ISSN 0974 – 5211

Journal of Natural Products

Volume 7 (2014)

www.JournalofNaturalProducts.Com

Research Paper

Chemical Composition of the Lipophilic Fraction of *Livistona australis* R.Br. Mart., (*Arecaceae*) Fruit Pulp and Evaluation of its Antioxidant and Antihyperlipidemic Activities

M.E.S. Kassem[1], M.S. Afifi[2]*, J.Y. Salib[3], O.K. Sakka[4], A.A. Sleem[5]

[1] Department of Phytochemistry and Plant Systematics, National Research Centre, El Tahrir st., Cairo, Egypt.

[2]Department of Pharmacognosy, Faculty of Pharmacy, Misr International University, Km.28 Cairo Ismailia road, Cairo, Egypt.

[3]Department of Chemistry of Tanning Materials, [5] Department of Pharmacology, National Research Centre, El Tahrir st., Cairo, Egypt

[4] Department of Chemistry, American University in Cairo, P.O. Box 74, New Cairo 11835, Egypt.

*Corresponding Author

(Received 15August 2014; Revised 22Sept.-17Nov. 2014; Accepted 30Nov. 2014; Re corrected 10 Feb. 2015)

ABSTRACT

The lipophilic fraction of the dried pulp of *Livistona australis* palm fruits (LALF) was investigated for its chemical constituents using gas chromatography-mass spectrometry (GC-MS) analysis. Analysis of LALF revealed that oleic acid is the major fatty acid (FA) component (59.05%) and that linoleic and palmitic acids constitute 0.79% and 20.59%, respectively. while, the diterpene phytol component constitutes (7.98%). Quantitative HPLC analysis of LALF for its fat-soluble vitamins D3, E and A concentrations were estimated to be 303.028, 0.28850 and 72.2296 IU/g LALF, respectively. Oleic acid (OA) was isolated and purified from LALF using chromatographic techniques. Its structure was elucidated using various spectroscopic methods (UV, ^{1}H-NMR and EI-MS). *In vivo* evaluation of antioxidant and antihyperlipidemic activities of OA and LALF was carried out. LALF as well as the isolated OA showed significant (P<0.05) increase in glutathione (GSH) and decrease in nitric oxide levels in CCl_4 induced oxidative stress in treated rats compared to control. LALF hypolipidemic effect had better profile compared to the standard drug (simvastatin). In addition, in-silico studies were performed to study the binding mode of oleic acid with inducible nitric oxide synthase (iNOS) active site.

Keywords: *L. australis;* Oleic acid; Antioxidant; Antihyperlipidemic; Antiulcer; In-silico docking.

INTRODUCTION

Livistona australis R. Br. (Cabbage-tree Palm) is growing up to about 25 m in height and 0.35 m in diameter. It has leaves plaited like a fan and during the summer, it bears flower spikes with sprigs of cream-white flowers and brown spherical berries. Phytochemical analyses of 125 species (*Arecaceae*) revealed presence of flavonoids, phenolic acids and carotinoids (Williams et al., 1973; Harborne, et al., 1974; Barron, et al., 1988). Some

species of the same genus (*Livistona chinenses*) are used in folk–medicine in Southern China for treating various tumors (Zhong and Dian, 1999). Previous chemical investigations on the leaves of *L. australis* resulted in the isolation of a new sulphated tricin glycoside together with 14 known flavonoids (Kassem, et al., 2012) and a new pyranone derivative, 3-hydroxy-2-(4-hydroxyphenyl)-6-methyl-4-H-pyran-4-one (El-Desouky, et al., 2009).

Nitric oxide is a radical substance that is synthesized by a family of enzymes named nitric-oxide synthases (NOS). The NOS family includes neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). iNOS is a part of the primary immune defense. It is expressed by the influence of proinflammatory cytokines or bacterial lipopolysaccharides (LPS), Ca^{2+}/calmodulin-independent and creates a high nitric oxide concentration (Moncada, et al., 1991; Alderton, et al.; Nathan and Xie, 1994). Overproduction of NO is linked to the pathogenesis of many diseases, for example, septic shock (Fortin, et al., 2010, Titheradge, et al., 1999), multiple sclerosis/Alzheimer's disease (Steinert, et al., 2010; Lassmann, 2011). However, NO forms nitroso adducts with various thiols, such as reduced glutathione (GSH). GSH is an antioxidant, involved in detoxification of xenobiotics and serves as a cofactor in isomerization reactions (Meisler, et al., 1983). Thus, the search for natural products that can effectively increase the levels of GSH is of great importance.

As part of our chemical investigations on natural product extracts of medicinal values (Kassem, et al., 2012; El- Desouky, et al.,2009) we studied the fruit pulp of *L. australis* cultivated in Egypt. In this work, the characterization of constituents of the lipophilic fraction of *L. australis* pulp (LALF) including fat-soluble vitamins (A, E and D_3) was studied. In addition, *in vivo* determination of the antioxidant activity (GSH and NO assays) of lipophilic fraction and the isolated component (oleic acid) was performed. Moreover, *in vivo* antihyperlipidemic activity of lipophilic fraction (Cholestrol, Triglycerides, HDL and LDL) was evaluated. In-silico docking studies were also performed to simulate the binding mode of oleic acid to iNOS. The relationship between the potential biological activities and the chemical composition of the LALF is also discussed.

MATERIAL AND METHOD

***Plant material*:** *L. australis* (Family *Arecaceae*) fruits were supplied by the Orman garden, Giza, Cairo. The plant was identified by Dr. Mohamed El Gibaly, Department of phytochemistry and plant systematics, National Research Centre, Cairo, Egypt. A voucher specimen (no. 950) has been deposited in the herbarium NRC. The pulp of the fruits was separated manually using a stainless steel knife, dried at 40°C for 24h, triturated in a mill and screened through 40 mesh sizes. The powdered sample was packed in polyethylene bags and stored in a refrigerator (≈4°C) until use.

