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Research Paper

Phytochemical and pharmacological studies of *Solanum elaeagnifolium* growing in Egypt.

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ABSTRACT

The aim of this study is the phytochemical investigation and the biological assay of *Solanum elaeagnifolium* Cav. The chemical investigation of the ethyl acetate and 25% methanol/ethyl acetate fractions of *Solanum elaeagnifolium* Cav led to the isolation of two compounds β-sitosterol-3-O-β–D-glucoside (4) and Quercetin-3-O-β-D-glucopyranoside (5) for the first time from the species as well as three known compounds, β-sitosterol (1), stigmasterol (2) and kaempferol (3). Structure elucidation was achieved using spectroscopic techniques, including 1D, 2D NMR and MS. Concerning the lipid fraction, linoleic acid (6.27%) and oleic acid (5.66%) are the major unsaturated fatty acids where palmitic acid (5.16%) represented the major saturated one. The total phytosterols was amounted 11.34%. The biological assay revealed that the alcoholic extract exhibited significant analgesic, anti-inflammatory, hepatoprotective and anti-oxidant activities.

Keywords: *S. elaeagnifolium;* Flavonoid; Antioxidant; Hepatoprotective; Anti-inflammatory activity.

INTRODUCTION

Among angiosperm families, the *Solanaceae* ranks one of the most important to human beings. Species of the family are used as foods (*Solanum tuberosum* L.; Potato, *Solanum lycopersicum* L; Tomato and *Solanum melongena* L.; the egg plant), drugs (*Nicotiana tabacum* L. and *Nicotiana rustica* L.) and as ornamentals (*Petunia hybrida* Hort.), (Symon, et al., 1991). The genus *Solanum* is the largest genera of the family solanaceae in the west side of the country (Collenette, 1999; Chaudhary, 2001). *Solanum* is a rich source for several classes of compounds such as alkaloids (Emmanuel, et al., 2006), steroids (Ferro, et al., 2005) and phenolic compounds (El-Sayed and Hassan, 2006). Many species of genus *Solanum* are used in the folk medicine of different countries, such as hypoglycemic (Kar, et al., 2006), hepatoprotective and hepatotonic (De Silva, et al., 2003; Son, et al., 2003), laxative, appetizer, cardiotonic (Mans, et al., 2004), antispasmodic, treatment of renal pain and epilepsy (Perez, et al., 2006). Zainul, et al., (2006) reported that the lipid soluble extract from the *Solanuim nigrum* leaves showed antinociceptive, anti-inflamatory and anti-pyretic properties.

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Concerning *Solanum elaeagnifolium*, the steroidal alkaloid solasodine used in the preparation of contraceptive and corticosteroid drugs has been commercially extracted from *S. elaeagnifolium* berries in India. Also, Argentina making it the most promising source among *Solanum* species investigated (Heap, et al., 2007)

Recent studies have identified other potential uses for *S. elaeagnifolium* as plant extracts have shown molluscicidal and nematicidal activity, as well as cancer-inhibiting activity (Heap, et al., 2007).

It is worthy to be note that there are a few reports on the chemistry of *Solanum elaeagnifolium* as well as the biological screening. Accordingly, in the present study, *S. elaeagnifolium* was phytochemically analyzed. Biological and pharmacological studies including the anti-inflammatory, analgesic, antioxidant, hepatoprotective activities of the extract were also evaluated.

MATERIALS AND METHODS

Plant material: The whole plant of *Solanum elaeagnifolium* (Coll. no. SAA-151) used in this study was collected once during the year 2010 from Sinai Peninsula (El-Arish), Egypt. The plant was kindly authenticated by Dr. Mohamed El-Gebaly, Department of Plant Taxonomy, Faculty of Science, Cairo University. The voucher specimen was deposited in the herbarium section of Pharmacognosy Department, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt under registration number SAA-151.

Fingerprinting of Solanum elaeagnifolium DNA by polymerase chain reaction: Total DNA was extracted from the *Solanum elaeagnifolium* using the Qiagen DNeasy (Qiagen Santa Clara, CA). Random Amplified Polymorphic DNA (RAPD) technique (Welsh and McClelland, 1990) provides an approach to find the genetic differences between species.

