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UPLC-PDA-ESI-MS/MS ANALYSIS, ISOLATION OF CHEMICAL CONSTITUENTS, CYTOTOXIC, ANTIOXIDANT, ANTIVIRAL AND ANTIMICROBIAL ACTIVITIES OF THE AERIAL PARTS OF LYCIUM SHAWII ROEM. & SCHULT

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ABSTRACT

Phytochemical investigation of petroleum ether and ethyl acetate fractions of the aerial parts of *Lycium shawii* Roem. & Schult led to isolation of seven known compounds including vanillic acid (**1**), luteolin (**2**), kaempferol (**3**), Diosmetin- 7- *O*- *β* – D – glucopyranosyl - (1'''→ 6'') – *β* – D – glucopyranoside (**4**), rutin (**5**), *β*-sitosterol (**6**) and *β*-amyrin (**7**)**.** Their structures were established on the basis of spectroscopic methods including MS, 1D and 2D NMR data and through comparison with published literatures. To the best of our knowledge compounds **1** - **4** were isolated for the first time from *Lycium shawii*. UPLC-PDA-ESI-MS/MS analysis in positive and negative modes were applied for the identification and chemical characterization of the phenolic compounds of the ethyl acetate fraction. It showed the presence of **32** compounds including phenolic acids, flavonoidal glycosides, flavonoid aglycones, epigallocatechin derivatives and polymethoxylated flavonoids. Biologically, the cytotoxic activity against A-549 (lung carcinoma), HepG-2 (liver carcinoma) and MCF-7 (breast cancer) cell lines were evaluated for petroleum ether, chloroform, ethyl acetate and butanol fractions of *L. shawii*. All the tested fractions showed significant activity and the most active was petroleum ether fraction with IC_{50} 5.01, 7.12, and 9.03µg/ml respectively. While the butanol fraction was more effective as antiviral agent against HAV-10 virus than ethyl acetate and petroleum ether fractions. In addition, the chloroform and ethyl acetate fractions exhibited DPPH scavenging potential stronger than that exerted by positive standard, ascorbic acid, with IC_{50} values of 7.6, 10.9 and 14.2 µg/ml respectively. However the petroleum ether fraction was inactive. Moreover, the antimicrobial activity of different fractions showed promising results and the best inhibitory activity was observed with chloroform fraction against *Escherichia coli* which is significantly stronger than that exerted by Gentamicin with inhibition zone diameters 20.3 mm and 19.9 mm respectively.

KEYWORDS: *L. Shawii*, UPLC-Mass analysis, phenolic compounds, antiviral, antioxidant, cytotoxic activities.

INTRODUCTION

Lycium (Boxthorn) is a genus of nightshade family [Solanaceae][1], it has about 100 species distributed throughout the tropical regions of the world.[2] The importance of plants belonging to the genus *Lycium* (*Solanaceae*) has increased rapidly in the last few years due to their contents of various classes of natural products as alkaloids, amides, peptides, flavonoids, coumarins, lignans, terpenoids, sterols, and steroids, organic acids and their derivatives, polysaccharides, carotenoids, nutrients, and essential oils[2] and glycosidic compounds[3] and because of Lycium species displayed many biological activities as antiviral, antioxidant[4-6], antimicrobial[6,7], antidiabetic[8-10], hepatoprotective[5], anticancer[11], hypolipidemic, hypotensive, glucosidase inhibition, and antiaging[1] activities. Few literature were reported on *L shawii* plant and showed isolation of *β*-sitosterol, atropine, trans-ferulic acid, 5- hydroxy ferulic acid, rutin and diosgenin.[1,5] In addition to some biological activities as antioxidant, cytotoxic and hepatoprotective, laxative, diuretic, hypotensive, antidiabetic and cure jaundice.[1,10,12] In Saudi Arabia *L. shawii* is used to treat mouth ulcer[13] and the aerial parts had antidiabetic[10], anti-toxoplasma[14], antiplasmodial, antitrypanosomal[14-16] and antimicrobial activities.[7] Because of the growing interest for isolation, analysis and identification of phenolic constituents of medicinal plants. and as nothing is reported on the phenolic constituents profile of *L. shawii* plant, the present study deals with the isolation and structural elucidation of seven known compounds, in addition to UPLC-PDA-ESI- MS/MS analysis of the ethyl acetate fraction and finally evaluation of biological activities of different fractions obtained from the aerial parts of *Lycium shawii* growing in Saudi Arabia.

EXPERIMENTAL SECTION

1. General experimental section

Evaporation of solvents was done using a Buchi rotary evaporator; EI/MS were carried out on Jeol JMS-AX

500, 70 ev and Shimadzu GC/MS-QP5050A, 70 ev; UPLC- ESI-MS-MS Positive and negative ion acquisition modes were carried out on a XEVO TQD triple quadruple instrument, Waters Corporation, Milford, MA01757 U.S.A, Mass Spectrometer, Column: ACQUITY UPLC – BEH C18 1.7µm - 2.1 × 50mm Column, Flow rate: 0.2 mL\min, Solvent system : consisted of (A) water containing 0.1 % formic acid (B) Methanol containing 0.1 % formic acid. UV lamp was used for TLC visualization UVP; circulating hot air oven W.T-binder 7200 (Germany) was used for drying and activation of silica gel plates; ^{1}H & ^{13}C NMR spectral analyses were obtained by: JEOL at 500, 125 MHz. and Bruker at 400 MHz. Chemical shifts were given in ppm with the TMS as internal standard.

1.1. Plant material

The fresh plant was collected from Al-Hada (Saudi Arabia) on March, 2010 and was kindly identified by Dr. Jakob Thomas, Professor in College of science, KSU. A voucher specimen (#15106) was deposited at the herbarium in the College of Pharmacy at King Saud University (KSU).

1.2. Extraction and fractionation of air dried aerial parts of *L. shawii*

About 450g of the air dried aerial parts of *L. shawii* were milled and extracted with 95% ethanol at room temperature. The crude extract was evaporated under vacuum, and the concentrated extracted (45.5g) was dispersed in water/methanol (9:1) and partitioned successively with petroleum ether, chloroform, ethyl acetate and *n*-butanol to obtain petroleum ether (12.5g), chloroform (8.5g), ethyl acetate (5.3g) and *n*-butanol (15.7g) fractions respectively.

1.2.1. Isolation of compounds 1-5 from ethyl acetate fraction of the aerial parts of *L. shawii*

About 4 gm of the ethyl acetate soluble fraction was subjected to silica gel column, elution was started with dichloromethane and the polarity was increased gradually using methanol. Fractions eluted with 2%, 3%, 4%, 9% and 6% MeOH/CH_2Cl_2 were separately collected and concentrated, then subjected to silica gel sub-columns for purification and the important sub-fractions were evaporated and subjected to crystallization from hot methanol to afford compounds **1**(15 mg), **2**(7mg), **3**(5mg)**, 4**(8mg) and **5**(5mg).

1.2.2. Isolation of compounds 6 and 7 from petroleum ether fraction of the aerial parts of *L. shawii*

About 10 gm of petroleum ether fraction was placed on the top of silica gel column. The elution was started with light petroleum then the polarity was increased gradually using dichloromethane and methanol respectively, the collected fractions were concentrated under reduced pressure, Compound **6** (4 mg) was isolated from fractions eluted with 70% CH_2Cl_2/ light pet. Fractions eluted with 60% CH_2Cl_2 / light pet. afforded 9 mg of white powder of compound **7**.