Material for chromatography: Silica gel G (70-230 mesh) for CC, precoated TLC plates (silica gel 60 GF254) (Merck Darmstadt, Germany), sephadex LH-20 (Pharmacia Uppsala, Sweden). Hexane: ethyl acetate: acetic acid (80:20:1) solvent system (S1) and 1% *p*-anisaldehyde in methanol-sulphuric acid (9:1, v/v) spray reagent (Fontana, et al., 2009) were used.

***Animals*:** Healthy male adult albino rats (120-150g) were used for the hyperlipidemic activity. Sprague-Dawley rats of both sexes, (120-130g) were used for the antioxidant assay. The animals were housed in-group of six per cage with free access to food and water *ad libitum* throughout the experiments. All animal procedures were performed in accordance to the Institutional Ethics Committee and in accordance with the

recommendations for the proper care and use of laboratory animals.(NIH publication No. 85-23, upon the human dose after conversion to that of the rat according to Paget and Barnes conversion tables (Paget and Barnes, 1964).

Drugs and chemicals: Blood reduced glutathione (GSH) level was estimated spectrophotometrically using a commercial kit (Biodiagnostic, Giza, Egypt). Biomerieux kits were used for the biochemical assessment of cholesterol, triglycerides, low-density lipoproteins and high-density lipoproteins levels. CCl_4 (BDH Chemicals, Poole, UK) was used to induce hepatic injury Silymarin (Sedico Pharmaceutical Co., Giza, Egypt) was dissolved in isotonic (0.9% NaCl) solution before use. Standard vitamins (A, E, D_3) were obtained from Sigma chemicals.

Extraction of lipophilic fraction: The dried and triturated pulp of *Livistona australis* (500g) was extracted with (2x2l) petroleum ether (60-80°C) by Soxhlet method. The solvent was distilled off under vacuum at low temperature (45^{o}C).

Saponification of lipofilic fraction and formation of fatty acid methyl esters: One gram of the petroleum ether extract was saponified according to the method of Nazif (2002) by refluxing with alcoholic potassium hydroxide (10%) for 2 h to yield the unsaponifiable matter (USM) as well as fatty acids (FA) fraction. The FA fraction was subjected to methylation according to the method of Hartman and Lago (1973) by refluxing with absolute methanol (50 ml) and sulphuric acid (3 ml) for 2 h to yield fatty acid methyl esters (FAME). The produced USM and FAME are kept in a desiccator for GLC analysis.

Oleic acid isolation and identification: Petroleum ether extract of *L. australis* (5g) was subjected to column chromatographic fractionation on silica gel using gradient elution with *n*-hexane-methanol (9:1 to 9:5, 500ml) to give 15 fractions (1-15). Fraction 6 (60 mg) was further purified on Sephadex LH-20 using gradient elution of chloroform-methanol (3:1 to 1:3, 300ml) to afford oleic acid (OA, 50mg). TLC was carried out on silica gel using solvent system S1. Detection was achieved by spraying with *p*-anisaldehyde reagent and heating for 3-5 min (Fontana, et al., 2009).

Apparatus: Shimadzu UV 240 (P/N 204-58000); EI-MS: Thermo Scientific spectrometer (70 eV); NMR: Jeol EX-500 spectrometer (^{1}H-NMR, 500 MHz, Japan). GC/MS: Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness).

Gas chromatographic–mass spectral (GC-MS) analysis for unsaponifiable matter: 1µl unsaponifiable matter sample was injected into gas chromatographic apparatus and separation was carried out on DB-5 fused silica (5% phenyl methyl polysiloxane). The carrier gas (helium) pressure was maintained at 13 psi and the column flow rate was 1 ml/min. Oven temperature was maintained at 50°C isothermal for 3 min, and then heated to 300°C at a rate of 5°C/min. The temperature of the injector during the injection was 220°C.

GC-MS analysis of fatty acid methyl esters: 1µl FAME sample was analyzed using the conditions except that the oven temperature was maintained at 150°C isothermal for 4 min, and then heated to 280°C at a rate of 5°C/min.

Extraction of vitamins A, D_3 and E: Five grams of the fruit pulp was extracted with *n*-hexane (2×50ml) for 1h with agitation in the dark under nitrogen-rich atmosphere to prevent the loss of tocopherols by oxidation from atmospheric oxygen. The resulting extract was concentrated under vacuum. 0.2g of the *n*-hexane extract as well as individual reference samples (Vitamin A, D_3 and E) was homogenized with methanol/water (98:2 v/v) and the mixture filtered with a micro-filter (45µm) and stored in amber colored glass bottle in refrigerator.

HPLC Chromatographic System and Conditions for Determination of Vitamins: One

microliter (1µl) aliquot of the sample was injected in HPLC PerkinElmer series 200 using the following conditions column Hypersil ODS (octadecylsiloxane) (C18,5 µm, 4.6×150 mm); mobile phase: methanol: water (98:2, v/v); Flow rate: 1ml/min. Total run time for the separation was approximately 10 min. The HPLC machine was coupled with a UV detector set at 254 nm. The vitamins were identified by matching their retention time data with those of the reference standards under the same conditions.