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 36ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

Ten primers was purchased from Operon Technologies Inc. (Almeda, California, USA), with the following sequences: B-04 (5'-GGACTGGAGT-3'), B-07 (5'-GGTGACGCAG-3'), B-10(5'-CTGCTGGGAC-3'), B-12(5'-CCTTGACGCA-3'), B-15(5'-GGAGGGTGTT-3'), A-01(5'-CAGGCCCTTC-3'), E-05(5'-TCAGGGAGGT -3'), C-05(5'-GATGACCGCC-3'), G-17(5'-ACGACCGACA-3'), G-18(5'-GGCTCAT GTG-3') were used for RAPD analysis

Electrophoresis and visualization of PCR products: The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5µg/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Polaroid camera (Fig. 1).

Extraction and isolation: 5kg powder of the whole plant of *S. elaeagnifolium* was extracted by maceration with 90% methanol till complete exhaustion, filtered, evaporated till dryness and weighed to give (220g) of the total extract.

The crude extract (180g) was slurred with a small portion of silica gel. The mixture was transferred to a top of a sintered glass Büchner filter funnel (30 X 15 cm) packed with 400g silica gel and connected to vacuum pump. Step gradient elution with a non-polar solvent (*n*-Hexane) with increasing the amounts of a polar solvent using EtOAc then MeOH to give nine successive fractions (2L each), monitored by

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TLC and concentrated. The similar fractions were collected together and subjected to further chromatographic purification.

Isolation of the major compounds from ethyl acetate and 25% methanol in ethyl acetate fractions: The fraction eluted using ethyl acetate and 25% methanol in ethyl acetate was concentrated to afford 40g of green residue. Purification of this fraction was carried out by flash column chromatography on silica gel. Elution was performed initially with n-hexane followed by gradient systems of n-hexane, ethyl acetate till 50% methanol in ethyl acetate. Fractions with the same TLC pattern were combined to afford four sub-fractions.

Fraction A eluted using hexane/ethyl acetate (80:20) showed one major spot, rechromatographed by flash column chromatography on silica gel using hexane/ethyl acetate (90:10) to afford compound 1 white crystalline needles (15mg) (R_f 0.49, 20% ethyl acetate/hexane).

Fraction B eluted using hexane/ethyl acetate (70:30) showed one major spot. Purification of this fraction was carried out by flash column chromatography on silica gel using hexane/ethyl acetate (8:2) to afford compound 2, a white colour powder (20mg) (R_f 0.48, 20 % ethyl acetate/hexane).

Fraction C eluted using hexane/ethyl acetate (10:90) showed two major spots. Purification of this fraction was carried out by Sephadex LH-20 using MeOH to afford compound 3 a yellow powder (10mg) (R_f 0.73, butanol/water/formic acid 4:5:1) and compound 4 which recrystallized from methanol, resulted in isolation a pure white powder compound 4 (20mg) (R_f 0.38, 10 % methanol/chloroform).

Fraction D eluted using methanol/ethyl acetate (5:95) showed one major spots. Purification of this fraction was carried out by Sephadex LH-20 using MeOH to afford a yellow powder compound 5 (120mg) (R_f 0.46, butanol/water/formic acid 4:5:1).

Investigation of the hexane fraction: About 1g of the hexane fraction of *S. elaeagnifolium* was refluxed for 6 hours with 0.5N alcoholic KOH (60ml) in a boiling water bath (Tsuda, et al., 1960). The saponified fraction was concentrated and the residue was diluted with an equal volume of distilled water and exhaustively extracted with ether. The combined ethereal fraction was washed several times with distilled water till free of alkalinity and dehydrated over anhydrous sodium sulfate. After evaporation of ether to dryness, the residue was kept for studying the unsaponifiable matter. The alkaline aqueous solution remaining after extraction of the unsaponifiable matter was acidified with hydrochloric acid to liberate the fatty acids which were extracted several times with ether. The combined ethereal fraction was washed several times with distilled water till free from acidity, then filtered over anhydrous sodium sulphate and the filtrate was evaporated to dryness. The residue was kept for studying the fatty acids contents.

Biological activity of the crude extract

Anti-inflammatory assay: Using the carrageenan-induced rat paw oedema test as described by (Winter, et al., 1962), thirty six male albino animals divided into six groups (each of six animals) were used. They were administered one single oral dose of the tested samples and the reference drug in specific doses. The negative control group received saline. One hour later all the animals had a subcutaneous injection of 0.1ml of 1% carrageenan solution in saline, in the right hind paw and 0.1% of saline in the left hind paw compared to Indomethacin (20mg/kg. b.wt.)

Four hours after drugs administration, the rats were sacrificed. Both hind paws were excised and weighed separately.

