E- ISSN: 2348-3962, P-ISSN: 2394-5583

Received on 10 November 2017; received in revised form, 13 November 2017; accepted, 18 November 2017; published 01 February 2018

LC/MS IDENTIFICATION OF PHYTOCONSTITUENTS OF THE METHANOLIC EXTRACT OF THE AERIAL PARTS OF ANTIGONON LEPTOPUS (HOOK & ARN)

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Keywords:

Antigonon leptopus Hook and Arn, Vine, Polygonaceae, LC/MS, Flavonoids, Phenolics, Hepatoprotective, Anti-diabetic, Anti-inflammatory

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ABSTRACT: Antigonon leptopus Hook & Arn (family Polygonaceae), also called: Chain of love, Queen's Wreath, and Mexican creeper is a fastgrowing vine with stems reaching up to 20 feet long. It has heart-shaped, green leaves climbing by tendrils which wrap around many types of supports. It is native to Mexico and commonly found in tropical Asia, Africa, the Caribbean, and the Americas. It possesses anticoagulant activity, analgesic, anti-thrombin, anti-inflammatory, anti-diabetic and antidepressant activities. Aerial parts of the vine have been used as hepatoprotective and for spleen disorders. During the present study, LC/MS experiment was conducted on the methanolic extract of the aerial parts of the vine. Results revealed the identification of total fifty compounds; flavonoids, phenolics, anthocyanins, and glycosides. Compounds were characterized by their retention times, mass spectra with comparison to online mass bank database. From the results, Antigonon leptopus can be incorporated in pharmaceutical supplements used to aid free radical-mediated disease; cancer, diabetes, inflammation and liver diseases.

INTRODUCTION: Despite the tremendous progress in the development of new synthetic drugs during the 20th century, plant-based drugs never totally lost their importance in the treatment of several ailments. *Antigonon leptopus*, is one of the species of belonging to the buckwheat family, Polygonaceae. It is native to Mexico, India and commonly found in tropical Asia, Africa, the Caribbean, and the Americas. It is known as the coral vine or Mexican creeper. It is a fast-growing vine with the stems reaching 20 feet long. It climbs by tendrils which wrap around many types of supports.



DOI:

10.13040/IJPSR.0975-8232.IJP.5(2).91-96

The article can be accessed online on www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(2).91-96

Long sprays of showy pink flowers appear in the summer and fall rapidly. Hot prepared tea from the aerial portion of A. leptopus is used for the prevention and treatment of cough, sore throat, flu, and menstrual pains. The flowers are used to treat high blood pressure. The leaves possess anticoagulant, analgesic, anti-thrombin, inflammatory, and anti-diabetic activities. Also, they have been used as hepatoprotective, treating asthma, liver and spleen disorders ¹⁻⁹. There is no such previous study on the exploration of bioactive compounds using LC/MS (Liquid Chromatography -Mass Spectrometry) of the methanolic extract of the aerial parts of the vine.

MATERIALS AND METHODS:

Plant Collection: Samples of *Antigonon leptopus* used in this study were collected from "El-Zohreya" Park, Cairo, Egypt during the flowering stage (Spring 2012).

A flowering branch was kindly authenticated by Mrs. Terase Labib, plant taxonomist of Orman Garden, Giza, Egypt. The voucher specimen has been deposited in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Cairo University "20.12. 15.1".

Preparation of the Methanolic Extract: The airdried powder of the aerial parts of A. leptopus (400 gm) was extracted using methanol 70 % with percolation (cold extraction technique) till exhaustion $(4 \times 4 \text{ L})$. The solvent was evaporated under reduced pressure at 60 °C, using a rotary evaporator and the residue was used for successive liquid-liquid fractionation. Total weight of the dried methanol residue was 60 g (15% w:w). The air-dried methanolic extract (60 g) was suspended in distilled water (100 ml) and subjected to liquidliquid fractionation with solvents of increasing polarity; petroleum ether (5 \times 500 ml), methylene chloride (4 \times 500 ml), ethyl acetate (6 \times 500 ml) and *n*-butanol (3 \times 500 ml) respectively. The solvents were evaporated in each case under reduced pressure at 45 °C using rotary evaporator. The dried solvent-free extractives were weighted.

Preliminary Phytochemical Screening: The methanolic extract was tested for carbohydrates, tannins, flavonoids, saponins, sterols, alkaloids, anthraquinones and cardiac glycosides ^{10, 11}.

LC-MS Analysis: Electrospray ionization (ESI) interfaced bruker daltonic esquire-LC amazon SL ion trap Mass Spectrometer (Bremen, Germany) and Dionex ultimate 3000 (Germany); composed of

the quaternary pump with an online degasser. A thermo-statted column compartment, photodiode array detector (DAD), an autosampler and Hystar software were used. (Environmental Studies and Research Institute. El-Sadat City. Egypt). Experimental conditions of LC/MS system were as follows: Dionex bounded silica C18 column, dimensions: 4.6×150 mm 3 um. The mobile phase was acetonitrile + 0.1% water (injector temperature set at 38 °C). The carrier gas was N₂ with a flow rate of 12 ml/min, and the injection volume was 20µl. The detector temperature was set at 350 °C.