CCl_4-induced hepatic injury: The rats were divided randomly into four groups of six rats each. Four groups received CCl_4 in olive oil (1:1, v/v) at a dose of 2.8 ml/kg. Starting on the first day of CCl_4 administration; group I animals received an oral dose of LALF (100mg/kg b.wt./day); group II animals received an oral dose of OA (125 mg/kg b.wt./day) while, group III received silymarin orally (25mg/kg b. wt.). In addition, group IV animals received the vehicle (olive oil) at 2.8 ml/kg followed 3 days later by an additional dose of 1.4ml/kg b. wt. olive oil. All treated rats were administered half the initial dose of CCl_4 (0.14ml/100g body weight) 3 days after the first administration of CCl_4 so as to maintain hepatic damage. Rats had free access to food and drinking water during the study. Drugs were given once daily orally for one week. At the end of experiments, blood samples were obtained from the retro-orbital vein plexuses, under ether anesthesia. Blood reduced glutathione (GSH) level was estimated spectrophotometrically (Beutler, et al., 1963) and glutathione levels were expressed as mg/dl. Nitric oxide estimated as nitrite, was determined in sera (Miranda, et al., 2001). Total nitrite level in serum expressed in µM, calculated using the standard curve constructed with the prepared serial dilutions of sodium nitrite.

***Screening of Antihyperlipidemic Activity*:** Thirty adult male rats were randomly divided into three groups each of six. The animals were fed for two months on high fat diet containing a basal diet supplement with 1% cholesterol, 0.2% cholic acid and 10% fat as previously reported (Ney, et al., 1988). Hyperlipidemia was assessed by measuring serum cholesterol level triglycerides and cholesterol fractions (HDL-c and LDL-c) following the methods previously reported (Ghasi, *et al.*, 2000; Bucolo and David, 1973; Varley, et al., 1983) at zero, one and two month's intervals. Group I animals fed on a high fat diet and served as the hyperlipidemic control; group II animals fed on a high fat diet and received an oral dose of LALF (100mg/kg b.wt./day) while group III animals fed on a high fat diet and received simvastatin orally (2mg/kg b.wt./day). At the end of the study, the animals were fasted over night and anaesthetized with ether, and blood samples collected from each animal from retro-orbital venous plexus through the eye canthus. The sera were isolated by centrifugation.

Software for docking Study: The docking program utilized for the visualization and molecular modeling of the compounds was Argus Lab 4.0.1(ArgusLab). Chem Draw Ultra was used to generate the 3D structure of the ligand (OA). ArgusLab implements an efficient grid-based docking algorithm, which approximates an exhaustive search within the free volume of the binding site cavity. In order to explore the conformational space, the program performs a geometry optimization fit of the flexible ligand (rings are treated as rigid) along with incremental construction of the ligand's torsions. Thus, docking occurs between flexible ligands and a rigid protein. The ligand orientation is determined by a shape scoring function based on Ascore and the final positions are ranked by lowest interaction energy values and final poses are ranked by lowest interaction energy values. Structure coordinates for iNOS were obtained from RCSB Protein Data Bank (PDB ID: 1NSI). The structure of iNOS was energy minimized using ArgusLab. The non-essential water molecules were removed and polar hydrogens were merged. Docking simulations were performed by selecting "Argus Dock" as the docking engine.

Statistical analysis: All results are expressed as means ±S.D. Comparison of the values between the vehicle and CCl_4 treated groups was done using student's *t*-test (Snedecor and Cochran, 1971). Multiple group comparisons were performed by ANOVA test followed by Duncan's multiple range test **(**John, et al., 2002). $P<0.05$ was considered statistically significant.

RESULTS

Extraction yield: *Livistona australis* yielded an oily liquid (12.5 % w/w at 28°C).

GC-MS analysis of USM: Unsaponifiable Matter (USM) and Fatty Acid Methyl Esters (FAME) were subjected to GC-MS analysis. The components were identified by comparing their retention times and mass fragmentation patterns with those of the data base libraries [Wiley (Wiley Int. USA) and NIST (Nat. Inst. St. Technol., USA)] and the published data (Adams, 1995). Quantitative determinations of (USM) and (FAME) were carried out based on computerized peak area measurements.

Spectroscopic data of Oleic acid: A pale yellow oily liquid, R_f 0.45 (S1), ($C_{18}H_{34}O_2$), positive EIMS at m/z 283.39 $[M+H]^+$, 264.38, 256.37, 222.34, 185.26, 167.24, 139.20, 125.21, 97.18, 83.18, 69.12; UV λ_{max} (MeOH) nm: 225; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 5.30-5.36 (2H, m); 2.26 (2H, t, *J*=7.34 Hz); 1.98-2.00 (4H, m); 1.57-1.60 (2H, m); 1.23-1.29 (20H, m); 0.85 (3H, t, *J*=6.04 Hz).

Determination of the Vitamins: Typical chromatogram obtained for the tested LALF is presented in Figure1. The retention times of Vitamins D3, E and A was about 1.34 min, 2.088 min and 5.395 min., with a relative area percentage 3.59, 2.15 and 4.14 and concentrations of 303.03, 0.29 and 72.23IU/g LALF in comparison to authentic standards injected individually.

Antioxidant activity: *In vivo* antioxidant potential of LALF and OA was assessed in CCl_4 induced oxidative stress in rats. Glutathione level in the blood of CCl_4 treated rats was reduced by 39.9% compared to vehicle treated group

Antihyperlipidemic activity: Petroleum ether extract of *L. australis* fruit pulps was *in vitro* investigated for its antihyperlipidemic activity. The palm oil was administrated once daily (100mg/Kg b. wt) to hypercholesterolaemic rats for 8 weeks.

