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Seasonal Variations and Identification of Biologically Active Constituents of *Lycium Shawii* Plant Roem. & Shult. (Family Solanaceae)

Fatma A. Ahmed [1], Nadia M. Abdallah[2], Magda Kamal Ezz[2] and Mayada M. El-Azab[1]

[1]Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt. [2]Biochemistry Department, Faculty of Science, Ain Shams University.

ABSTRACT

The high percentage of water content of *Lycium shawii Roem. & Shult.* leaves and stem observed in winter season affected the metabolic processes in plant. The percentage of total carbohydrates of *L. shawii* plant reached its maximum values in summer decreasing gradually in spring to reach the lowest percentage leaves in autumn. Investigation of the free sugars showed that, plant leaves contained arabinose, ribose, fructose, galactose, glucose and sucrose, while plant stem was deficient in ribose and sucrose. The highest amount of total nitrogen and protein content was recorded in winter. Amino acid analyzer showed that, *L. shawii* plant contains 16 and 15 free amino acids, in addition to 15 and 16 amino acids in protein hydrolysates in leaves and stem, respectively. GLC analysis revealed that, *L. shawii* plant contains 10 fatty acids: 8 saturated and 2 unsaturated as well as 10 hydrocarbons beside three sterols for both leaves & stem. The percentage of total flavonoids, tannins, saponins and alkaloids increased in plant leaves than stem. Chromatographic methods revealed the separation of nine biologically active constituents from *L. shawii leaves*. Identification of the chemical composition as well as the physico-chemical properties of the nine active substances was carried out using ultraviolet, ^{1}H-NMR, ^{13}C-NMR spectral data and mass spectroscopy, the separated compounds are; 2, 3 dihydroxy benzoic acid**,** quercetin, gallic acid**,** rutin, *p*-coumaric acid, ferulic acid, quercetin 3-methoxy glucoside, quercetin 3, 7 diglucoside and quercetin 3-O-*β*- glucoside.

Keywords:*Lycium shawii*, chemical composition, flavonoids, phenolic acids.

1. INTRODUCTION

The Egyptian deserts are very rich in medicinal plants belonging to many families. Solanaceae (Nightshade or Potato family) resembles one of the largest families in the plant kingdom that is rich in medicinal plants. The family consists of approximately 98 genera and some 2,700 species **(Olmstead & Bohs, 2007).** Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The chemical constituents present in the plant play a significant role in the identification of crude drugs **(Akindele & Adeyemi, 2007).** Medicinal plants play a major role in the treatment of human diseases and

have various effects on living systems. Family Solanaceae is one of the largest family in plant kingdom including cultivated, wild plants **(Boulos, 2002)**, *Solanum elaeagnifolium* belonging to this family growing in Egypt contain quercetin-3-O-*β*-Dglucopyranoside and kaempferol **(Badawy *et al.*, 2013)** their chemical structures were elucidated using spectroscopic techniques. **Chung *et al*., 2013** isolated two new glycosidic constituents from fruits of *Lycium chinense*. So it is of interest to choose *Lycium shawii* Roem. & Shult as an herbal plant belongs to this family. *L. shawii* is a thorny perennial shrub growing in Al-Arish. The aim of the study is identification of sugars, amino acids, fatty acids and biologically active constituents of the plant.

2. MATERIALS AND METHODS

2.1. Plant Materials

The fresh leaves & stem of *Lycium shawii Roem. & Shult.* were collected seasonally from Al- Arish habitat during the period of investigation 2012.