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The percentage of oedema produced and that of oedema inhibition due to drug administration were, respectively calculated as follows:

Oedema = (Wt of right paw - Wt. of left paw) ×100/Wt. of left paw

% Oedema inhibition = (Mc – Mt) × 100/Mc

- Where, Mc is the mean oedema in control rats and Mt is the mean oedema in drug-treated animals.

The analgesic activity: Swiss male albino mice (20-25g) were acclimatized to the laboratory conditions for at least one hour before testing. The analgesic activity was estimated using acetic acid induced writhing test, the extract was administrated orally at a dose of (200mg/kg b.wt.). Thirty minutes later 0.6% acetic acid was injected intraperitonial (0.2ml/mouse) each mouse was then placed in an individual clear plastic observation chamber and the total number of writhes/minute was counted for each mouse (koster, et al., 1959).

Hepatoprotective activity: Liver damage in rats was induced according to the method of (Hernandez, et al., 1997) by i.p. injection of 5ml/kg of 25 % carbon tetrachloride (CCl_4) in liquid paraffin. Seventy-two hours after administration of CCl_4, blood samples were withdrawn to be used for the biochemical study. Adult male albino rats of sprangedawely strains (130-140g) were randomly divided into 5 groups each of 10 animals. The ethanol extract of the plant (100mg/kg b.wt.), as well as, the standard drug (Sylimarin, 25mg/kg b.wt.) were separately administered daily for one week before and one week after liver damage. A group of animals were kept untreated (receiving only saline) and served as a negative control.

Biochemical studies were carried out; followed by an overnight fast; whole blood was obtained from the retro-orbital venous plexus through the eye canthus of anaesthetized rats. The blood samples were collected at zero time, after one week of receiving the tested drug, 72 hours after induction of liver damage then after a week of treatment with the tested samples and standard. Serum was isolated by centrifugation and divided for analysis of aspartate amino-transferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP) enzymes.

Antioxidant effect: TLC-based 1,1- diphenyl-2-picrylhydrazyl radical (DPPH) autographic chemical assay was used. Total ethanolic extract and the isolated compounds were dissolved in DMF at a concentration of 2mg/ml. A 4µl volume of each compound was applied in the form of a spot (4-5 mm in diameter) on silica gel plates. The residual DMF was removed under vacuum (15-20 min). A similar amount of Vitamin E in DMF was used as positive antioxidant control. The radical-scavenging effects of the isolated compounds were detected on the TLC plate using a spray reagent composed of a 0.2% (w/v) solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) in MeOH. The plate was observed 30 min after spraying. Active compounds were observed as yellow spots against a purple background. Relative radical-scavenging activity was assigned as “strong” (compounds that produce an intense bright yellow zone), “medium” (compounds that produce a clear yellow spot), “weak” (compounds that produce a weakly visible yellow spot), or “not active” (compounds that produce no sign of any yellow spot) (Takamatsu, et al., 2003). Vitamin E was taken as positive antioxidant control which produced an intense bright yellow zone.

Equipments and apparatuses: The laboratory procedures performed in the course of this research were carried out by using the following equipments and apparatuses.

Melting points were determined by using Electrothermal1 A9000, The ^{1}H and ^{13}C NMR spectra were recorded using JEOL Ex-500 MHz and Ex-125 MHz, spectrometer respectively. The mass spectra were carried on JEOL model JMS-AX500.

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Authentic reference materials for TLC (β-Sitosterol, Stigmasterol, Kaempferol, Quercetin) were obtained from Merk Co. Darmstadt, Germany (Batch number 715439). Silica gel 60-120 mesh (Fluka) for fractionation was obtained from (Sigma-Aldrich, chemicals-Germany) and silica gel (70-230) for column chromatography was obtained from (Merk). TLC analyses were carried out on Precoated silica gel GF_{245} chromatoplates (Merk). Sephadex LH-20 for final purification was purchased from Pharmacia Fine Chemicals AB (Uppsala, Sweden).

Experimental animals: Albino mice (25-30g) and adult male albino rats of Sprague Dawley strain (120-150g) were utilized for assessment of the different pharmacological effects, were obtained from the animal house (Animal's treatment control protocol approved by Animal Right Committee 2003, National Research Center, Giza, Egypt).

RESULTS AND DISCUSSION

Fingerprinting of S. elaeagnifolium DNA by RAPD: The banding profile produced by the ten decamer primers used in RAPD analysis of *S. eleagnifolium* Cav. cultivated in Egypt is illustrated in (Fig.1). It showed distinguishable bands and generated 69 fragment patterns. RAPD fragments showed 9 bands by B-07 and E-05, 8 bands by B-10 and A-01, 7 bands by B-12, G-17, and G-18, 5 bands by B-04 and B-15, and 4 bands by C-05.