Compound 1 [Vanillic acid]: (15 mg), off white flakes with R_f value 0.47 (solvent system CH_2Cl_2: CD_3OD; 9: 1); ^{1}H-NMR (CD_3OD, 400 MHz): δ_H 7.58 (1H, s, H-2), 7.53 (1H, d, *J* = 8.5, H-6), 6.90 (1H, d, *J* = 8.5 Hz, H-5), 3.85 (3H, s, OCH_3); ^{13}C-NMR (CD_3OD, 125 MHz); δ_C 123.9 (C-1), 115.2 (C-2), 151.8 (C-3), 147.8 (C-4), 115.2 (C-5), 121.1 (C-6), 55.6 (C-7), and 167.2 (C-8); EI-MS *m/z*: 168 [M^+], 153 [M^+-CH_3], 123 [M^+ COOH] and 108 [M^+-CH_3-COOH].

Compound 2 [Luteolin]: (7 mg), yellow amorphous powder; R_f value of 0.74 in solvent system benzene : Ethyl acetate : Formic acid (5:4:0.5); The ^{1}H -NMR (CD_3OD, 500 MHz): δ_H 6.52 (1H, s, H-3), 6.18 (1H, d, *J*=1.75 Hz, H-6), 6.42 (1H, d, *J*=1.75 Hz, H-8), 7.36 (1H, d, *J*= 2.0 Hz, H-2`), 6.98 (1H, d, *J*= 8.6 Hz, H-5`) and 7.34 (1H, dd, *J*= 2.0, 8.6 Hz, H-6`); $^{13}C$-NMR ($CD_3OD$, 125 MHz): $\delta_C$ 160.0 (C-2), 103.1 (C-3), 182.5 (C-4), 158.1 (C-5), 101.9 (C-6), 165.0 (C-7), 101.8 (C-8), 164.7 (C-9), 103.0 (C-10), 118.5 (C-1`), 112.4 (C-2`), 145.7 (C-3`), 149.6 (C-4`), 115.9 (C- 5`) and 122.3 (C-6`); EI-MS *m/z* : 286 [M^+], 258, 228, 153, 135, 134, 96.

Compound 3 [Kaempferol]: (5 mg), yellow powder, R_f 0.6 [EtOAc: MeOH: H_2O (30:5:1)]; ^{1}H NMR (400 MHz, DMSO) δ_H 12.5 (1H, br. s, OH-5), 8.04 (2 H, d, *J*= 8.8, H-2`, 6`), 6.92 (2H, d, *J*=8.8, H-3`, 5`), 6.44 (1H, d, *J*=1.8 Hz, H-8) and 6.19 (d, *J*= 1.8, H-6); ^{13}C-NMR (DMSO, 125 MHz): δ_C 160.0 (C-2), 136.0 (C-3), 176.2 (C-4), 161.0 (C-5), 98.5 (C-6), 164.2 (C-7), 93.8 (C-8), 156.5 (C-9), 103.4 (C-10), 122.0 (C-1`), 129.8 (C-2`, 6`), 115.8 (C-3`, 5`), 159.5 (C-4`); EI-MS *m/z* 286, 153 and 121.

Compound 4 [Diosmetin- 7- *O*- *β* -D- glucopyranosyl-(1'''→6'')-*β*-D- glucopyranoside]: (8 mg) yellow residue, R_f 0.62 [butanol: acetic acid: water, (24:10:1)]; UV λ max; MeOH: (nm) 253, 269 sh., 344. ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm) 7.48 (1H, dd, 2.0, 8.8, H-6`), 7.61 (1H, d, 2.0, H-2`), 7.10 (1H, d, 8.8, H-5`), 6.83 (1H, s, H-3*)*, 6.81 (1H, d, 2.0, H-8), 6.48 (1H, d, 2.0, H-6), 5.06 (1H, d, *J* = 7.2, glucosyl H-1``), 3.88 (3H, s, OCH_3-4`), 4.15 (br d H-1```), 3.0- 4.0 ppm (the remaining sugars protons).^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm) 162.9 (C-2), 105.4 (C-3), 181.9 (C-4), 161.0 (C-5), 99.8 (C-6), 164.1 (C-7), 94.8 (C-8), 156.9 (C-9), 104.1 (C-10), 122.1 (C-1`), 113.8 (C-2`), 146.7 (C-3`), 151.3 (C-4`), 55.8 (OCH_3-4`), 112.2 (C-5`), 119.0 (C-6`), 100.4 (C-1``), 73.3 (C-2``), 75.4 (C-3``), 70.5 (C-4``), 76.1 (C-5``), 68.6 (C-6``). 103.1 (C-1```), 73.0 (C-2```), 72.9 (C-3```), 69.6 (C-4```), 76.4 (C-5```), 64.1 (C-6```); ESI/MS (Positive ion) *m/z* 625 [M^+ + H] for $C_{28}H_{32}O_{16}$, 463 [M-Glu + H]$^+$, 301 [M^+ - Glu –Glu +H]$^+$.

Compound 5 [Rutin]: (5 mg), yellow powder, R_f 0.37 [EtOAc : MeOH: H_2O (30:5:4)]; ^{1}H NMR (500 MHz, DMSO): δ_H 7.69 (IH, H-2` ), 7.65 (1H, d, *J*=7.5 Hz, H-6`), 6.9 (1H, d, *J*=7.5 Hz, H-5`), 6.30 (1H, s, H-8), 6.20 (1H, s, H-6), 5.50 (1H, d, *J*=7.5 Hz, H-1``), 5.10 (1H, s, H-1```) and 12.5 (1H, br. s, OH-5), 3.30-3.8 (remaining

protons of sugar moieties), 1.10 (3H, s, CH_3-6```); ^{13}C-NMR (DMSO, 125 MHz): δ_C 159.3 (C-2), 135.6 (C-3), 179.3 (C-4), 162.3 (C-5), 99.9 (C-6), 166.0 (C-7), 94.9 (C-8), 158.4 (C-9), 105.6 (C-10), 123.6 (C-1`), 116.0 (C-2`), 145.8 (C-3`),149.8 (C-4`), 117.7 (C-5`), 123.1 (C-6`), 104.8 (C-1``), 75.7 (C-2``),78.1 (C-3``), 71.5 (C-3``), 77.1 (C-5``), 68.5 (C-6``), 102.4 (C-1```),72.1 (C-2```), 72.3 (C-3```), 73.9 (C-4```), 69.7 (5```), 17.9 (C-6```); EI-MS *m/z* 302 [M- (Glu + rha)]$^+$.

Compound 6 [*β*-sitosterol]: (4 mg) white powder; R_f 0.53 [EtOAc: Hex: (1: 3)]**;** EI-MS *m/z*: 414 [M]$^+$, 396, 381, 329, 303, 255, 213, 145.

Compound 7 [*β*-amyrin]: (9 mg) white powder; R_f 0.72 [EtOAc: Hex: (1: 3)]**;** EI-MS *m/z*: 426 [M]$^+$, 396, 297, 257, 203, 189, 147, 133, 103, 89, 73.