2.2. Methods

2.2.1. Eco-physiological study including determination of the percentage of plant water content for leaves & stem of *L. shawii* **(Rowell, 1994)**. Determination of certain pharmacopoeial constants of plant materials, including inorganic (ash) and organic matter **(Brower and Zar, 1984)**, acid-soluble and acid-insoluble ash, water-soluble and water-insoluble ash **(Askar and Treptow, 1993)** and crude fibers (**British pharmacopoeia, 1980).**

2.2.2. Investigation of metabolic products including determination of total carbohydrates, soluble and insoluble carbohydrates **(Chaplin & Kennedy, 1994)**, free and combined sugars were investigated using paper chromatography on Whatmann No. 1 paper chromatography by the descending technique using the solvent system [n-butanol- acetic acid- water (4:1:5)] alongside with authentic sugars and spraying reagent aniline-hydrogen phthalate **(Stahl, 1969)**.

2.2.3. Total nitrogen and protein contentin leaves & stem of *L. shawii* was determined using Kjeldahl method **(James, 1995).** Free amino acids and protein-amino acids were accomplished according to **Pellet and Young, (1980)** using Amino Acid Analyzer (Beakman system 7300 High Performance analyzer).

2.2.4. Total lipids content, unsaponifiable and saponifiable matter of leaves & stem of *L. shawii* were determined using Gas Liquid chromatography (GLC) GCV Pye-Unicam **(Farag *et al.*, 1986).**

2.2.5. Phytochemical study including preliminary phytochemical screening, including steam distillation of volatile oils **(Balbaa, *et al.*, 1981),** test for carbohydrates and/or glycosides, and resins **(Balbaa, 1986)**, saponins **(Hostettmann, *et al.*, 1991)**, tannins **(Trease, 1966),** flavonoids **(Geissmann, 1962),** sterols and terpenes **(Fieser and Fieser, 1959)** and test for alkaloids **(Woo *et al.*, 1977)**.

2.2.6. Investigation of total active materials, including estimation of total flavonoids of leaves & stem of *L. shawii* in four seasons were determined spectrophotometrically and calculated as quercetin **(Karawaya & Aboutable, 1982)**. Estimation of total tannins using gravimetric method (Copper acetate method) according to **(Ali, *et al.*, 1991),** estimation of total saponinsa ccording to **Okwu & Ukanwa (2007)** and estimation of total alkaloids (Gravimetric method) were carried according to the meth described by **(Woo, *et al.*, 1977)**.

2.2.7. Investigation of flavonoids and phenolic acids of of *L. shawii* leaves were done using chromatographic investigation using paper chromatography, column chromatography **(Markham, 1982)** of polyamide column **(Markham & Mabry, 1975**) and SephadexLH-20 (Pharmacia) column chromatography **(Liu, *et al.*, 1989).**Identification techniques of flavonoids by chemical analysis **(Harborne, 1984).** Complete elucidation of the flavonoids include physical methods: ultraviolet (UV) **(Markham, 1982)**, nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) measurements using a Jeol Ex-500 spectroscopy; 500MHz (^{1}NMR), 125 MHz (^{13}C-NMR) or Joel JNM-EX 270 spectroscopy; 270 MHz (^{1}H-NMR), 67.5 MHz (^{13}C-NMR) **(Shen, *et al.*, 1993)**. Mass Spectrometry **(Andersen** & **Markham, 2006)** using EI-MS, model Finnigan-Mat SSQ 7000 spectrometer and ESI-MS: LCQ Advantage Thermo Finnigan spectrometers were used.

3. RESULTS AND DISCUSSION

3.1. Seasonal variations in *Lycium shawii Roem. & Shult.* leaves and stem contents are summarized in table (1). Results indicated that, the percentage of water content, total nitrogen, total protein and total lipid reached maximum values in winter and minimum values in summer for both leaves and stem due to, water supplement in winter (rainfall), which lead to normal plant growth. The percentage of inorganic matter (ash), acid soluble ash, acid insoluble ash, water soluble ash, water insoluble ash and crude fibers reached maximum values in summer and minimum values in winter for both leaves and stem. This may be due to the increase of total ion accumulation as a result of increasing soil moisture stress and soil salinity, which agreed with the results obtained by **Larcher, (1995)** & **Smith, (2009)**.