Analysis of well characterized marker compounds, through deoxy-ribonucleic acid (DNA), is now the most popular method for the authentication and identification of herbal materials (Shinde and Dhalwal, 2010). Therefore the DNA fingerprinting of *S. elaeagnifolium* Cav. is done as a contribution to the morphological identification and characterization of the plant. This finding indicates that the analysis of RAPD data can select the use of primers B-07 and E-05 for the selective discrimination of *S. eleagnifolium* Cav. growing in Egypt.

GC/MS analysis

Unsaponifiable matter analysis: Using GC/MS analysis, qualitative and quantitative assay of the hydrocarbons, sterols and triterpenoids was based on comparison of the retention times and peak area measurement of their peaks with those of the available authentic samples.

The percentage of total identified phytosterols was (11.34%). γ - sitosterol was prevailing in the plant of *S. elaeagnifolium* (5.36%). Meanwhile, campesterol was (ca. 3.720%) and stigmasta-5,24(28)-dien-3-ol (2.25%). Squalene was also detected as triterpene (1.59%). Vitamine E which represented the natural antioxidants was Ca. (4.48%).

Concerning hydrocarbons, heptacosane was the predominant hydrocarbon amounting to (7.96%). Other hydrocarbons, identified in appreciable quantities, were n-Octacosane (2.90%) and Nonadecane (1.02%).

Fatty acid methyl ester analysis: GC/MS analysis for methyl ester revealed that, the major fatty acid constituent in the plant is E form of linoleic acid (6.27%), oleic acid (5.66%), palmitic acid (5.16%) and Z form of linoleic acid (3.20%).

Identification of the major compounds

Compound 1: was obtained as white needle crystals, soluble in n-hexane, m.p. 140-141°C, it gives positive Libermann test indicating the sterols and/or triterpenes skeleton. The mass spectrum (EI-MS) of compound 1 showed a molecular ion peak at m/z 414 calculated for the molecular formula $C_{29}H_{50}O$, in addition to the following characteristic peaks at 396 [M^+-H_2O], 303 [M^+-111], and 255 [M^+-side chain-H_2O].

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Further, evidence for its characterization came from its ^{1}H NMR spectrum which exhibited a broad signal at δ 5.3 and a multiple at 3.5 corresponding to H-6 olefinic proton and H-3α proton respectively. Rest of protons appeared in the high field region in between δ 0.67-2.0 ppm (Parveen, et al., 2011). From the above findings and by comparison with the published data as well as m.p. and co-chromatography, compound 1 was identified as β-sitosterol (Fig.2).

Compound 2: was obtained as white powder, soluble in n-hexane, m.p. 165-166°C, it gave positive tests for sterols and or triterpenes. The mass spectrum (EI-MS) showed a molecular ion peak at m/z 412 calculated for the molecular formula $C_{29}H_{48}O$, in addition to the following characteristic peaks at 351, 300 and 213.

The ^{1}H NMR spectrum showed signals at δ 3.5 (1H, m, H-3), 5.4 (1H, bd, *J* = 6.0 Hz, H-6), 5.1 (1H, AB, q, H-22), 5.18 (1H, AB, q, H-23), 0.69 (3H, s, H-18), 1.04 (3H, s, H-19), 1.08 (3H, d, *J* = 3.9, H-21), 0.84 (3H, d, *J* = 3.3, H-26), 0.80 (3H, d, *J* =4.2, H-27), 0.82 (3H, t, H-29)

The ^{13}C-NMR spectrum of compound 2 revealed the presence of 28 signals which are assignable for the sterol nucleus. Signals appearing at δ ppm 12.0, 12.3, 21.2, 19.4, 18.9 and 11.9 correspond to the methyl carbons C-18, C-19, C-21, C-26, C-27 and C-29, respectively. Signals at δ 28.9, 31.9, 24.4, 25.4, 129.2, 31.9 and 31.64 corresponding to C-2, C-7, C-11, C-15, C-23, C-25 and C-28 were also demonstrated by the spectrum. Moreover, signals at δ 42.2, 31.9, 50.1, 39.7, 31.6, 40.5, 138.3, 39.8, 56.7 and 55.9 corresponding to C-4, C-8, C-9, C-10, C-12, C-20, C-22, C-13, C-14 and C-17 were clear and signals δ at 37.2, 28.9, 140.7, 121.7, 31.6, 51.2. corresponding to C-1, C-3, C-5, C-6, C-16, C-24. From the above findings which confirmed by HSQC and by comparison with published data (Nurettin and Cemalettin, 1996) as well as m.p. and co-chromatography compound 2 was identified as stigmasterol (Fig. 2).

