiotis ST (1999) Variation in essential oil yield and composition of Gretan *Vitex agnus-castus* L. fruits. *Journal of Essential Oil Research* **11**, 599-605

Tomada M, Shimizu N, Oshima Y, Takahasi M, Murakanu M, Hikino H (1987) Hypoglycaemic activity of twenty plant mucilages and three modified products. *Planta Medica* **52**, 8-12

Wohaieb SA, Godin DV (1987) Alteration in free radical tissue defence mechanism in streptozotacine induced diabetes in rats. Effect of insulin treatment. *Diabetes* **36**, 1014-1018

Wolff SP (1993) Diabeties mellitus and free radicals. *British Medical Bulletin* **49**, 642-652

Zoghbi MG, Andrade EH, Maia JG (1999) The essential oil of *Vitx agnus-castus* L. growing in the Amazon region. *Flavour and Fragrance Journal* **14**, 211-213