1.3. UPLC-PDA-ESI-MS-MS analysis of the ethyl acetate fraction of *L. shawii*

The sample (100 µg/mL) solution was prepared using high performance liquid chromatography (HPLC) analytical grade solvent of MeOH, filtered using a membrane disc filter (0.2µm) then subjected to LC-ESI-MS analysis. Samples injection volumes (10µL) were injected into the UPLC instrument equipped with reverse phase C-18 column (ACQUITY UPLC - BEH C18 1.7µm particle size - 2.1 × 50 mm Column). Sample mobile phase was prepared by filtering using 0.2µm filter membrane disc and degassed by sonication before injection. Mobile phase elution was made with the flow rate of 0.2mL/min using gradient mobile phase comprising two eluents: eluent A is H_2O acidified with 0.1% formic acid and eluent B is MeOH acidified with 0.1% formic acid. Elution was performed using the following gradient: 20% B, 0–1 min; 20%–90% B, 1–18 min; 20% B, 18–20 min. The parameters for analysis were carried out using negative and/or positive ion mode as follows: source temperature 150°C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 450 °C, cone gas flow 50L/h, and desolvation gas flow 900 L/h. Mass spectra were detected in the ESI negative and /or positive ion mode between *m/z* 50–900. The peaks and spectra were processed using the Maslynx 4.1 software and tentatively identified by comparing its retention time (Rt) and mass spectrum with reported data. For fragmentation collision energy 40 ev was used.

1.4. Biological activities

All the biological activities were carried out at Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University, Cairo, Egypt.

1.4.1. Cytotoxic activity[17]

The cytotoxic effect and of petroleum ether, chloroform, ethyl acetate and butanol fractions of the aerial parts of *L. shawii* was investigated at different concentrations, 50, 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39µg/ml using Sulfo-rhodamine B-assay where the cells were seeded in 96-well plates (5,000 cells per well). Cells were fixed and stained according to published protocols[17] for a 10-day period. Optical density readings were carried out at 450 nm. The tested cells were breast carcinoma (MCF-7), lung carcinoma (A-549) and hepatocellular carcinoma (HepG-2), IC_{50} was defined as the drug concentration required to reduce fluorescence to 50% of that of the control and the results were reported in Table 2 and Figure 3.

1.4.2. Antioxidant activity[18]

2, 2 diphenyl-1-picrylhydrazyl (DPPH) tests were used for evaluation of antioxidant activity of petroleum ether, chloroform, ethyl acetate and butanol fractions of aerial parts of *L. chawii* was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University *by* the DPPH free radical scavenging assay in triplicate and average values were considered (Figures 4 and 5).

DPPH Radical Scavenging Activity[18]

Methanol solution (0.004%w/v) of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals was freshly prepared and stored at 10ºC in the dark. A methanol solution of the test extract was prepared. A 40µL aliquot of the methanol solution was added to 3 ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515nm was determined continuously, with data being recorded at 1 min intervals until the absorbance reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$\% \text{ Antioxidant activity (E)} = ((Ac–At)/Ac)\times 100$$

Where: Ac was the absorbance of methanolic DPPH solution (control) and At was the absorbance of the extract sample + DPPH.

Extracts concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentages against extract concentration.

1.4.3. Antiviral activity[19]

For the first time the antiviral activity of petroleum ether, chloroform, ethyl acetate and butanol fractions of aerial parts of *L. shawii* was tested at a concentration of 200µg/ml against HAV-10 virus using cytopathic effect inhibition assay[19] and the results were reported in Table 3. Briefly monolayers of 10,000 vero cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24h at 37ºC in a humidified incubator with 5%CO2. The plates were washed with fresh DMEM and challenged with 104 HAV-10 virus doses and simultaneously the cultures were treated with two-fold serial dilutions of tested fractions in fresh maintenance medium and incubated at 37ºC for 3 days. An infection control as well as untreated vero cells control was made in the absence of tested fraction. Six wells were used for each concentration of the tested fraction. Every 24 h the

observation under the inverted microscope was made until the virus in the control wells showed complete viral-induce cytopathic effects (CPE). Antiviral activity was determined by the inhibition of cytopathic effect compared to control i.e., the protection offered by the tested extract to the cells was scored.[20] Ribavirin, was used as a positive control under this assay system.[21] After the incubation period, the media was aspirated, and then the cells were stained with a 0.1% crystal violet solution for 4 h. The stain was removed and the plates were rinsed using tap water until all excess stain was removed. The plates were allowed to dry for 24 h and then glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), at 620 nm. Viral inhibition rate was calculated as follows: [(ODtv-ODcv)/(ODcd-ODcv)] × 100% Where ODtv, ODcv and ODcd indicate the absorbance of the tested extracts with virus infected cells, the absorbance of the virus control and the absorbance of the cell control, respectively.

1.4.4. Antimicrobial activity

Agar diffusion method[22]

The antimicrobial activity of petroleum ether, chloroform, ethyl acetate and butanol fractions of aerial parts of *L. shawii* were evaluated at a dose of 50µg/ml by agar diffusion method against fungi, *Asperagillus fumigates* (RCMB 02568), *Syncephalastrum racemosum* (RCMB 05922), *Geotricum candidum* (RCMB 05097), *Candida albicans* (RCMB 05036); Gram positive bacteria, *Streptococcus pneumonia* (RCMB 010010), *Bacillus subtillis* (RCMB 010067) and Gram negative bacteria *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052) was evaluated using agar diffusion technique. and the results were reported in Table 4. The tested fungi were *Asperagillus fumigates* (RCMB 02568), *Syncephalastrum racemosum* (RCMB 05922), *Geotricum candidum* (RCMB 05097) and *Candida albicans* (RCMB 05036); Gram positive bacteria *were Streptococcus pneumonia* (RCMB 010010) and *Bacillus subtillis* (RCMB 010067) while Gram negative bacteria were *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052). Amphotricin, ampicillin and gentamicin were used as standard antifungal and antimicrobial agents; the diameter of zone of inhibition was measured in mm. The inhibition diameters of test fractions were expressed as mean standard deviation. Group comparisons were done using one way analysis of variance (ANOVA) followed by Waller-Duncan Post Hoc test. A value of $P<0.05$ was considered statistically significant. The microorganisms were obtained from Regional Centre of Mycology and Biotechnology, Al-Azhar University.