Compound 3: was obtained as yellow powder, m.p. 276-278°C, It exhibited positive tests for flavonoids (yellow color with $ALCL_3$, and ammonia vapors. The ^{1}HNMR spectrum showed meta coupled doublets at δ 6.1 (1 H, d, *J* = 1.8 Hz, H-6) and 6.4 ppm (1 H, d, *J* = 1.8 Hz, H-8). Also ortho doublet at δ 6.9 ppm (2 H, d, *J* = 7.8 Hz, H-3`,H-5`) and ortho doublet at δ 8.1 ppm (2H, d, *J* = 7.5, Hz, H-2', H-6') (Harborne, et al., 1975).

From the previous spectral data and by comparison with published data (Mabry, et al., 1996) the structure of compound 3 (Fig. 2) was established as the 3, 5, 7, 4', tetrahydroxy flavone (Kaempferol). This was confirmed through direct comparison with an authentic sample m.p. and co-chromatography.

Compound 4: was obtained as white amorphous powder, m.p. 270-273°C, insoluble in chloroform and methanol, soluble in chloroform-methanol 1:1, it gave positive Libermann test indicating the sterols and/or triterpenes skeleton and Molisch's test for carbohydrates and/or glycosides.

The mass spectrum (EI-MS) of the aglycone of compound 4 was similar to this of compound 1 showed a molecular ion peak at m/z 414 calculated for the molecular formula (M^+-$C_6H_{10}O_5$) $C_{29}H_{50}O$, in addition to the following characteristic peaks at 396 [M^+-H_2O], 381[M^+-H_2O-CH_3], and 255 [M^+-side chain-H_2O].

The ^{1}H-NMR spectrum of compound 4 revealed the presence of a C-24 ethyl sterol nucleus by the appearance of 6 methyl groups, two singlets at δ 0.65 (3H, s), 0.96 (3H, s), assigned for Me-18 and Me-19, respectively, three doublets at δ 0.80 (3H, d, *J* = 4.2 Hz), 0.9 (3H, d, *J* = 6.3 Hz), 0.99(3H, d, *J* = 6.6 Hz) assigned for Me-26, Me-27 and Me-21 respectively and one triplet at δ 1.16 (3H, t, *J* = 6.3, 7.5 Hz) assigned for Me-29 which is characteristic for C-24 ethyl sterols. In addition to the

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appearance of a signal at 3.10 (1H, m, H-3) and a signal at 5.33 (1H, brs) corresponding to the olefinic proton at H-6. Finally, the anomeric proton appearing at 4.22 (1H, d, *J* = 7.8 Hz) indicated a β-linkage.

The ^{13}C-NMR spectrum of compound 4 revealed the presence of 35 signals; 29 signals of which are assignable for the C-24 ethyl sterol nucleus and the other 6 signals correspond to the sugar moiety. Signals appearing at δ ppm 11.6, 19.6, 18.6, 18.9, 19.1 and 11.7 correspond to the methyl carbons C-18, C-19, C-21, C-26, C-27 and C-29, respectively. The methyl group at C-29 appeared as a signal at δ ppm 11.7 thus confirming that this C-24 ethyl sterol is the β-epimer (β-sitosterol nucleus, c.f. α-epimer in which this methyl group appears at δ 12.3 as in clionasterol). Signals at δ ppm 29.2, 39.5, 20.6, 23.8, 25.5, 28.7 and 22.6 corresponding to C-2, C-7, C-11, C-15, C-23, C-25 and C-28 were also demonstrated by the spectrum. Moreover, signals at δ 31.4, 36.2, 36.8, 35.5, 33.3, 41.8, 49.6, 45.1, 56.2 and 55.4 corresponding to C-8, C-10, C-12, C-20, C-22, C-4, C-9, C-13, C-14 and C-17 were clear. The presence of a sugar moiety attached to C-3 was verified by the occurrence of a signal at δ ppm 76.9, which is downfield shifted from the aglycone value at (δ 73.0). The olefinic carbons at C-5 and C-6 were verified by the presence of signals at δ 140.4 and 121.1. Finally, the anomeric carbon appearing at δ 90.6 confirms the β linkage of the sugar moiety, the 5 carbons of which appeared at δ 70.1, 73.4, 68.1, 76.6 and 61.1 assigned for C-2', C-3', C-4', C-5' and C-6'.