The percentage of (total and soluble) carbohydrates reached maximum values in summer and minimum values in autumn for both leaves and stem. Meanwhile**,** the percentage of insoluble carbohydrates reached its maximum values in autumn for leaves and stem; this is due to increasing in soil moisture stress at studied habitat. These results are in agreement with those obtained by **El – Monayeri, *et al.*, (1981).**

The highest amount of total nitrogen and protein content was recorded in winter which may be due to the increase in metabolic rate of the studied plant as a result of high water resources of the soil during winter than during that of dry period in summer season which is in agreement with **Stocker's (1960)** who reported that, the metabolic rate increased in the presence of high water resources, total N_2 and protein contents of *L. shawii* decreased during the ripping month (summer) may be attributed to the decrease in the water content**,** which was found to be linked with an accumulation of some amino acids (e**.**g**.** proline), this may play an important role in increasing cell osmoregulation **(Ali, *et al.*, 1992).**

In the present study, the percentage of total lipids reached their maximum values in plant leaves and stem during winter, which decreased directly in spring & autumn samples giving its minimum values in summer in plant leaves and stem, this may be due to the increase in carbohydrate concentration which is converted to lipids by oxidation reaction. This result is in agreement with **Meyer and Anderson, (1952).**

3.2. Free and combined sugars in leaves and stem of *L. shawii* are illustrated in table (2). Chromatogram of free sugars in leaves contained arabinose, ribose, fructose, galactose, glucose and sucrose, while plant stem was deficient in ribose and sucrose. The hydrolysable combined sugars leaf extract revealed the presence of arabinose**,** ribose, xylose, fructose, galactose and glucose. Meanwhile plant stem contained arabinose, fructose, galactose and glucose.

Figure (1) revealed that, *L. shawii* Roem. & Shult. plant contains 16 and 15 free amino acids in leaves and stem, respectively. The exception was absence of methionine in the plant stem**.** The highest concentration in the leaves was proline and histidine in stem. Plant leaves and stem protein hydrolysates gave 15 and 16 amino acids in leaves and stem, respectively. The exception was absence of valine in the plant leaves**.** The highest content of the protein amino acids in plant leaves was argenine and in plant stem was histidine.

3.3. Determination of fatty acids contents of plant leaves and stem were determined using GLC (Table 3), revealed the existence of 8 saturated fatty acids beside four unsaturated fatty acids for both leave and stem. Table (4) showed that, there were 10 hydrocarbons beside three sterols for both leaves and stem. The highest concentrations of the hydrocarbon were hexacosane (10.65%) in leavesand nonacosane (26.31%) in the stem**.** On the otherhand**,** the lowest concentrations of the hydrocarbon were nonadecane for both leaves and stem. Meanwhile, the highest concentration of the sterol was cholesterol for both leaves and stem (12.31 & 10.12 %), respectively and the lowest concentration was β-sitosterol for both leaves and stem (7.80 & 4.54 %), respectively.

3.4. Rajan*, et al.*, (2011) reported that phytochemical evaluation was performed for qualitative detection of various chemical constituents which aid in tracing the presence of active entity that elicit a major pharmacological response. The literature collection, pertaining to this investigation indicates that, flavonoids and other plant phenolics are reported to have multiple biological activities in addition to their antioxidants or free radical terminators activity **(Bendini*, et al.*, 2006)**. Therefore, it is worthwhile to determine their total amount in the selected plant for the present study. The preliminary phytochemical screening for *L. shawii* (Table 5) showed the presence of flavonoids, phenolic compounds, alkaloids, saponins and tannins. **Kayani *et al.*, (2007)** recorded that, these secondary metabolites are chemicals produced by means of secondary reactions resulting from primary carbohydrates, amino acids and lipids. Total active compounds in leaves of *L. shawii* plant are shown in table (6) which revealed the percentage of total flavonoids increased gradually from autumn, summer, and winter to spring in leaves and stem. This quantitative variation of the flavonoids must be derived from induction by environmental factors to which the plant is subjected. In plants, flavonoids and phenolics appear to have diverse functions, including functioning as antioxidants, superoxide radical scavengers, chelators mediating mineral uptake, enzyme inhibitors and regulators, redox dispersal mechanisms by insects and animals.