RESULTS AND DISCUSSION

1. Phytochemical results

1.1. Characterization of the isolated compounds

Compound 1: EI-MS spectrum of compound **1** gave a molecular ion peak at *m/z* 168 $[M]^+$ which corresponding to molecular formula $C_8H_8O_4$, in addition to fragment at *m/z* 153 $[M^+-CH_3]$, 123 $[M^+-$ COOH] and 108 $[M^+-CH_3-$COOH]. The ^{1}H-NMR, ^{13}C-NMR and DEPT 135 data indicated the presence of one methoxy group (δ_H, 3.85 s, 3H; δ_C: 55.6); ABX spin system at (δ_H 6.90 d, *J*= 8.5; δ_C: 115.3), (δ_H: 7.53 d, *J*= 8.5 Hz; δ_C: 121.1) and (δ_H: 7.58, s, 1H, δ_C: 115.2) for H-5, H-6 and H-2 respectively. ^{13}C-NMR show also two oxygenated aromatic carbons at δ_C: 151.8 and 147.8 in addition to one carboxylic acid carbonyl (δ_C: 167.2). The HHCOSY data shows the correlation of H-5 with H-6 while the HMBC correlation (Figure 2) confirmed the position of methoxy group at C-3 from the correlation of the protons of methoxy group with carbon at δ_C: 151.8. From the above NMR and Mass data and through comparison with literature[23] compound **1** was identified as vanillic acid and this is the first time to isolate vanillic acid from the aerial parts of *L. Shawii*. Vanillic acid have been shown to exhibit various pharmacological activities. It has hepatoprotective effect as it suppresses hepatic fibrosis in chronic liver injury[24,25], anti-sickling and anthelmintic activities, It is also found to be an inhibitor of snake venom 5'-nucleotidase.[26] Vanillic acid has a protective effect against heart disease[27] and anti-inflammatory effect[28]

Compound 2: The EI-MS spectrum of compound **2** exhibited a molecular ion at *m/z* 286 $[M^+]$ with fragments at*m/z* 258 $[M^+-CO]$, 153 [benzoyl moiety of ring A] and 135 [for cinnamoyl moiety of ring B]. ^{1}H NMR spectrum showed signals for ABX spin system of ring B at δ_H 7.36 (1H, d, *J*=2 Hz), 7.34 (1H, dd, *J*=, 8.6, 2 Hz) and 6.98 (1H, d, *J*=8.6 Hz) for H-2`, H-6` and H-5 respectively. It also showed a singlet signal at δ_H 6.52 for H-3. The other two protons were at δ_H 6.42 (1H, d, *J*=1.7 Hz) and 6.18 (1H, d, *J*=1.7 Hz) for H-8 and H-6 respectively. The ^{13}C NMR spectra showed the carbonyl signal at δ_C 182.7 (C-4) and C-3 signal at δ_C 103.1. The oxygen containing carbons showed the downfield signals at δ_C 165.0 (C-7), 164.7 (C-9), 160.0 (C-2), 158.1 (C-5), 149.6 (C-4`) and 145.7 (C-3`) for oxygen carrying carbons. By comparison of MS and ^{1}H & ^{13}C-NMR of compound **2** with literature[29] compound **2** could be identified as 3', 4', 5, 7-tetrahydroxyflavone (luteolin). And this compound was detected in UPLC Ms Ms profile as shown in Table 1. To our knowledge, it is the first time to isolate luteolin from the aerial parts of *L. Shawii*.

Compound 3: The EI-MS spectrum of compound **3** exhibited a parent ion peak at *m/z* 286 in accordance with the molecular formula $C_{15}H_{10}O_6$ suggesting a tetrahydroxyflavonol. Other Mass fragments at *m/z* 153 and 121 indicated the presence of two hydroxyl groups at ring A and one hydroxyl at ring B, respectively. The ^{1}H and ^{13}C NMR data of compound **3** show the presence of AA`BB` spin system of ring B from the signals at δ_H 8.04 (*d*, 8.8, 2 H, H-2`, 6`), 6.92 (*d*, 8.8, 2H, H-3`, 5`) and δ_C 129.8 (C-2`, 6`), 115.8 (C-3`,5`). In addition, the other ^{1}H and ^{13}C NMR signals at δ_H 6.19 (*d*, 1.8, H-6), 6.44 (*d*, 1.8. H-8), δ_C 98.5 (C-6) and 93.8 (C-8) revealed

the presence of AX spin system in ring A. Thus from the above data and current literature, compound **3** was identified as kaempferol[29] and it is the first report to isolate this compound from *L. shawii*.

Compound 4: The ^{1}H NMR and ^{13}C NMR spectra of compound **4** showed the presence of methoxyflavone nucleus with the presence of ABX spin system in ring B which is in a good agreement with diosmetin[30], the remaining ^{1}H NMR and ^{13}C NMR signals especially the signals at δ_H 5.06 (1H, d, J = 7.2, glucosyl H-1``), 5.15 (br d glucosyl H-1```) and δ_C 100.4 (C-1``), 103.1 (C-1```), 68.6 (C-6``), 62.1 (C-6```) showed the presence of two glucose moieties. The position of the first glucose was determined to be at C-7 from the HMBC correlation, Figure 2 of the anomeric proton (H- 1``) with C-7 at $\delta_C$ 164.1 while the second glucose was found to be at C-6`` and this from the downfield shift of C-6`` at δ_C 68.6. The ESI (Positive ion) confirmed the above suggestion as it showed a protonated molecular ion peak at *m/z* 625 $[M + H]^+$ for $C_{28}H_{32}O_{16}$ and fragment ions at *m/z* 463 $[M - glu + H]^+$ and 301 $[M - glu - glu + H]^+$ confirmed that the structure is diosmetin-7-O- *β*-D glucopyranosyl-*β*-D-(1```- 6``) glucopyranoside and this is the first time to isolate this compound from nature and from *L. shawii* but it was previously detected in LC/MS/MS analysis of phenolic compounds of *Chrysanthemum morifolium.*[31]

Compound 5: The ^{1}H NMR spectrum of compound **4** showed the presence of five signals for aromatic protons at δ_H 7.69 (IH, H-2`), 7.65 (1H, d, 7.5 Hz, H-6`), 6.9 (1H, d, 7.5Hz, H-5`), 6.30 (1H, s, H-8), 6.20 (1H, s, H-6). The ^{1}H NMR spectrum also supported the presence of rhamnose and glucose moieties from the anomeric protons at δ_H 5.50 (1H, d, 7.5 Hz, H-1``), 5.10 (1H, s, H-1```) for glucose and rhamnose respectively. A methyl group of rhamnose was observed at δ_H 1.10. The rest of protons in the sugar moieties resonated between 3.30-3.80 ppm. The ^{13}C NMR spectrum showed the presence of 15 carbon signals due to the flavonol skeleton and 12 carbons for sugar moieties. By careful investigation of ^{1}H and ^{13}C NMR, DEPT spectra in addition to HMBC correlations, figure 2, and through comparison with literature[29], compound **5** was identified as rutin. And this compound was detected in UPLC Ms/Ms profile of phenolic compounds shown in table 1.