The glycoside (5mg) was refluxed with 5ml 2N HCL in 50% methanol for 1 hour. After hydrolysis, the solution was diluted with water and the released aglycone was extracted with chloroform. The chloroform extract was concentrated. Identification of the aglycone was done by TLC alongside with authentic references. The aglycone was corresponding to β-Sitosterol. The sugar moiety was identified as D-glucose by PC pattern alongside with authentic and aniline phthalate as spraying agent. By comparing this data with the published ones (Goad, et al., 1997, Sangs, et al., 2002), compound 4 was identified as β-sitosterol-3-O-β–D-glucoside (Fig. 2). Identification was confirmed by its co-chromatography. Compound 4 not reported previously in *S. elaeagnifolium.*

Compound 5: was obtained as yellow powder, soluble in methanol, m.p. 255-257°C, It gave positive tests for flavonoids (Seikel, 1962).

The mass spectrum (EI-MS) showed a parent ion at m/z 302 which is in a good accordance with the aglycone (M^+-$C_6H_{10}O_5$) of this compound and fragments at m/z 285, 273, 153, 150 and 137.

The ^{1}H-NMR spectrum confirmed flavonol structure and displayed the presence of protons at δ 6.2 (1H, d, *J* = 2.0 Hz, H-6), δ 6.4 (1H, d, *J* = 2.0 Hz, H-8), δ 7.5 (1H, d, *J* = 2.3 Hz, H-2'), δ 6.8 (1H, d, *J* = 9.2 Hz, H-5') and δ 7.5 (1H, dd, *J* = 2.3, 9.2 Hz, H-6'). An anomeric proton signal of the compound appeared at δ 5.4 (1H, d, *J* = 7.4 Hz, Glu-H-1) and the resonances in the region of δ 3.1-3.6 (6H, m, Glu-H) suggested the presence of glucopyranose unit (Liu, et al., 2010).

The ^{13}C-NMR spectrum of this compound showed signals for five (C-7, C-5, C -4′, C-3′ and C-3) carbons with OH at δ 164.5, 161.5, 148.8, 145.2 & 133.9 and C=O carbon at δ 177.9 in addition to the other characteristic chemical shift for carbon at δ 156.7 (C-2), 156.9 (C-9), 122.1 (C-6′), 121.7 (C-1′), 116.7 (C- 5′), 115.5 (C-2′), 104.5 (C-10), 101.4 (Glu-C-1), 99.1 (C-6), 94.0 (C-8), 78.0 (Glu-C-5), 76.8 (Glu-C-3), 74.5 (Glu-C-2), 70.3 (Glu-C-4) and 61.3 (Glu-C-6) (Jin, et al., 2009).

After acid hydrolysis of compound 5, TLC of the aglycone was done alongside with authentic references. It was identified as quercetin. Concerning the glycone part,

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by PC alongside with authentic and aniline phthalate as visualizing agent, it was found to be glucose.

The NMR and (EI-MS) data led to the identification of the compound 5 (Fig. 2) as quercetin 3-O-β-D-glucopyranoside. Compound 5 had not previously been isolated from *S. elaeagnifolium*.

Biological evaluation

The anti-inflammatory activity: The anti-inflammatory activity (Table-1) of the extract of *S. eleaegnifolium* was evaluated on carrageenan-induced rat hind paw oedema model. The extract (200mg/kg) has been found to possess significant anti-inflammatory activity on the tested experimental model.

The analgesic activity: The analgesic activity (Table-2) of the extract of *S. eleaegnifolium* was estimated using acetic acid induced writhing test. The extract (200mg/kg) has a significant analgesic activity on the tested experimental model.

Antioxidant activity: Biological evaluation of the total extract and the isolated compounds revealed that compound 3 and 5 possesses "strong" anti-oxidant activity while compound 1, 2, 4 had low or not active as anti-oxidant. This related to the flavonoidal and phenolic nature of compound 3 and 5.

Hepatoprotective activity: The data obtained were analyzed using student's t- test where means of the treated groups were compared to that of the control group for each variable. Results obtained for the ethanolic extract are recorded in (Table-3).