Table (1): Seasonal variations in *Lycium shawii* leaves and stem contents

Item (%)	Leaves				Stem			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Water content	21.45	17.31	14.17	20.00	17.25	15.30	13.05	16.12
Total ash	18.51	20.25	24.85	22.67	13.10	13.19	14.49	14.16
Organic matter	81.49	79.75	75.15	77.33	86.90	86.81	85.51	85.84
Acid soluble ash	10.25	11.31	14.26	12.82	11.09	11.14	12.01	11.82
Acid insoluble ash	8.26	8.93	10.59	9.85	2.01	2.05	2.48	2.33
Water soluble ash	5.93	6.53	8.36	7.82	11.11	11.18	11.66	11.44
Water insoluble ash	12.58	13.72	16.49	14.85	1.99	2.01	2.83	2.72
Crude fibers	12.90	13.23	15.23	14.01	29.03	29.96	32.50	30.32
Total carbohydrate	33.06	33.06	34.33	29.12	27.62	29.64	30.18	28.51
Soluble carbohydrate	15.67	13.91	16.21	9.33	11.05	12.81	14.37	9.21
Insoluble carbohydrates	17.39	19.15	18.12	19.79	16.57	16.83	15.81	19.30
Total nitrogen	2.32	2.11	1.50	1.83	1.84	1.76	1.46	1.62
Total protein	14.50	13.19	9.38	11.44	11.50	11.00	9.13	10.13
Total lipids	3.43	2.97	2.66	2.78	1.50	0.91	0.65	0.78

Table (2): Free and combined sugars in leaves & stem of *L. shawii* plant

Sugars	Free sugars		Combined sugars	
	Leaves	Stem	Leaves	Stem
Arabinose	+ ve	+ ve	+ ve	+ ve
Ribose	+ ve	- ve	+ ve	- ve
Xylose	- ve	- ve	+ ve	- ve
Fructose	+ ve	+ ve	+ ve	+ ve
Galactose	+ ve	+ ve	+ ve	+ ve
Glucose	+ ve	+ ve	+ ve	+ ve
Sucrose	+ ve	-ve	-ve	-ve

+ ve= positive results -ve = negative results

Table (3): Fatty acids in leaves & stem of *L. shawii* plant using GLC.

Number of carbon atoms	Systemic name	Trivial name	RT	Relative conc. (g %)	
				Leaves	Stem
C:10	Decanoic	Capric	9.12	7.21	19.41
C:11	Henedecanoic	Undecylic	10.25	5.99	16.50

C:12	Dodecanoic	Lauric	11.92	-	1.30
C:13	Tridecanoic	Tridecylic	12.65	9.92	12.21
C:14	Tetradecanoic	Myristic	14.65	22.19	17.09
C:15	Pentadecanoic	Pentadecylic	16.03	1.51	-
C:16	Hexadecanoic	Palmitic	17.18	16.12	13.29
C:17	Heptadecanoic	Margaric	20.18	7.74	12.97
C18:0	Octadecanoic	Stearic	21.12	12.73	3.21
C18:1	Cis-9-Octadecanoic	Oleic	21.73	11.91	-
C18:2	Cis, cis-9, 12-Octadecanoic	Linoleic	23.17	-	2.11
C18:3	Cis-9, 12, 15-Octadecatrienoic	Linolenic	25.23	4.69	1.91

T: retention time/minute.