Compounds 6 and **7:** By comparison of EI-MS with literatures, TLC and Co-TLC with authentics, compounds **6** and **7** were identified as *β*-sitosterol and *β*-amyrin respectively.[32]

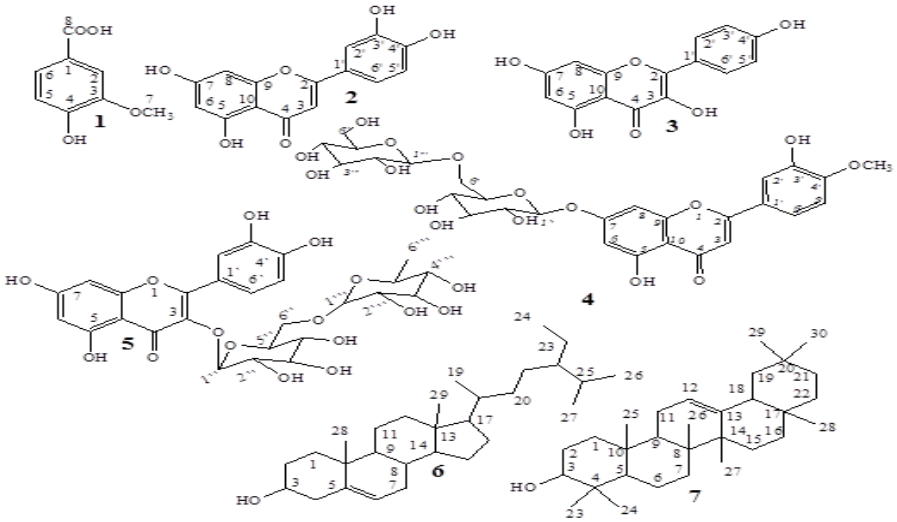

Figure (1): Structure of compounds 1-7.

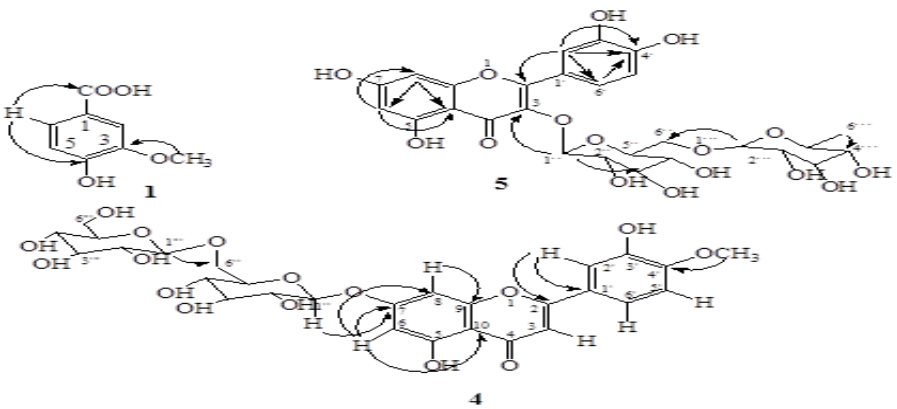

Figure (2): Important HMBC correlations of compounds 1, 4 and 5.

1.2. Identification of phenolic constituents of the ethyl acetate fraction of *L. shawii* by UPLC-PDA-ESI-MS/MS

Phenolic constituents of the ethyl acetate fraction of *L. shawii* were identified for the first time by UPLC-using photodiode array detector and electrospray mass spectrometry [ESI-MS/MS] in both negative and positive ionization modes and the results reported in (Table 1) showed the presence of **32** compounds and the proposed structures of these compounds were identified by fragmentation pattern using ESI-MS-MS.

1.2.1. Identification of phenolic acids

Peak **1** with deprotonated molecular ion peak at *m/z* 353 and MS^2 at *m/z* 191[M − H − caffeoyl]$^+$, 179 [M − H − quinic]$^+$ was tentatively identified as chlorogenic acid.[33, 34] Peak **2** which showed a molecular ion peak [M-H]$^+$ at *m/z* 167 and MS^2 fragmentations at *m/z* 152[M-H-CH_3]$^+$, 123 [M-H-CO_2]$^+$ and 108[M-H-CH_3-CO_2]$^+$ was identified as vanillic acid [34] and it was isolated in this work as a pure compound (compound **1**). In addition peak **3** was identified as caffeic acid from MS^1 ions at *m/z* 179[M-H]$^+$, MS^2 at *m/z* 135 [M-H - CO_2]$^+$, while peak **5** was identified as *p*-coumaric acid from MS^1 at *m/z* 163[M-H]$^+$ and MS^2 at *m/z* 119 [M-H-CO_2]$^+$.[33, 34] Furthermore peak **10** was identified as syringic acid from MS^1 at *m/z* 197[M-H]$^+$ and MS^2 at *m/z* 182 [M-H-CH_3]$^+$, 167 [M-H-2CH_3]$^+$, 153 [M-H-CO_2]$^+$.[33,34] Caffeic, chlorogenic, vanillic and *p*-coumaric acids were previously detected in *Lycium barbarum Lycium chinense*[35, 36]

1.2.2. Identification of flavonoidal glycosides

Ten flavonoidal glycosides were identified in the ethyl acetate fraction of *L. shawii*, where the peak **4** showed a molecular ion peak at *m/z* 609 [M-H]$^+$ and generated MS^2 fragment ions at *m/z* 463, and 301 corresponding to the loss of 146 amu [M-rha]$^+$ and loss of 308 amu [M-glu-rha]$^+$, the fragments ion at *m/z* 301, 151 corresponded to the quercetin aglycone, so this compound was identified as rutin,[33] Rutin was previously detected in *Lycium barbarum* and *Lycium chinense*[35,36] and it was isolated in this work as pure compound (compound **5**), while the signal of peak **9** detected at *m/z* 625 [M+H]$^+$ with daughter ions at *m/z* 463 [M+H-glu]$^+$ and 301 [M+H-glu-glu]$^+$ was attributed to diosmetin-7-*O*-diglucoside[31] and the identification of this compound was confirmed by the 1D and 2D NMR data for the isolated compound (compound **4**). Peaks **11**, **12** and **13** with pseudomolecular ion peaks at *m/z* 449 [M+H]$^+$, 447 [M-H]$^+$and 449 [M+H]$^+$ and MS^2 fragments at *m/z* 287 [M+H-162]$^+$, 285 [M-H-162]$^+$ and 303 [M+H-146]$^+$ were identified respectively as kaempferol-3-*O*-glucoside, luteolin-7-*O*-glucoside, quercetin-3-*O*-rhamnoside and diosmetin-7-*O*-glucoside.[31] Other peaks number **14** and **16** had the same deprotonated ions at *m/z* 331 [M-H]$^+$ with daughter fragment ions at *m/z* 269 with loss of 162 amu [M-H-glu]$^+$ and *m/z* 285 with loss of 146 amu [M-H-rha]$^+$ and were identified as apigenin-7-*O*-glucoside and kaempferol-3-*O*-rhamnoside respectively.[31] In addition the peak **17** with a protonated ion at *m/z* 595 [M+H]$^+$ and MS^2 fragments at *m/z* 463 [M+H-132]$^+$ and *m/z* 301 [M+H-162-132]$^+$ was detected as diosmetin -7-*O*-arabinoglucoside.[31] Peak **18** with pseudomolecular ion peak at 463 [M+H]$^+$ and MS^2 fragment at *m/z* 301 [M+H -162]$^+$ were identified as diosmetin-7-*O*-glucoside.[31] Furthermore peak **19** had a protonated ion at *m/z* 579 [M+H]$^+$ and MS^2 fragments at *m/z* 285 [M+H -132-162]$^+$ and was identified as acacetin -7-*O*-arabinoglycoside.[31]