Generally from the biological evaluation of isolated metabolites revealed that compound 3 and 5 possesses "strong" anti-oxidant activity while compound 1, 2 and 4 had low or not active as anti-oxidant. The anti-oxidant and hepatoprotective activities may be related to the flavonoidal and phenolic nature of compound 3 and 5. (Hopia and Heinonen, 1999). Concerning the anti-inflammatory effect may be connected with the total phytosterols (Perez, 2001).

CONCLUSION

It can be conclude from these studies that, five compounds (β-sitosterol, stigmasterol, kaempferol, β-sitosterol-3-O-β–D-glucoside, Quercetin-3-O-β-D-glucopyranoside have been isolated from the ethyl acetate and 25% methanol/ethyl acetate fractions of *S. elaeagnifolium*. As far as the available literature is concerned, the last two are reported for the first time in these species. Concerning the lipid fraction, linoleic acid and oleic acid are the unsaturated fatty acids where palmitic acid represented the major saturated one. The total phytosterols was amounted as 11.34%. Assessment of the pharmacological potentialities of the alcoholic extract of *S. elaeagnifolium* revealed that extract exhibited remarkable analgesic, anti-inflammatory, antioxidant and hepatoprotective activities. Biological evaluation of isolated metabolites revealed that compound 3 and 5 possesses "strong" anti-oxidant activity while compound 1, 2 and 4 had low or not active as anti-oxidant.

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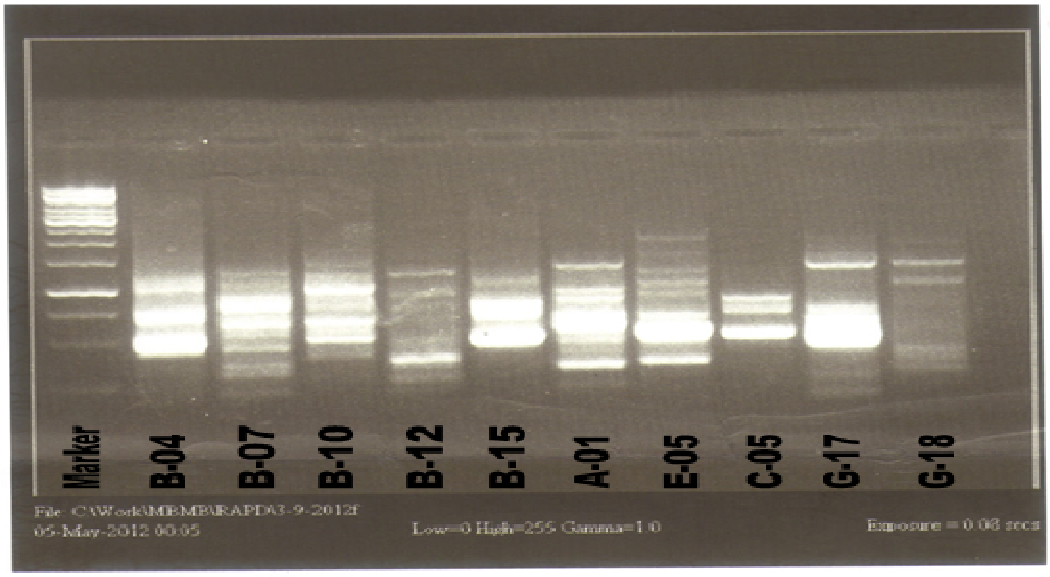
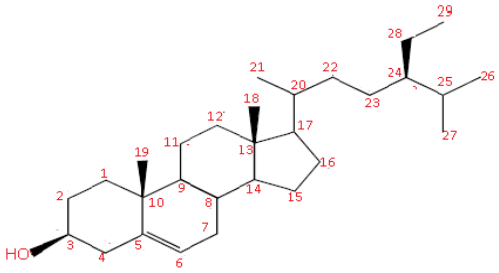
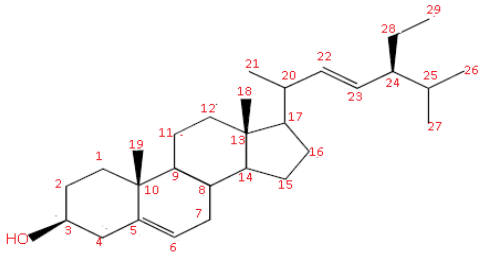


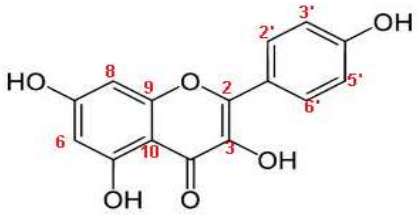
Figure-1: Gel electrophoresis of the RAPD-PCR products for *S. elaeagnifolium* using ten decamer primers (B-04, B-07, B-10, B-12, B-15, A-01, E-05, C-05, G-17 and G-18).