Docking Study: Inducible NOS (iNOs) is a homodimer possessing a bidomain structure. The oxygenase domain contains binding sites for cofactors haem, tetrahydrobiopterine (BH_4) and the substrate L-arginine. The reductase domain located on the other side of the enzyme reveals binding regions for cofactors FAD, FMN, NADPH (Li, et al., 1999). Most of the reported iNOS inhibitors interact with arginine-binding site of the oxygenase domain. The promising reduction in nitric oxide concentration induced by oleic acid prompted us to perform in-silico docking studies to simulate the possible interactions between oleic acid & amino acid residues within the iNOS (PDB ID: 1NSI) active site. Structure of the iNOS active site was optimized by molecular mechanics, using the UFF method (Casewit, et al., 1992) implemented in Argus Lab 4.0.1. Flexible ligand docking was performed with help of Argus Lab 4.0.1.Optimized docking conformations of the ligand at the iNOS active site were examined. The binding site consists of ARG 266, GLN 263, PRO 350, GLU 377, ASN 370, VAL 352 & GLY 371 amino acids. Our results have revealed that one oxygen atom in oleic acid forms two hydrogen bonding interactions with basic Arg-266 residue present in the backbone of iNOS (2.95, 2.76A° respectively). The second oxygen atom in oleic acid shares two hydrogen-bonding interactions with Arg-266 & Gln-263 (2.64 & 2.54A° respectively). Oleic acid does not share any H bonding interactions with neither heme nor tetrahydrobiopterin (Fig.2). Binding energy of -11.76kcal/mol was obtained for oleic acid.

DICUSSION

The analysis of *Livistona australis* unsaponifiable fraction revealed the presence of a series of *n*-alkanes (12.3%) ranging from heptadecane (C_{17}) to squalene (C_{30}) which is the predominant triterpene homologue and dotriacontane (C32). Significant amounts of *n*-fatty alcohols (25.26%) ranging from *n*-pentadecanol (C_{15}) to *n*-octacosanol (C_{28}) were present. The most abundant compound was phytol **(**C_{20}) (7.98%), a diterpene alcohol which is considered as a precursor for the manufacture of vitamin E and possesses anti-inflammatory and redox-protective pharmacological activities (Silva et al., 2013). The only *n*-aldehyde detected was Vitamin A aldehyde (0.37%) which is important for the maintenance of the immune system and good vision.

Fourteen fatty acids were identified in LALF, which were grouped as saturated 21.22%, monounsaturated 72.58% and polyunsaturated 1.05% (Table 2). Palmitic ($C_{16:0}$) acid (*m/z:* 74, 87, 143, 270) was the principal saturated fatty acid in LALF representing 20.95% of the total fatty acids concentration (FAME). Although, there is convincing evidence that palmitic acid and saturated fatty acids contribute to an increased risk of developing cardiovascular diseases (Hays, 1995). Previous researches reported that it has no effect on serum lipoprotein profiles (serum total cholesterol, LDL and HDL) in the presence of recommended intakes of linoleic acid (Clandinin, et al., 1999). LALF was found to be highly rich in the monounsaturated fatty acid, oleic ($C_{18:1}$) acid (*m/z*: 55, 155, 199, 312) which is well known as the omega-9 fatty acid and constitutes about 59.05% of the total identified compounds of (FAME) in this oil. Undoubtedly, oleic acid has been proven to have cholesterol-lowering properties, a decrease in the LDL-$_C$ (Reaven, et al., 1991; Grundy, 1986; Mensink and Katan, 1987) and protective effects against cardiovascular complications of diabetes since glutathione (GSH), total lipid and triacylglycerol (TAG) levels are beneficially affected (Emekli-Alturfan, et al., 2010). Oleate prevented the stimulated depletion of GSH without any change in the activity of antioxidant enzymes, which suggest an antioxidant mechanism by which oleate may exert direct vascular atheroprotective effects (Massaro et al., 2002). Linoleic acid ($C_{18:2}$) (0.75%) was also found among the polyunsaturated fatty acids in LALF, which plays an important regulatory role in human cholesterol metabolism and decreases the LDL-c (Reaven, et al., 1993; Reaven, et al., 1993). On the other hand, the identified sterols are among the most predominant compounds in the (USM) fraction of LALF where, campesterol (1.64%) and β-sitosterol (7.7%) (m/z: 145, 213, 396, 414), were present (Table 2). β-Sitosterol and campesterol, are the most common plant sterols, the structural similarity of plant sterols to cholesterol enables them to compete with cholesterol (Reaven, et al., 1993; Reaven, et al., 1993).This competition reduces dietary and bilary cholesterol absorption in the gastrointestinal tract (Reaven, et al., 1993). Decreased cholesterol absorption up- regulates LDL-$_C$ receptor concentration (Reaven, et al., 1993) and therefore decreases LDL-$_C$ serum level.

The chromatographic behavior, UV absorption, EIMS and ^{1}H NMR spectrum, showed that these data are in accordance with those reported for OA (Arudi, et al., 1983; Adas, et al., 1998; Knothe and Kenar, 2004)

Vitamins are well-known group of organic compounds that are essentially required in minute amounts for health, normal growth & maintenance of human bodies. Fat soluble vitamins mainly include A, E, D, K. Reversed-phase HPLC technique well suited for vitamin analysis. Vitamin E (α-tocopherol) is capable of scavenging free radicals, which protects phospholipids & cholesterol against oxidation and subsequent breakdown to potentially harmful chemically reactive products (Gray, et al., 1996). It also inhibits oxidative modification of LDL that is responsible for development & progression

of atherosclerosis (Munteanu, et al., 2004).

LALF and OA administered at doses of 100 and 125 mg/kg b. wt. increased GSH by 60.3% and 61.7%, respectively, compared to control group while GSH was raised to (65.4%) in positive group (Table 3). Thus, LALF and OA showed significant activity expressed by the counterbalance of the depleted GSH level induced by oxidative stress. This indicates that they can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects (Maritim, et al., 2003; Rajasekaran, et al., 2005).

Nitrite concentration in serum was markedly raised in CCl_4-treated rats compared to control, in accordance with other studies (Muriel 1998). Nitrite is generated by inflammatory cytokines due to the over expression of inducible nitric oxide synthase (iNOS) after the administration of CCl_4 (Mizumoto, et al., 1997; Iwai, et al., 2002). Treatment with LALF and OA at doses of 100 and 125 mg/kg significantly protected rats against CCl_4 induced elevation in NO level, resulting in percentage suppression of 53.2 and 62.3% respectively, compared to control group CCl_4. Also, silymarin significantly (P<0.05) lowered the production of NO level by 67.9% compared to CCl_4 control group (Table 3).