1.2.3. Identification of flavonoidal aglycones

Seven flavonoidal aglycones were detected in the ethyl acetate fraction of *L. shawii*. Peaks **20**, **21** and **22** were characterized as flavonols, based on comparison of their characteristic fragment ions with previous literatures as follow, quercetin [MS^1 at *m/z* 301 {M-H}$^+$, MS^2 at *m/z* {179,151}][33,37], isorhamnetin [MS^1 at *m/z* 317{M+H}$^+$, MS^2 at *m/z* 302, 243, 151}][38] and kaempferol [MS^1 at *m/z* 287{M+H}$^+$, MS^2 at *m/z* {259, 241, 213, 165, 153, 121}][33] respectively. While the other four peaks **23**, **24**, **27** and **30** were identified as flavones including luteolin [MS^1 at *m/z* 287 {M+H}$^+$, MS^2 at *m/z* {259, 241, 179,161, 153, 136}][31,33, 39], diosmetin [MS^1 at *m/z* 301 {M+H}$^+$, MS^2 at *m/z* {181,153}][31], apigenin [MS^1 at *m/z* 271 {M+H}$^+$, MS^2 at *m/z* {243, 225, 197, 163, 121 }][31,39] and 7 or 5-hydroxyflavone [MS^1 at *m/z* 239 {M+H}$^+$, MS^2 at *m/z* {163 (M-ring B), 121, 105}][40] respectively. To our knowledge, luteolin and kaempferol were isolated as pure compounds in this study and for the first time from *L. shawii*. Luteolin, kaempferol, apigenin, and quercetin were previously detected in *Lycium barbarum* and *Lycium chinense*.[35,36]

1.2.4. Identification of methoxylated flavonoids

Genera of family solanaceae are characterized by the presence of polymethoxylated flavonoids.[401,42] UPLC-PDA-ESI- MS-MS analysis of the ethyl acetate fraction of *L. shawii* helped us in identification of four methoxylated flavonoids including peak **25** which was identified as trihydroxy dimethoxy flavone from the protonated molecular ion peak at *m/z* 331 with daughter ions at *m/z* 316 [M+H -CH_3]$^+$, 301 [M+H -2 CH_3]$^+$, 197 and 123.[43,44] While peak number **26** with molecular ion peak at *m/z* 359 [M+H]$^+$ and product ion peak at *m/z* 344 [M-CH_3]$^+$ and *m/z* 285 [M+H-15-60]$^+$ which showed the loss of methoxy groups so peak number **26** was assigned as tetramethoxy monhydroxy flavone.[43, 44] In addition peak **31** with pseudomolecular ion peak at *m/z* 299 [M+H]$^+$ was identified as apigenin 5, 7-dimethyl ether from MS^2 fragment ions at *m/z* 284 [M-CH_3]$^+$, 281 [M-H_2O]$^+$, 123 and the important fragment at *m/z* 181 which confirm the presence of 5 and 7 dimethoxy groups.[43,44] Trimethoxyflavone was identified from the peak **32** with deprotonated molecular ion at *m/z* 311 and fragments ions at *m/z* 296 [M-H-Me]$^+$, 281 [M-H-2Me]$^+$ and by comparison with literature[43,44]

1.2.5. Identification of other phenolic compounds

Peak **7** displayed a $[M-H]^+$ or $[M+H]^+$ at *m*/z 305 and 307 respectively and showed MS^2 ions at *m/z* 261, 221, 219, 179, 167, 165 which is similar pattern to reported fragmentation of epigallocatechin[37,45,46], while peak **15** was identified as epigallocatechin-methyl gallate according to the mass spectrum at *m/z* 471 $[M-H]^+$ with a distinctive MS^2 fragment at *m/z* 305 which indicated the loss of methylgalloyl moiety $[M-166-H]^+$. Other MS^2 fragment at *m/z* 185, 168, 139 so it is consistent with epigallocatechin methyl gallate.[37,45,46]

1.2.6. Unidentified compounds

Finally peaks **6**, **8**, **28** and **29** with pseudomolecular ion peaks at *m/z* 595 $[M+H]^+$, 667 $[M+H]^+$, 413 $[M+H]^+$ and 339 $[M-H]^+$ and MS^2 ions at *m/z* [544, 519, 396, 339 (100%), 109], [620, 562, 331, 260], [395, 332, 323, 294, 251] and [251, 264] respectively could not be identified.

Table (1): Phenolic metabolites of ethyl acetate extract of *L. shawii* using UPLC-DAD/ESI/MS/MS in positive and /or negative ionization modes

Peak No.	R_T (min)	Name of compound	Molecular weight	Observed *m/z* [M+H] /[M-H]	Major fragments *m/z*	Ref
1	0.223	Chlorogenic acid	354	-/353	191, 179	[33, 34]
2	0.388	*Vanillic acid	168	-/167	152, 123, 108	[34]
3	0.455	Caffeic acid	180	-/179	135 [M-H-CO_2]	[33, 34]
4	0.477	*Rutin	610	-/609	463, 301, 255, 179, 163, 151	[33]
5	0.773	*P*-coumaric acid	164	-/163	119 [M-H-CO_2]	[33, 34]
6	2.309	Unknown	594	595/-	544, 519, 396, 339 (100%), 109	
7	5.311	Epigallocatechin	306	307/305	261, 221, 219, 179, 167, 165	[37, 45, 46]
8	5.574	Unknown	666	667/-	620, 562, 331, 260	
9	7.735	*Diosmetin-7-O-diglucoside	624	625/-	463, 301	[31]
10	7.44	Syringic acid	198	-/197	182, 167	[33, 34]
11	7.445	Kaempferol-3-O-glucoside	448	449/447	285, 255, 179, 151	[31]
12	7.640	Luteolin-7-O-glucoside	448	-/447	285 [[M-H-162]	[31]
13	7.788	Quercetin-3-O rhamnoside	448	449/447	303/301[M+H-146] 179, 151	[31]
14	7.888	Apigenin-7-O-glucoside	432	-/431	269 [M-H-162]	[31]
15	8.011	Epigallocatechin methyl gallate		-/471	305 [M-MG-H], 185, 168, 139	[37, 45, 46]
16	8.705	Kaempferol-3-O-rhamnoside	430	-/431	285 [M-H-rha]	[31]
17	8.953	Diosmetin-7-O-arabinoglucoside	594	595/-	301, (100%), 254, 132	[31]
18	9.545	Diosmetin-7-O-glucoside	462	463/-	301 (100%), 255, 243	[31]
19	9.257	Acacetin-7-O-arabinoglucoside	578	579/-	285 {M+H-arab-glu), 179	[31]
20	9.809	Quercetin	302	-/301	179, 151	[33, 37]
21	10. 111	Isorhamnetin	316	317/-	271, 243, 168, 135	[38]
22	10.352	*Kaempferol	286	387/285	258, 213, 165, 137, 153, 121	[33]
23	10.500	*Luteolin	286	287/285	258, 241, 179, 151, 136, 121	[31, 33, 39]
24	11.100	Diosmetin	300	301/	282, 252, 211, 163, 133, 121, 105	[31]
25	11.215	Trihydroxy-dimethoxy flavone	332	-/331	316 (M -CH_3), 301 (M+H -2 CH_3), 197 and 123.	[43, 44]
26	14.009	Tetramethoxy monohydroxy flavone	358	359/-	344 (M-CH_3), 285 (M+H-CH_3-2OCH_3),	[43, 44]
27	15.490	*Apigenin	270	271/-	269, 255, 238, 162, 121	[31, 39]
28	16.183	Unknown	412	413/-	395, 332, 323, 294, 251, 239	
29	16.400	Unknown	340	339/-	251, 264	
30	16.493	5 or 7 Hydroxyflavone	238	239/-	137, 163 [M-ring B]	[40]
31	16.588	Apigenin-5, 7 dimethyl ether	298	299/-	284 (M-CH_3), 281 (M-H_2O), 181, 123	[43, 44]
32	16.917	Trimethoxy flavone	312	-/311	296 (M-H-Me), 281 (M-H-2Me),	[43, 44]