Sample Preparation: 0.9 gm of the crude methanolic prepared was dissolved in 1 ml methanol. The sample was filtered by teflon 0.22 membrane filter, then 100 μ m of the sample was dissolved in 1ml of methanol to carry the analysis.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening: The preliminary phytochemical study revealed that the methanolic extract of *Antigonon leptopus* contains carbohydrates, tannins, flavonoids, saponins, sterols, and anthraquinones.

LC-MS Analysis: The results of the LC/MS analysis revealed the identification of fifty compounds from the methanolic extract of *Antigonon leptopus*. The compounds were flavonoids, flavones, flavonols, flavanones, biflavonoid, phenolics, glycosides, anthocyanins, and amino acids. Two types of experiment were carried out to achieve the objectives of this study.

TABLE 1: COMPOUNDS IDENTIFIED FROM LC/MS ANALYSIS OF METHANOLIC EXTRACT OF ANTIGONON LEPTOPUS

S. no.	RT	Molecular	Molecular	Compound	Class /	Area	References
	(min)	weight	formula	identification	biological use	%	
1	0.1	316	$C_{16}H_{12}O_7$	Rhamnetin	Flavonol	0.2	Mass bank ID:
					Hepatoprotective		PR020009
					-reduces liver enzymes		
2	0.2	228	$C_{14}H_{12}O_3$	Resveratrol	Polyphenol "Stilbenoid"	0.7	Mass bank ID:
					-Cardioprotective		CE000097
3	0.4	493	$C_{23}H_{25}O_{12}$	Malvidin-3-O-beta-D-	Anthocyanin	7	Mass bank ID:
				glucoside	-Antioxidant		PR020065
4	0.4	610	$C_{27}H_{30}O_{16}$	Luteolin-3',7-di-O-	Flavone	0.2	Mass bank ID:
				glucoside	-Antioxidant		PR020059
5	1.2	286	$C_{16}H_{14}O_5$	Sakuranetin	Flavanone	0.1	Mass bank ID:
					-Antimicrobial		PR020021
					-Antimutagenic		
6	1.3	655	$C_{29}H_{35}O_{17}$	Malvin	Anthocyanin	0.3	Mass bank ID:
					-Antioxidant		PR020064
7	1.5	331	$C_{17}H_{15}O_7$	Neohesperidin	Flavanone	0.1	Mass bank ID:

				dihydrochalcone	-Decreases gastric mucus and ulcers		PR020039
8	1.8	302	$C_{15}H_{10}O_7$	Quercetin	Flavonoid -Antioxidant -Prevents lipid peroxidation	4.2	Mass bank ID: CE000171
9	1.8	300	$C_{16}H_{12}O_6$	Kaempferide	Flavonol -Antioxidant	0.2	Mass bank ID: PR020013
10	1.9	450	$C_{21}H_{22}O_{11}$	Marein	Phenol "Chalconoid" -Antioxidant	1.8	Mass bank ID: PR020071
11	1.9	464	$C_{21}H_{20}O_{12}$	Isoquercitrin	Flavonoid -Antioxidant -Antinflammatory -Antimicrobial	4	Mass bank ID: PR040188
12	2.1	331	$C_{17}H_{15}O_7$	Malvidin	Anthocyanin -Antioxidant	0.7	Mass bank ID: PR020010
13	2.8	448	$C_{21}H_{20}O_{11}$	Quercetrin	Flavonoid -Antiviral -Antioxidant -Hypoglycaemic -Hypolipidaemic	2.1	Mass bank ID: PR020079
14	2.9	594	$C_{27}H_{30}O_{15}$	Saponarin	Flavone -Hypoglycaemic -Hepatoprotective -Antioxidant	0.8	Mass bank ID: PRO030006
15	3.1	165	C ₉ H ₁₁ NO ₂	L-Phenyl alanine	Amino-acid Essential amino acid -Treats depression, arthritis	0.2	Mass bank ID: KO003668
16	3.3	204	$C_{11}H_{12}N_2O_2$	L-tryptophan	Amino-acid -Essential amino acid -Treats anxiety, depression and premenstrual syndrome	0.5	Mass bank ID: KNA001015
17	3.3	448	$C_{21}H_{20}O_{11}$	Luteolin-7-O-glucoside	Flavone -Antioxidant -Antinflammatory -Antibacterial	0.2	Mass bank ID: PR040140
18	12.1	318	$C_{15}H_{10}O_8$	Myricetin	Flavonoid -Antioxidant -Protects against DNA damage	0.2	Mass bank ID: PR020006
19	15.9	862	$C_{42}H_{38}O_{20}$	Sennoside A	Senna Glycoside	3	Mass bank ID: TY000200
20	16.4	286	$C_{15}H_{10}O_6$	Luteolin	-Purgative Flavonoid -Antiallergic -Antinflammatory	0.5	Mass bank ID: TY000143
21	19.5	608	$C_{28}H_{32}O_{15}$	Flavocommelin	Flavone -Antioxidant	0.5	Mass bank ID: TY000133
22	19.7	286	$C_{15}H_{10}O_6$	Flavanone	Flavanone -Antimicrobial	0.1	Mass bank ID: PR020003
23	20.9	462	$C_{22}H_{22}O_{11}$	Diosmetin-7-O-beta-D- glucopyranoside	Flavone -Antioxidant -Anticancer -Phytoestrogen fights breast cancer	0.8	Mass bank ID: TY000189
24	21.3	300	$C_{16}H_{12}O_6$	Chrysoeriol	Flavone -Antioxidant	1.1	Mass bank ID: TY000126
25	21.4	284	$C_{16}H_{12}O_5$	Acacetin	-Antinflammatory Flavone -Dietary estrogen -Antidiabetic "α-glucosidase	3.1	Mass bank ID: TY000176