Treatment with LALF and OA as well as the silymarin significantly depressed increase in serum nitric oxide level. These results suggest that they ameliorate CCl_4-induced hepatic damage by enhancing the anti-oxidative defense system and reducing the inflammatory signaling pathways. Consequently, the results indicate that LALF and OA might have a role in inhibition of inducible nitric oxide synthase (iNOS) in serum, which were significantly increased by CCl_4 injection (Mizumoto, et al., 1997).

The LALF induced significant reduction of LDL-c (50%) and triglycerides (43.6%) in addition to significant increase in HDL-c (1.1%) with a risk factor of 1.1, compared to 8.5 and 0.5 for the control group and simvastatin treated group (2mg /Kg b.wt) respectively. This showed that administration of LALF (100mg/kg b.wt./day) to high-fat diet fed rats for a period of 4 weeks induced significant reduction in cholesterol, triglyceride and LDL-$_C$ levels in addition to significant increase in HDL-$_C$ in hypercholesterolaemic rats (Table 4). The reduction in serum cholesterol and triglycerides were 50% and 43.6%, respectively while reduction in LDL-$_C$ was 71.2% with a significant increase in HDL-$_C$ by 101% (% of change calculated in regards to the effect at zero time after 8 weeks of the administration). The standard drug showed significant reduction in serum cholesterol, triglycerides and LDL-$_C$ level by 60%, 57% and 85%, respectively with a significant increase in HDL-$_C$ by 103%. It has been shown that the results of *L. australis* were comparable to previous reports (Reaven, et al., 1991; Grundy, 1986; Mensink and Katan, 1987). However, more analysis deems necessary to estimate constituents, which may contribute to the biological activity.

CONCLUSION

Oleic acid constitutes the principle component among the fatty acid contents of LALF. Oleic acid, linoleic acid and phytol inhibited hyperlipidemia probably through oxidative reaction. LALF content of vitamin E may play a role as a strong antioxidant in inhibiting lipid peroxidation and protecting against oxidative degradation of the biologically active substances. The *in vivo* antioxidant potential of OA was simulated by in-silico docking study showing the binding mode of oleic acid as competitive inhibitor with nitric oxide synthase (iNOS) active site. Hence, this study holds great implications for drug designing from natural products.

Acknowledgement: We are grateful to Prof. Amin El Ansary, Departement of Phytochemistry and Plant Systematics, National Research Centre, Dokki, Cairo, Egypt, for running the GC-MS and EIMS.

REFERENCES

Adams, R.P., (1995): Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy. Allured Publishing Corporation, Carol stream, Illinois. USA.

Adas, F., Berthou, F., Picart, D., Lozac'h, P., Beaugé, F., Amet, Y., (1998): Involvement of cytochrome P450 2E1 in hydroxylation of oleic acid in human and rat liver microsomes. *J. Lipid Res.*, 39:1210-1219.

Alderton, W.K., Cooper, C.E., Knowles, R.G., (2001): Nitric oxide synthases: structure, function and inhibition. *Biochem. J.*, 357(3): 593-615.

ArgusLab, M.A. Thompson, Planaria Software, Seattle, WA 98155.

Arudi, R.L., Sutherland, M.W., Bielski, B.H.J., (1983): Purification of oleic acid and linoleic acid materials. *J. Lipid Res.*, 24: 485-488.

Barron, D.,Va rin L., Ibrahim, R.K., Harborne, J.B., Williams, C.A. (1988): Sulphated flavonoids-an update. *Phytochemistry*, 27: 2375-2395.

Beutler, E., Duron, O., Kellely, B., (1963): Improved methods for determination of blood glutathione. *J. Lab. Clin. Med.*, 61: 882-888.

British Medical Association (BMA) medical dictionary (2002): Dorling Kindersley Book, London.

Bucolo, G., David, H., (1973): Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.*, 19: 476-482.

Casewit, C.J., Colwell, K.S., Rappe, A.K., (1992): Application of a universal force field to organic molecules. *J. Am. Chem. Soc.*, 114, 10035-10046.

Clandinin, M.T., Cook, S.L., Konrad, S.D., Goh, Y.K., (1999): The effect of palmitic acid on lipoprotein cholesterol levels & endogenous cholesterol synthesis in hyperlipidemic subjects. *Lipids*, 34(1):S121-24.

Davies, N.M., Wallace, J.L., (1997): Non-steroidal anti-inflammatory drug induced gastro- intestinal toxicity: New insights into an old problem. *Am. J. Gastroenterol*, 32: 127-133.

El- Desouky, S.K., Kassem, M.E.S., Al-Fifi, Z.I.A., Gamal El-Deen, A.M., (2009): A new pyranone derivative from the leaves of *L. australis*. *Nat. Prod. Comm.*, 4: 499-500.

Emekli-Alturfan, E., Kasikci, E., Yarat, A., (2010): Effects of oleic acid on the tissue factor activity, blood lipids, antioxidant and oxidant parameters of streptozotocin induced diabetic rats fed a high cholesterol diet. *Med. Chem. Res.*, 19: 1011-1024.

Fontana J.D., Zagonel, G., Vechiatto, W.W., Costa, B.J., Laurindo, J.C., Fontana, R., Pelisson, L., Jorge, B.H., Lanças, F.M., (2009): Simple TLC-Screening of Acylglycerol Levels in Biodiesel as an Alternative to GC Determination. *J. Chrom. Sci.*, 47:845-846**.**

Fortin, C.F., McDonald, P.P., Fulop, T., Lesur, O., (2010): Sepsis, leukocytes, and nitric oxide (NO): an intricate affair. *Shock*, 33(4): 344-352.