* Compounds isolated in this work

2. Biological results

2.1. Cytotoxic effect

The cytotoxic effect of petroleum ether, chloroform, ethyl acetate and butanol fractions of the aerial parts of *L. shawii* and the IC_{50} were investigated against A-549 (lung carcinoma), HepG-2 (liver carcinoma), and MCF-7 (breast cancer) cell lines using Sulfo-rhodamine B-assay The results, table 2 and figure 3, showed that the petroleum ether fraction had potent cytotoxic activity against all tested cell lines with IC_{50} 5.01, 7.12, and 9.03 µg/ml respectively followed by butanol extract with IC_{50} 7.3, 9.13, and 12.1 µg/ml respectively. The lowest

activity was observed against MCF-7 (breast cancer) and was exerted by the ethyl acetate fraction with IC_{50} 32.4 µg/ml. To our knowledge, it is the first time to evaluate the cytotoxic activity of different fractions obtained from the ethanolic extract of the aerial parts of *L. Shawii* growing in Saudi Arabia against A-549 (lung carcinoma), HepG-2 (liver carcinoma) and MCF-7 (breast cancer) cell lines.

2.2. Antioxidant Assay

The antioxidant activity of different fractions was determined *by* the DPPH free radical scavenging assay in triplicate and the average values were considered. As shown in Figures 4 and 5. The scavenging effects of petroleum ether, chloroform, ethyl acetate and butanol fractions of the aerial parts of *L. shawii* on DPPH radical were in the following order chloroform fraction > ethyl acetate fraction > butanol fraction, where the chloroform and ethyl acetate fraction exhibited antioxidant activity stronger than that exerted by ascorbic acid which was used as a positive standard. The Antioxidant activity of *L. shawii* extract may be due to the presence of phenolic and flavonoidal compounds.[47]

2.3. Antiviral activity

Hepatitis A virus infections are often asymptomatic, but result occasionally in jaundice, fever, fatigue and malaise. It is estimated that about 1.5 million clinical cases of hepatitis A occur annually.[48] This results in mortality rates up to 5.4% in old aged over 50.[49] Superinfections with HAV in patients chronically infected with hepatitis B or C are believed to increase morbidity and mortality.[50-52] And as there is an increasing need for substances with antiviral activity. So, the antiviral activity of petroleum ether, chloroform, ethyl acetate and butanol fractions of aerial parts of *L. shawii* were evaluated for the first time against hepatitis A virus (HAV-10) and our results showed that the butanol fraction exhibit strong antiviral activity followed by ethyl acetate fraction which showed moderate activity then petroleum ether fraction with weak activity. In this assay the chloroform fraction showed no activity, and the results were reported in table 3.

2.4. Antimicrobial activity

The antimicrobial activity of the petroleum ether, chloroform, ethyl acetate and butanol fractions of the aerial parts of *L. shawii* against fungi, *Asperagillus fumigates* (RCMB 02568), *Syncephalastrum racemosum* (RCMB 05922), *Geotricum candidum* (RCMB 05097), *Candida albicans* (RCMB 05036); Gram positive bacteria, *Streptococcus pneumonia* (RCMB 010010), *Bacillus subtillis* (RCMB 010067) and Gram negative bacteria *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052) was evaluated using agar diffusion technique. The chloroform, ethyl acetate and butanol fractions were effective against *Asperagillus fumigates, Syncephalastrum racemosum, Geotricum candidum, Streptococcus pneumonia, Bacillus subtillis* and *Escherichia coli* bacteria with significant zone of inhibition, but lacking activity against *Candida albicans* and *Pseudomonas aeruginosa*, the best antibacterial effect was obtained on *Escherichia coli* (20.6 mm) which is better than that of the standard antibiotic Gentamicin (19.9) and was exerted by the chloroform extract, while petroleum ether fraction showed no activity against all tested microorganisms and all the results are summarized in Table 4**.**

Table (2): The cytotoxic effect of petroleum ether, chloroform, ethyl acetate and butanol fractions of the aerial parts of *L. shawii* against A-549, HepG-2 and MCF-7 cell lines at different concentrations

Sample conc. (µg/ml)	% of Viability											
	A-549 cell line				MCF-7 cell line				HepG-2 cell line			
	P	C	E	B	P	C	E	B	P	C	E	B
0	100	100	100	100	100	100	100	100	100	100	100	100
1.56	80.54	96.73	97.04	91.42	89.46	95.82	99.08	96.57	86.38	93.31	92.72	95.79
3.125	68.92	89.21	90.62	78.93	81.52	88.29	97.26	89.22	76.24	87.67	80.95	83.48
6.25	37.58	78.58	78.31	52.96	63.74	79.45	91.43	74.16	54.19	72.34	68.43	69.52
12.5	24.97	56.82	67.85	35.41	32.82	64.76	68.97	48.51	23.96	51.82	46.84	27.13
25	13.59	29.34	48.91	20.67	19.88	38.54	58.24	30.97	15.08	30.98	31.22	18.64
50	4.82	13.89	26.54	9.25	7.97	19.73	30.49	11.82	6.43	15.27	14.61	8.56

***P,** petroleum ether fraction**; C,** Chloroform fraction**; E,** Ethyl acetate fraction**; B,** butanol fraction

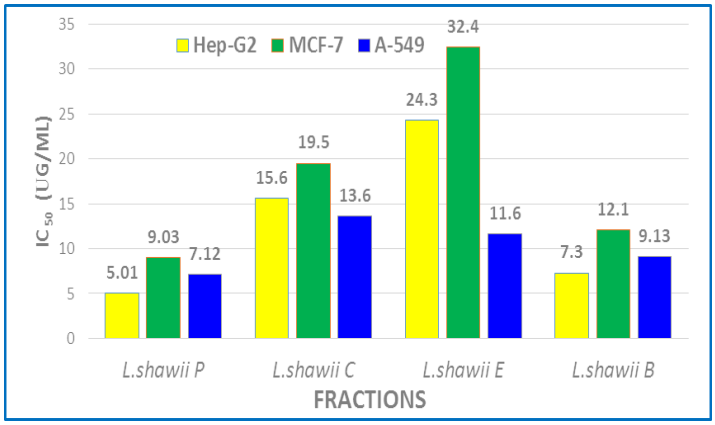

Figure (3): IC_{50} in µg/ml of cytotoxic effect of Petroleum ether (P), Chloroform (C), Ethyl acetate (E) and Butanol (B) fractions of the aerial parts of *L. shawii* against A-549, HepG-2 and MCF-7 cell lines.