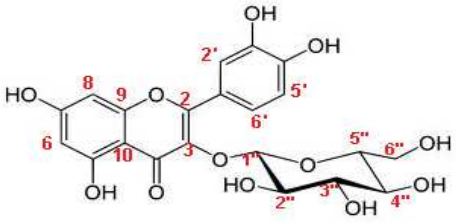
Compound 1: β-Sitosterol



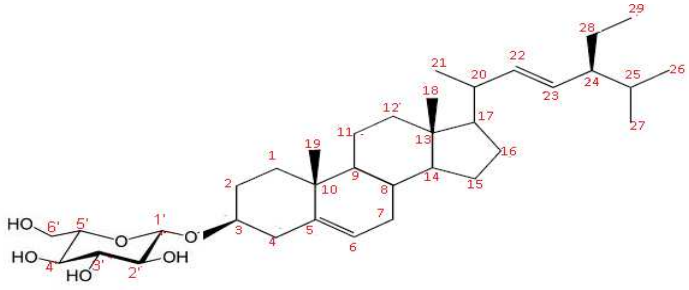
Compound 2: Stigmasterol



Compound 3: Kaempferol



Compound 5: Quercetin-3-O-β-D-glucopyranoside



Compound 4: β-Sitosterol-3-O-β–D-glucoside

Figure- 2: The suggested structures of the isolated compounds from *Solanum elaeagnifolium*.

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Table-1: Acute anti-inflammatory activity of the alcoholic extract of *S. elaeagnifolium* plant and indomethacin drug in male albino rats (N=6).

Group	Dose in mg/kg. b.wt.	% Oedema		Potency[1]
		Mean ± S.E.	% of Change	—
Control	1 ml Saline	59.4±1.6	—	—
Ethanolic extract of *S. elaeagnifolium*	100	36.7±1.2*	38.22	0.62
Indomethacin	20	22.6±0.4*	61.95	1

- [1]Potency calculated as compared to the standard anti-inflammatory drug Indomethacin. *Significantly different from control group at P <0.01. S.E. = standard error, % of change is calculated as regards to the control group.

Table-2: Effect of alcoholic extract of *S. elaeagnifolium* plant on number of abdominal constrictions and acetic acid in mice (n=6).

Group	Dose mg/kg b.wt.	Number of Abdominal Constrictions	% inhibition	Potency[1]
Control	1 ml saline	46.9±1.3	——	——
Ethanolic extract of *S. elaeagnifolium*	100	26.2±0.4*	44.14	0.73
Indomethacin	20	18.4±0.3*	60.77	1

- [1]Potency calculated as compared to the standard anti-analgesic drug Indomethacin. *Significantly different from control group at P <0.01, S.E. = standard error, % of inhibition is calculated as regards to the control group.

Table-3: Effect of ethanolic extract (50 mg/kg) of *S. elaeagnifolium* plant and silymarin drug on serum enzymes level (AST, ALT and ALP) of liver damaged rats (n=6).

Test	AST (U/L)			
	Zero	7d	72h	7d
Control	44.6±2.1	43.9±1.8	156.3±4.8*	164.4±5.4 Ω *
Ethanolic extract of *S. elaeagnifolium*	46.2±1.9	45.7±1.6	86.4±2.9*	72.9±2.6 Ω *
Silymarin	45.3±1.6	44.9±1.7	68.2 ±2.3*	43.7±1.5 Ω *
Test	**ALT (U/L)**			
Control	38.9±1.3	38.1±1.6	149.4±4.2*	154.9±5.1 Ω *
Ethanolic extract of *S. elaeagnifolium*	41.1±1.4	42.7±1.2	78.9±2.9*	69.1±2.3 Ω *
Silymarin	39.8±1.2	38.4±1.3	49.8±1.6*	38.2±1.1 Ω *
Test	**ALP (KAU)**			
Control	7.3±0.1	7.4±0.1	48.6±1.8*	59.7±2.1 Ω *
Ethanolic extract of *S. elaeagnifolium*	7.2±0.1	7.1±0.1	26.4±0.9*	22.6±0.4 Ω *
Silymarin	7.4±0.1	7.2±0.1	18.3±0.4*	7.6±0.1 Ω

- * Statistically significant from zero time at P <0.01.
- Ω Statistically significant from 72h after CCl_4 at P < 0.01.

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