Ghasi, S., Nwobodo, E., Ofili, J.O., (2000): Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed Wistar rats. *J. Ethnopharmacol.*, 69: 21-25.

Gray, J.L., Gomaa, E.A., Buckley, D.J., (1996): Oxidative quality & shelf life of meats. *Meat Sci.*, 43:111-23.

Grundy, S.M., (1986): Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *New England J. Med.*, 314: 745-748.

Harborne, J.B., Williams, C.A., Greenham, J., (1974): Distribution of charged flavones and caffeyl shikimic acid in Palmae. *Phytochemistry*, 13: 1557-1559.

Hartman, L., Lago, R.C.A., (1973): Rapid preparation of fatty acid methyl esters from lipids. *Lab. Pract.*, 222: 475- 476.

Hays, K.C., (1995) Saturated fats & blood lipids: new slant on an old story. *Can. J. Cardiol.*, 11:39G-46G.

Iwai, S., Karim, R., Kitano, M., Sukata, T., Min, W., Morimura, K., Wanibuchi, H., Seki, S., Fukushima, S., (2002): Role of oxidative DNA damage caused by CCl_4-induced liver injury–enhancement of MeIQ-induced glutathione S-transferase placental form-positive foci in rats. *Cancer Lett.*, 179:15-24.

Kassem, M.E.S., Shoela, S.b, Marzouk, M.M., Sleem, A.A., (2012): A sulphated flavone glycoside from *Livistona australis* and its antioxidant and cytotoxic activity. *Nat. Prod. Res.*, 26 (15): 1381-1387.

Knothe, G., Kenar, J.A., (2004): Determination of the fatty acid profile by ^{1}H-NMR spectroscopy, *Eur. J. Lipid Sci. Techn.*, 106(2): 88-96.

Lassmann, H., (2011): Mechanisms of neurodegeneration shared between multiple sclerosis and Alzheimer's disease. *J. Neural Transm*., 118: 747-752.

Li, H., Raman, C.S., Glaser, C.B., Blasko, E., Young, T.A., Parkinson, J.F., Whitlow, M., Poulos, T.L., (1999): Crystal structures of zinc-free and –bound heme domain of human inducible nitric-oxide synthase. Implications for dimer stability and comparison with endothelial nitric-oxide synthase. *J. Biol. Chem.*, 274: 21276-21284.

Maritim, A.C., Sanders, R.A., Watkins, J.B., (2003). Diabetes, oxidative stress and antioxidants. *J. Biochem. Mol. Toxicol.*, 17:24-38.

Massaro, M., Carluccio, M.A., Paolicchi, A., Bosetti, F., Solaini, G., De Caterina, R., (2002): Mechanisms for reduction of endothelial activation by oleate: inhibition of nuclear factor-kappaB through antioxidant effects. *Prostaglan. Leu. Esse. Fatty Acids*, 67(2-3):175-81.

Meisler, A., Anderson, M.E., (1983): Glutathione. *Ann. Rev. Biochem*., 52: 711-760.

Mensink, P., Katan, M.B., (1987): Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men & women. *Lancet*, 1:122-125.

Miranda, K.M., Espey, M.G., Wink, D.A., (2001): A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, 5: 62-71.

Mizumoto, M., Arii, S., Furutani, M., Nakamura, T., Ishigami, S., Monden, K., Ishiguro, S., Fujita, S., Imamura, M., (1997): NO as an indicator of portal hemodynamics and the role of iNOS in increased NO production in CCl_4-induced liver cirrhosis. *J. Surg Res.*, 70:124-133.

Moncada, S., Palmer, R.M., Higgs, E.A., (1991): Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev*., 43: 109-142.

Munteanu, A., Zingg, J.M., Azzi, A., (2004): Anti-atherosclerotic effects of vitamin E-myth or reality. *J. Cell. Mol. Med.*, 8: 59-76.

Muriel, P., (1998): Nitric oxide protection of rat liver from lipid peroxidation, collagen accumulation and liver damage induced by CCl_4. *Biochem Pharmacol.*, 56: 773-779.

Nathan, C., Xie, Q. W.J., (1994): Regulation of biosynthesis of nitric oxide. *Biol. Chem*. 269:13725-28.

Nazif, N.M., (2002): Phytoconstituents of *Z. spina-christi* fruits & their antimicrobial activity. *Food Chem.*, 76: 77-81.

Ney, D.M., Laska, J.B., Shinnck, E.F., (1988): Induction of hyperlipidemia. *J. Nutrition*, 118:1455-1462.

Rafter, J.A., Abell, M.L., Braselton, J.P., (2002): Multiple comparison methods for means. *Soc. Indust. Appl.Math.*, 44 (2): 259-278.

Rajasekaran, S., Sivagnanam, K., Subramanian, S., (2005): Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. *Pharmacol. Rep.*, 57(1): 90-96.

Reaven, P.D., Parthasarathy, S., Grasse, B.J., Miller, E., Steingerg, D., Witztum, J.L. (1993): Effects of oleate-enriched, linoleate-enriched diet on the susceptibility of low-density lipoprotein to oxidative modification and hypercholesterolemic subjects. *J. Clinical Invest.*, 91:668-676.

Reaven, P.D., Grass, B.J., Tribble, D.L., (1993): Effects of linoleate-rich and oleate-rich diets in combination with α-tocopherol on the susceptibility of low density lipoproteins (LDL) and LDL subfractions to oxidative modification in human. *Arterioscler. Thromb. Vasc. Biol.*, 14: 557-566.

Reaven, P.D., Parthasarathy, S., Grasse, B.J., (1991): Feasibility of using an oleate-enriched diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. *Amer. J. Clin. Nutrition*, 54: 701-706.

Snedecor, W.G., Cochran, G.W., (2010): Statistical methods, 10th Ed. Lowa state, Uni. press, USA.