3.5. The percentage of total tannins reached maximum values in summer and minimum values in winter. The percentage of total saponins reached maximum values in summer coinciding with drought and high rate of evaporation and minimum values in spring. **Inderjit and Foy, (1999)** reported that, differential synthesis and/or accumulation of saponins and their aglycones are observed in different plant tissues and organs depending upon species or genotype, age and environmental conditions The percentage of total alkaloids reached to highest values in winter (2.58 & 1.86 %) and lowest values in summer (1.51 & 0.44 %) for both leaves and stem, respectively.

Table (4): Hydrocarbons and sterols in leaves & stem of *L. shawii* plant using GLC

Number of carbon atoms	Name	RT	Relative conc. (g %)	
			Leaves	Stem
Hydrocarbons				
C:12	n-Dodecane	7.25	-	-
C:14	n-Tetradecane	7.91	-	-
C:16	n-Hexadecane	8.22	6.99	6.08
C:17	n-Heptadecane	9.10	-	-
C:18	n-Octadecane	10.83	-	-
C:19	n-Nonadecane	11.27	3.20	1.39
C:20	n-Eicosane	13.42	7.31	8.62
C:21	n-Heneicosane	14.33	5.21	4.98
C:22	n-Docosane	15.20	7.00	8.50
C:23	n-Tricosane	15.95	-	-

C:24	n-Tetracosane	16.42	6.57	5.31
C:25	n-Pentacosane	17.01	-	-
C:26	n-Hexacosane	17.62	10.65	7.30
C:27	n-Heptacosane	18.48	-	2.06
C:28	n-Octacosane	20.00	5.00	-
C:29	n-Nonacosane	20.73	7.41	26.31
C:30	n-Triacontane	21.25	9.75	8.31
Sterols				
C:27	Cholesterol	25.98	12.31	10.12
C:27	Stigmasterol	27.23	10.78	6.47
C:29	β-sitosterol	30.75	7.80	4.54

RT: retention time/minute.

Table (5): Total active compounds in leaves & stem of *L. shawii* plant

Item (%)	**Leaves**				**Stem**			
	Winter	**Sprin g**	**Summer**	**Autumn**	**Winter**	**Sprin g**	**Summer**	**Autumn**
Total flavonoids	2.02	2.19	1.53	1.28	0.50	0.61	0.36	0.11
Total alkaloids	2.58	2.33	1.51	1.89	1.86	1.38	0.44	0.93
Total saponins	7.06	5.15	9.68	8.61	5.36	4.13	7.30	6.10
Total tannins	3.14	3.31	3.44	3.34	3.05	3.13	3.26	3.21