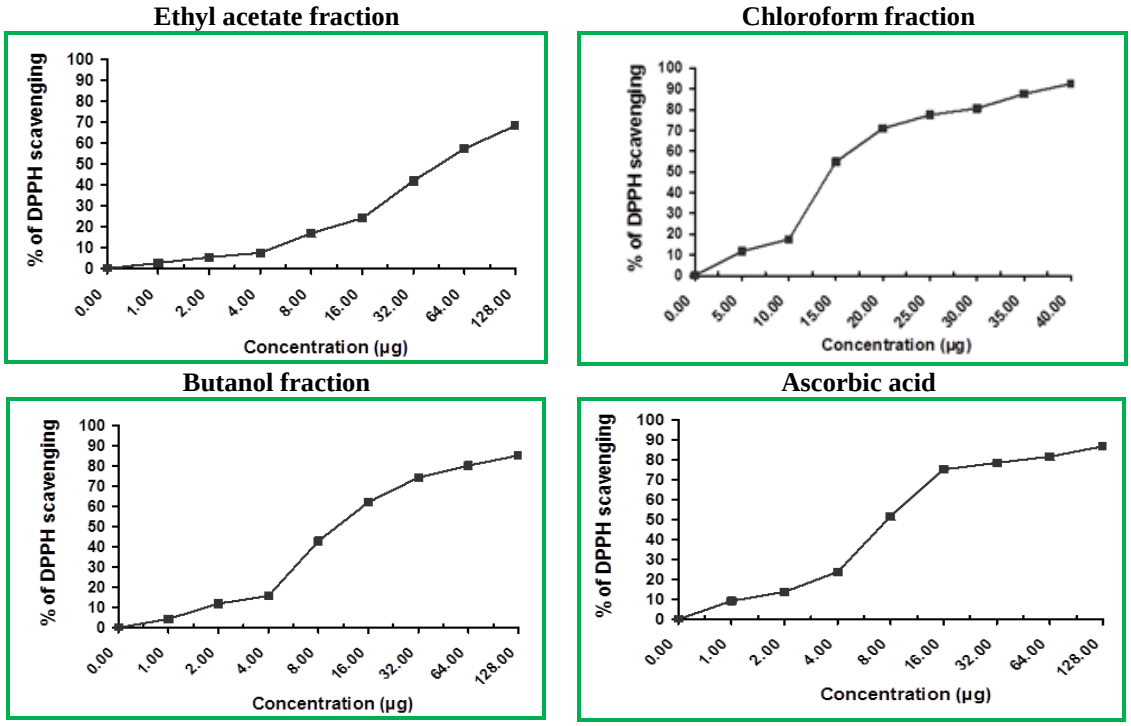

Figure (4): Antioxidant effects of different fractions of aerial parts of *L. shawii* on DPPH free radicals

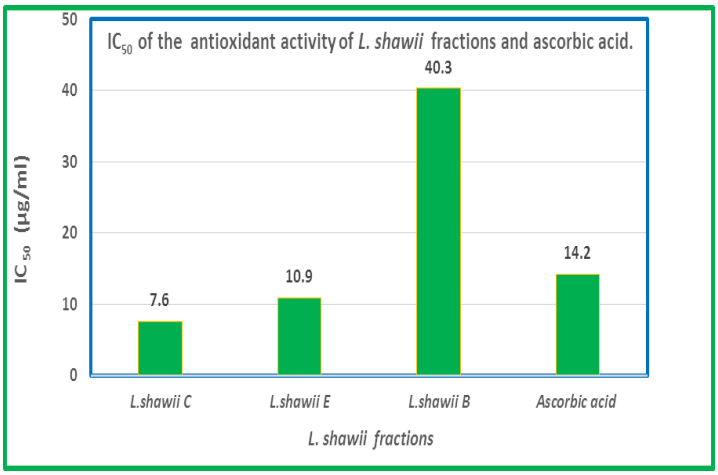

Figure (5): IC50 in µg/ml of antioxidant activity of chloroform (C), ethyl acetate (E), butanol (B) fractions of the aerial parts of L. shawii and ascorbic acid using DPPH free radicals

Table (3): Antiviral activities of petroleum ether, chloroform, ethyl acetate and butanol fractions of the aerial parts of *L. shawii* on HAV-10 virus (tested at 200 µg/ml).

Extract	Antiviral effect on HAV-10 virus
Ethyl acetate fraction	++
Petroleum ether fraction	+
Butanol fraction	+++
Chloroform fraction	- ve
+++: Strong ++: Moderate +: Weak -ve: No antiviral activity was detected	

Table (4): Antimicrobial activity[Diameters (mean ± SD) of inhibition zones] of petroleum ether, chloroform, ethyl acetate and butanol fractions of the aerial parts of *L. shawii*.

Sample tested Microorganism	**Ethyl acetate fraction**	**Pet ether fraction**	**Butanol fraction**	**Chloroform fraction**	**Standard**
Fungi:	**Inhibition zone diameters (mm)**				
					Amphotricin B
Asperagillus fumigates	16.3 ± 0.63	NA	14.6 ± 0.72	18.8 ± 0.58	23.7 ± 0.1
Syncephalastrum racemosum	14.6 ±1.2	NA	13.2 ± 0.72	16.3 ± 0.63	19.7 ± 0.2
Geotricum candidum	19.6 ± 0.63	NA	17.5 ± 0.38	22.6 ± 0.72	28.7 ± 0.2
Candida albicans	NA	NA	NA	NA	25.4 ± 0.1
Gram positive bacteria:					**Ampicillin**
Streptococcus pneumonia	20.3 ± 1.2	NA	16. ± 0.63	21.8 ± 0.44	23.8 ± 0.2
Bacillus subtillis	21.4 ± 0.58	NA	18.2 ± 1.2	22.6 ± 0.58	32.4 ± 0.3
Gram negative bacteria:					**Gentamicin**
Pseudomonas aeruginosa	NA	NA	NA	NA	17.3 ± 0.1
Escherichia coli	18.7 ± 0.72	NA	13.6 ± 0.58	20.6 ± 0.58	19.9 ± 0.3

*__NA__= no activity; Extract and used standards were tested at a dose of 50µg; Values are mean of triplicate readings (mean ± S.D).

CONCLUSION

The overall results of this study indicate that, vanillic acid (**1**), luteolin (**2**), kaempferol (**3**), Diosmetin- 7- *O*- *β* – D – glucopyranosyl - (1'''→ 6'') – *β* – D – glucopyranoside (**4**), rutin (**5**), *β*-sitosterol (**6**) and *β*-amyrin (**7**) have been isolated from the ethyl acetate and petroleum ether fractions of *L. shawii*, compounds **1**-**4** and **7** were isolated for the first time from *L. shawii*. UPLC/MS/MS analysis of ethyl acetate fraction led to the identification of **28** phenolic compounds from **32** detected ones. The high contents of phenolic compounds in the extracts, may contribute to the high antioxidant activities of this plant. The strong antiviral effect of butanol and ethyl acetate fractions is in a good agreement with the current literatures of hepatoprotective effect of some Lycium species as *Lycium chinense* and this may be due to the presence of vanillic acid which reported to suppress hepatic fibrosis in chronic liver injury. In addition, the cytotoxicity assay indicated the potential of this medicinal plant as a source of anticancer therapeutic compounds and this need further investigation.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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