Steinert, J.R., Chernova, T., Forsythe, I.D., (2010): Nitric Oxide in brain function, dysfunction and dementia. *Neuroscientist*, 16: 435-452.

Titheradge, M.A., (1999): Nitric oxide in septic shock. *Biochim. Biophys. Acta*, 1411:437-455.

Varley, H., Gowenlock, A.H., Bell, M., (1983): Practical Clinical Biochemistry, 5th ed. Heinemann Medical Book, London, pp. 625-685.

Williams, C.A., Harborne, J.B., Clifford, H.T., (1973): Negatively charged flavones and tricin as chemosystematics markers in the Palmae. *Phytochemistry*, 12: 2417-2430.

Zhong, Y., Dian, D.C., (1999): An Encyclopedia of Traditional Chinese Medicinal Substances. The Jiangsu College of New Medicine (Ed.), 7th ed., Shanghi Science Technology Press, 2: 2459.

Table- 1: Chemical composition of unsaponifiable matters of *Livistona australis*.

No.	Name of compound	R_t	Area%	M^+
1	Methyl- 9,12,15-octadecatrienoate	0.14	0.69	292
2	n-Pentadecanol	0.67	0.53	228
3	9-Octadecenamide,	0.68	0.49	281
4	Vitamin A aldehyde =Retinal	0.72	0.35	284
5	Heptadecane	0.73	0.37	240
6	10- Heptadecen-8-ynoic acid, methyl ester, (E)	0.77	0.53	278
7	1-Nonadecene	0.78	1.05	266
8	Nonadecane	0.79	0.57	268
9	n-Eicosane	0.80	0.50	282
10	n-octadecane	0.81	0.51	254
11	1,1-Dimethoxydecane	0. 82	4.32	202
12	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	0.82	0.61	278
13	Geranyl isovalerate	0.84	0.53	238
14	1-Octadecanol	0.87	2.88	270
15	1-Eicosanol	0.88	2.98	298
16	9-Hexadecenoic acid	0.91	0.53	254
17	hexadecanoic acid Methyl ester	0.93	1.16	270
18	Octadecenoic acid	0.95	0.55	282
19	Isochiapin	0. 96	1.81	346
20	n- docosane	0.97	0.51	310
21	1-Docosanol	0.98	0.95	326
22	Isophytol	0.99	1.43	296
23	Phytol	1.	7.98	296
24	trans-Geranyl geraniol	1.02	2.85	290
25	Dotriacontane	1.05	1.04	450
26	Hexadecanoic acid, ethyl ester	1.07	0.58	284
27	Ethyl isoallocholate	1.11	1.52	436
28	Heptacosane	1.12	2.26	380
29	1-Heptacosanol	1.18	2.11	396
30	Squalene	1.27	6.06	410
31	Octacosanol	1.36	3.55	410
32	Lucenin 2	1.40	0.41	610
33	Campesterol	1. 43	1.64	400
34	β-Sitosterol	1. 48	7.77	414

R_t = retention time relative to phytol , M^+= molecular ion peak

Table-2: Chemical composition of saponifiable matters of *Livistona australis*

No.	Name of compound	R_t	Area%	M^+
1	Dodecanoic acid, methyl ester (CAS)	0.33	0.13	214
2	9-Hexadecenoic acid, methyl ester, (Z) = palmitoleic acid methyl ester	0.73	1.11	268
3	Hexadecanoic acid, methyl ester = Palmitic acid, methyl ester	0.80	20.59	270
4	*Cis*-10-Heptadecenoic acid, methyl ester = margaric acid methyl ester	0.84	0.20	282
5	11-Octadecenoic acid, methyl ester	0.94	0.08	296
6	9-Octadecenoic acid, methyl ester(Z) = Oleic acid, methyl ester	1	59.05	296
7	9- Octadecenoic acid (E), methyl ester = Elaidic acid, methyl ester	1.01	11.63	296
8	Methyl 9-cis,11- transoctadeca dienoate	1.02	0.26	295
9	9- Octadecenoic acid (Z), ethyl ester = Oleic acid, ethyl ester	1.03	0.10	310
10	9,12- Octadecadienoic acid (Z,Z), methyl ester = Linoleic acid, methylester	1.04	0.79	294
11	Octadecanoic acid, 9,10- dihydroxy, methyl ester,	1.05	0.26	330
12	11-Eicosenoic acid, methyl ester	1.11	0.17	324
13	Octadecanoic acid, 10-oxo, methyl ester	1.12	0.24	312
14	Eicosanoic acid, methyl ester = Archidic acid methyl ester	1.13	0.34	326

- R_t = retention time relative to methyl oleate M^+ = molecular ion peak

Table-3: Antioxidant activity of LALF and OA in carbon tetrachloride-treated albino rats