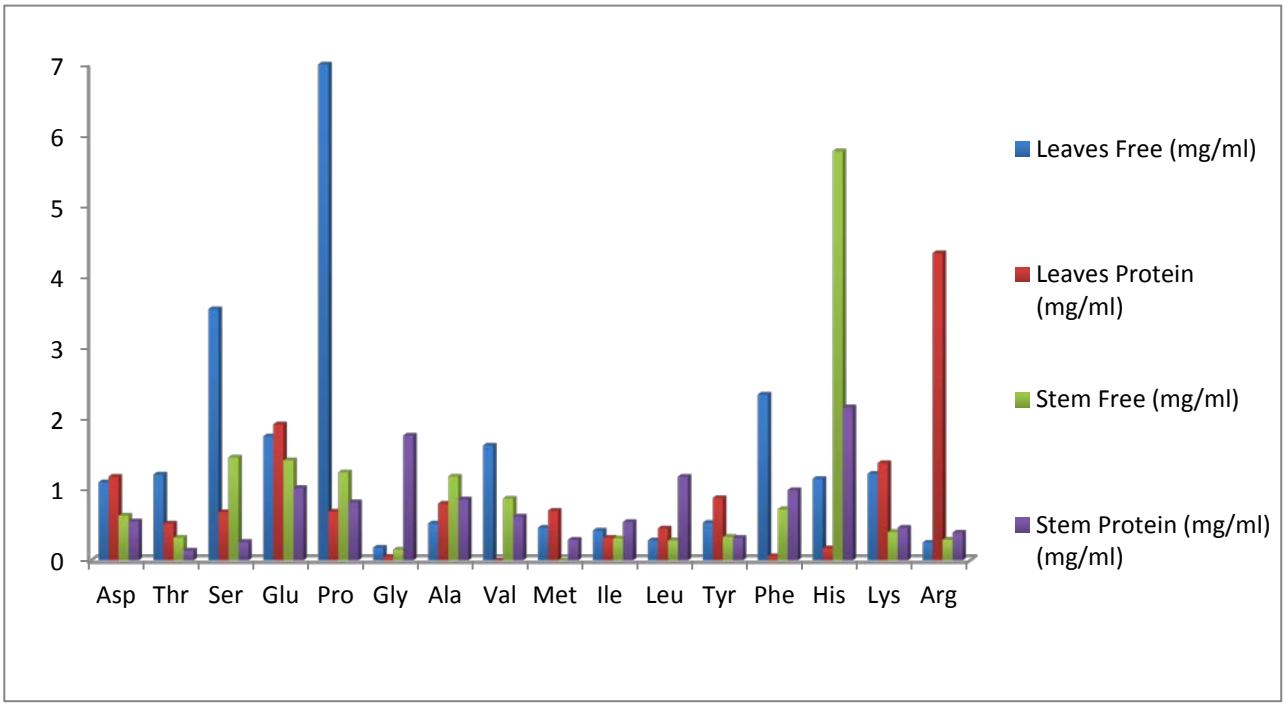


Fig (1): Free and protein amino acids in leaves & stem of *L. shawii* plant.

Separation of flavonoids and phenolic acids

Nine compounds were separated from the leaves of *L. shawii* plant (Table 6). The plant leaves powder was extracted with 70% methanol to produce crude extract, and then fractionated by using different solvents from low polarity to high polarity, starting with diethyl ether and ending with water to produce 5 fractions. Diethyl ether fraction was subjected to preparative paper chromatography (3MM) using system BAW (4:1:5) for 24 hours, given one major band. The band was eluted separately with hot methanol / H_2O (1:1v/v), to isolated (Compound 1). Chloroform fraction was applied in polyamide column (10% MeOH/ H_2O) to isolate (Compound 2). Ethyl acetate fraction, was loaded on polyamide column, to produced three main fractions, first one subjected to preparative paper chromatography (3MM) to isolate (Compound 3 & 4), second fraction placed in a polyamide column (80% MeOH/H_2O) to isolate (Compounds 5) and third fraction placed in a polyamide column (90% MeOH/ H_2O) to isolate (compounds 6 & 7). Methanol fraction was applied in polyamide column (80% MeOH/H_2O) to isolated (Compound 8). Water fraction subjected to preparative paper chromatography (3MM) to isolate (Compound 9).

Identification of isolated flavonoids and phenolic acids

Table (6): Compounds separated from the leaves of *L. shawii* plant.

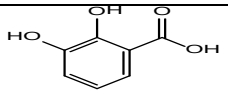
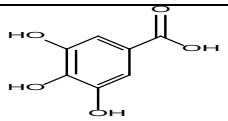
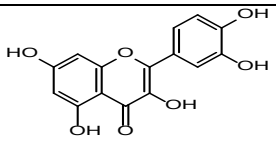
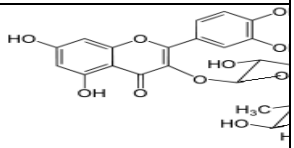
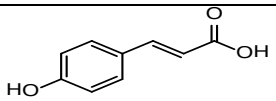
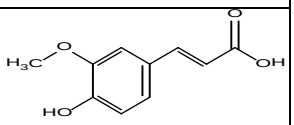
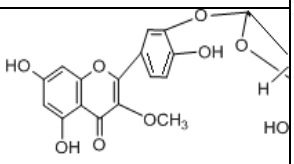
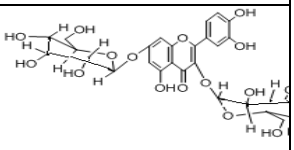
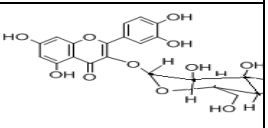
Compound No.	Name	Structure
1	2, 3 dihydroxy benzoic acid	
2	Gallic acid	
3	Quercetin	
4	Rutin	
5	*ρ*-Coumaric acid	
6	Ferulic acid	
7	Quercetin 3-methoxy glucoside	
8	Quercetin 3,7 diglucoside	
9	Quercetin 3-O-*β*- glucoside	

Table (7): UV Spectral data, λ_{max}(nm) of some of the isolated compounds from the leaves of *L. shawii* plant.

Comp. no.	UV Shift reagents					
	MeO	**NaOMe**	**$AlCl_3$**	**$AlCl_3$/ HCl**	**NaOAc**	**NaOAc+ H_3BO_3**
1	285, 308					
2	272, 335	275, 345				
3	258, 267sh, 298sh, 360	274, 324, 409	275, 304sh, 333sh, 429	269, 300sh, 358sh, 403	269, 323, 373	261, 301sh, 379
4	257, 267sh, 295sh, 360	275, 320sh, 415	275, 305sh 335sh, 340	275, 305sh, 345sh, 420	270, 320sh, 380	260, 300sh, 380
6	285, 312	250sh, 290,345	-	-	-	-
7	258, 264sh, 359	272, 327, 402	263, 300sh, 366, 405sh	264, 300sh, 360, 402sh	269, 321, 369	262, 370
8	258, 267sh, 355	270, 308sh, 405	270, 309sh, 436	266, 303sh, 357sh, 406	261, 310sh, 387	263, 306sh, 377
9	256, 267sh, 298sh, 359	272, 326, 408	275, 304sh, 333sh, 429	269, 300sh, 358sh, 403	271, 323, 373	262, 301sh, 379

Table (8): ^{1}H-NMR Spectral data in (δ ppm) of some of the isolated compounds from the leaves of *L. shawii.*

Comp. no.	Signals of ^{1}H-NMR spectrum in DMSO-d_6 δ (ppm)
1	δ 6.8 (t,*J*=7.5 Hz, H-5), δ 7.1 (dd, *J*= 7.5 Hz and *J*=2.5 Hz, H-6) and δ 7.4 (dd, *J*= 7.5 Hz and *J*= 2.5 Hz, H-4).
2	6.98 (s, H-2 and H-6).
3	δ 7.6 (1H, dd, *J*= 8.5, 2.3 Hz, H-2`), δ 7.5 (1H, dd, *J*= 8.5Hz, H-6`), δ