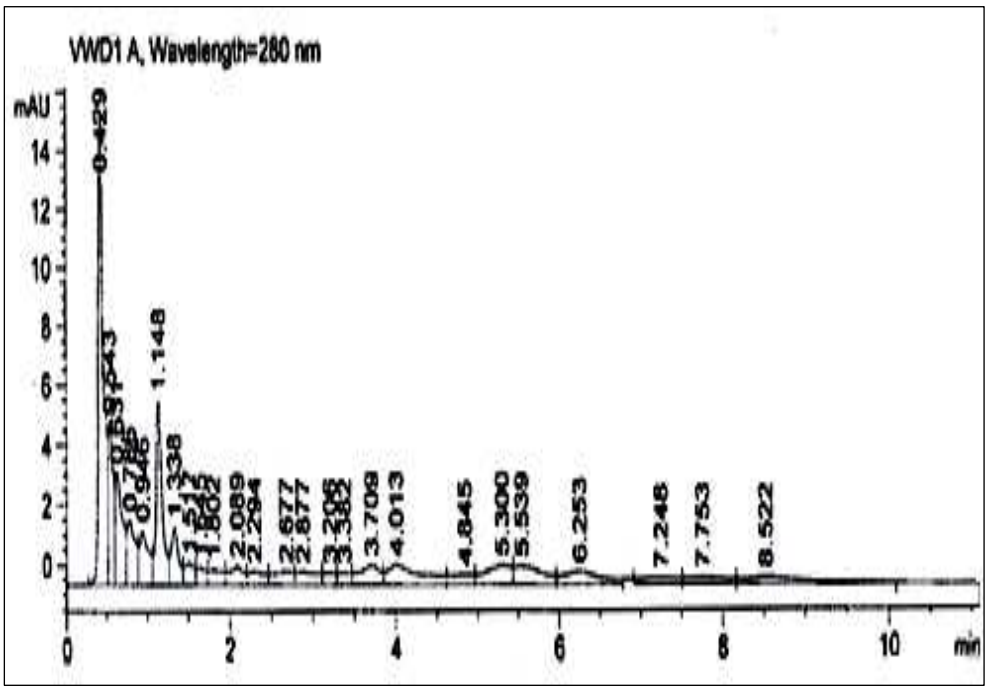
Group	Reduced glutathione (mg %) Mean ± SD (mg/dl)	Nitric oxide (µM) Mean ± SD
Vehicle	36.1 ± 1.1	53.7 ± 1.6
CCl_4 (control)	21.7 ± 0.4*	521.2 ± 15.3
CCl_4+LALF 100mg/kg	34.8 ± 0.6* (60.3%)	243.7 ± 9.4 (-53.2%)
CCl_4+OA 125mg/kg	35.1± 0.8* (61.7%)	196.4± 8.7* (-62.3%)
CCl_4+Silymarin 25mg/Kg	35.9 ± 0.9* (65.4%)	167.2 ± 9.4 (- 67.9%)

- Statistical difference between the vehicle and CCl_4 treated group was done using student's *t-test*.
- Inter group comparison was done using one-way ANOVA & Duncans test.*$P<0.05$was considered significant.

Table-4: TC, TG, LDL-C, HDL-C concentrations & R.F in Hypercholesterolaemic rats.

Parameter studied (mg/dl)	Time of treatment		
	O week	4week	8week
Total cholesterol (TC)			
Hypercholesterolaemic Group	212.1 ± 8.6	212.7 ± 9.2	239.4 ± 11.2
Livistona australis group	220.4 ± 9.3	161.2 ± 7.6	104.3 ± 4.5
Simvastatin group	218.3 ± 9.8	132.4 ± 7.2	85.7 ± 3.3
Triglyceride (TG)			
Hypercholesterolaemic group	172.3 ± 6.4	183.4 ± 7.1	189.1 ± 8.3
Livistona australis group	162.4 ± 7.2	123.7 ± 5.1	91.6 ± 3.7
Simvastatin group	169.8 ± 6.5	96.3 ± 4.1*	73.1 ± 2.4*
LDL-$_C$			
Hypercholesterolaemic group	155.5 ± 6.3	164.7 ± 6.8	180.4 ± 8.3
Livistona australis group	155.3 ± 6.8	106.3 ± 4.2	44.7 ± 2.9
Simvastatin group	164.7 ± 6.9	78.0 ± 2.6*	24.7 ± 0.8*
HDL-$_C$			
Hypercholesterolaemic group	19.1 ± 0.7	20.3 ± 0.9	21.2 ± 0.8
Livistona australis group	19.6 ± 0.8	30.2 ±1.1	41.3* ± 1.9
Simvastatin group	19.6 ± 0.8	35.1 ± 1.3*	46.4± 1.7*
Risk factor (R.F)			
Hypercholesterolaemic group	8.3	8.1	8.5
Livistona australis group	**7.9**	**3.5**	**1.1**
Simvastatin group	8.4	2.2	0.5

- Dose of L.aust. oil (100mg/kg b.wt.
- Inter group comparison was done using one way ANOVA and Duncans test.
- *$P< 0.05$ was considered significant. Low-density lipoprotein cholesterol (LDL-$_C$), High-density lipoprotein cholesterol (HDL-$_C$).



P

LC chromatogram of fat-soluble vitamins of LALF.

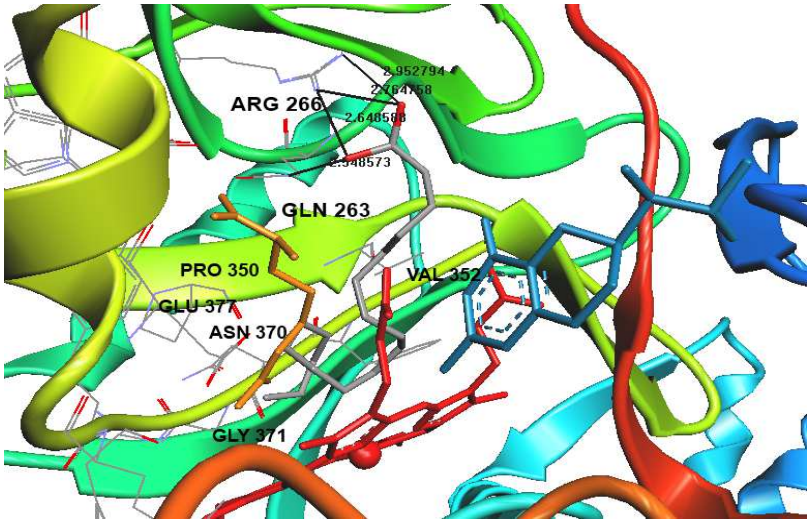


Figure-2: Oleic acid (*grey*) docked into the active site of inducible nitric oxide synthase (PDB ID: 1NSI).

- Hydrogen bonding interactions are shown in black. L-Arginine (orange), Heme (red) and Tetrahydrobiopterin (blue) are included in the figure.