	6.89 (1H, d, *J*= 8.5 Hz, H-5`), δ 6.4 (1H, d, *J*= 2.5 Hz, H-6) and δ 6.2 (1H, d, *J*= 2.5 Hz, H-8).
4	δ 7.6 (1H, d, *J* = 2.5 Hz, H-2), δ 7.5 (1H, dd, *J*=8.5, 2.5 Hz, H-6`), δ 6.8 (1H, d, *J*= 8 Hz, H-5`), δ 6.4 (1H, d, *J*=1.5 Hz, H-8), δ 6.2 (1H, d, *J*= 1.5Hz, H-6), δ 5.3 (1H, d, *J*= 8 Hz, H-1`` glucose), δ 4.5 (1H, d, *J*=2.5 Hz, H-1```rhamnose), δ 3.4 (m, remaning sugar protons) and δ 0.8 (3H, d, *J*=6 Hz, CH_3rhamnose).
6	δ 12.2(broad, s, COOH), δ 9.15 (s, OH), 8.9 (s, OH), δ 7.5 (2H, d, *J*=17Hz, H-2 and H-7), δ 7.15 (1H, d, *J*=2.5Hz, H-2), δ 7.09 (1H, dd, *J*=7.5 Hz and 2.5 Hz, H-6), δ 6.95(1H, d, *J*=7.5Hz, H-2), δ 6.25 (1H, d, *J*=17Hz, H-8) and δ 3.85 (3H, s, OCH_3).
7	δ 8.37(1H, d, *J*=2Hz,H-2`), δ 7.26(1H, d, *J*=7.7Hz, H-6`), δ 6.77 (1H, d, *J*=8.4Hz, H-5`), δ 6.33(1H, s, H-8), δ 6.13(1H, s, H-6), δ 5.11(1H, d, *J*=7.0Hz,H-1``), δ 3.60(3H, s, OCH_3) and δ 3-4 (5H, Multiplet).
8	δ 7.55(2H, dd, *J*=2.5Hz,H-2`, *J*=7.5Hz,H-6`), δ 7.15 (1H, d, *J*=8.5Hz,H-5`), δ 6.80(1H,d, *J*=2Hz, H-8), δ 6.45(1H, d, *J*=2Hz, H-6), δ 5.32(1H, d, *J*=8Hz, H-1``), δ 4.85(1H, d, *J*=8Hz, H-1```) and δ 3-3.8(10H, Multiplet).
9	δ 7.66 (1H,d, *J*=8.5Hz, H-6`), δ 7.55 (1H, d, *J*=2Hz, H-2`), δ 6.80 (1H, d, *J*=8.5Hz, H-5`), δ 6.37 (1H, d,*J*=2Hz,H-8), δ 6.17 (1H, d, *J*=2Hz, H-6), δ5.37 (1H, d, *J*=7.5Hz, H-1``) and δ 3-4 (5H, Multiplet).

Table (9): ^{13}C-NMR spectral data of some of the isolated compounds from the leaves of *L.shawii.*

Comp. no.	**Chemical shifts in δ (ppm)**
1	δ (ppm): 172.83(C=O),121.4(C-1),151.06(C-2),146.63(C-3),119.7(C-4),113.3(C-5),121.4(C-6).
2	δ (ppm): 120.6(C-1);108.8(C-2 and C-6);145.5(C-3 and C-5);138.1(C-4); 167.7(C-7).
3	δ (ppm): 175.7(C-4),163.9(C-7),160.7(C-5),156.2(C-9),146.9(C-4`),147.6(C-2),145.0(C-3`),135.6(C-3),122.0(C-1`),120.0(C-6`),115.6(C-5`),115.3(C-2`),103(C-10),98.2(C-6),93.4(C-8).
4	δ (ppm): 146.9(C-2),135.5(C-3),175.8(C-4),160.7(C-5),98.2(C-6),163.9(C-7),93.3(C-8),156.2(C-9),103.1(C-10),122.1(C-1),115.3(C-2`),145.0(C-3),147.6(C-4`),115.6(C-5`),120.0(C-6`), sugar moiety, 101.5(C-1``),74.3(C-2``),75.9(C-3``),70.2(C-4``),76.2(C-2```),71.0(C-3```),72.2(C-4```),69.1(C-5```),181.0(C-6```).

Table (10): Mass spectrometry data of the isolated compounds from the leaves of *L. shawii.*

Comp. No.	**Ion peak *m/z***
1	*m/z* % : 154 (M^+, 9), 136 (37), 110 (100), 92 (14), 81 (15), 64 (42), 52 (17), 44 (61).
2	Molecular ion peak at 170 *m/z*
3	Base peak [M-H] at 301 *m/z*
4	610 (M^+, glucose), *m/z* 464 (M^+, rhamnose), *m/z* 302 (quercetin).
6	M^+ *m/z* 194, M^+ -15 *m/z* 179, C_6H_6*m/z* 77
7	Base peak [M-H] at 477 *m/z*

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