26	21.4	7.50		D.L.	inhibitor"	0.7	M 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
26	21.4	558	$C_{30}H_{22}O_{11}$	Fukugetin	Flavonoid	0.7	Mass bank ID:
					-Antibacterial		TY000181
27	21.5	270	СПО	Anigonin	-Hypoglycaemic Flavonoid	3.7	Mass bank ID:
21	21.5	270	$C_{15}H_{10}O_5$	Apigenin	-Inhibits skin and	3.7	PR020018
					thyroid carcinogenesis		PK020018
28	21.7	181	$C_9H_{11}NO_3$	L-tyrosine	Amino-acid	0.8	Mass bank ID:
20	21.7	161	$C_9\Pi_{11}NO_3$	L-tyrosine	-Non-essential amino	0.8	KNA00264
					acid		KNA00204
					-The precursor to		
					neurotransmitters and		
					hormones		
29	22.1	464	$C_{21}H_{20}O_{12}$	Quercetin-3-beta-O-	Flavonoid	1.2	Mass bank ID:
			- 21 20 - 12	galactoside	-Antinflammatory		PR020075
				5	-Antiproliferative		
30	22.2	314	$C_{17}H_{14}O_6$	Velutin	Flavonoid	1.5	Mass bank ID:
					-Antinflammatory		FIO00265
					-Antifungal		
31	22.4	284	$C_{16}H_{12}O_5$	Biochanin A	Flavone	2.3	Mass bank ID:
					-Phytoestrogen		PR0000261
					Inhibits prostate cancer		
32	22.8	448	$C_{21}H_{20}O_{11}$	Maritimein	Phenol	0.4	Mass bank ID:
					-Antioxidant		PR020067
33	25.3	290	$C_{15}H_{14}O_6$	Epicatechin	Flavonol	0.5	Mass bank
					-Antioxidant		ID: CE000092
34	26.4	418	$C_{20}H_{18}O_{10}$	Juglanin	Flavonoid	0.1	Mass bank
					-Inhibits hepatic		ID: Ty000222
					diseases and hepatitis		
		-0.4	~ ** ^		viruses		
35	26.5	286	$C_{15}H_{10}O_6$	Kaempferol	Flavonol	2.6	Mass bank
					-Antioxidant		ID: CE000175
36	26.6	578	CILO	V a aman fa nitnin	-Antinflammatory Flavonol	1.0	Maaa hauli
30	20.0	3/8	$C_{27}H_{30}O_{14}$	Kaempferitrin	-Insulinomimetic	1.0	Mass bank
37	27.0	328	$C_{18}H_{16}O_{6}$	Salvigenin	-insumnommenc Flavonoid	1.7	ID: TY000224 Mass bank
31	27.0	326	$C_{18}\Pi_{16}O_{6}$	Sarvigenin	-Immunomodulatory	1./	ID : Ty000249
					-Antitumor		ID . Ty000247
38	27.3	286	$C_{15}H_{10}O_6$	Fisetin	Flavonol	0.4	Mass bank ID:
30	27.3	200	C151110O6	1 iscuii	-Antioxidant	0.1	FIO00086
					-Enhances memory		11000000
39	27.5	374	$C_{16}H_{22}O_{10}$	Swertiamarin	Glycoside "Secoiridoid"	0.3	Mass bank ID:
			- 10 22 - 10		-Hepatoprotective		Ty000077
					-Antioxidant		J
40	27.5	300	$C_{16}H_{12}O_6$	Diosmetin	Flavonoid	8.5	Mass bank
			10 12 0		-Antioxidant		ID: Ty000129
					-Anticancer		
					-Cytoprotective		
41	27.5	272	$C_{15}H_{12}O_5$	Naringenin	Flavonoid	2.8	Mass bank
					-Antioxidant		ID: PR020017
					-Neuroprotective		
					-Regulate HepG2		
42	28.0	300	$C_{16}H_{12}O_6$	Hispidulin	Flavone	0.1	Mass bank
					-Fights pancreatic		ID: Ty000233
4.0	00.0	705	G 11 0	D	cancer	0.1	3.6
43	28.2	592	$C_{28}H_{32}O_{14}$	Fortunellin	Flavonoid	0.1	Mass bank
					-Antioxidant		ID: PR020026
4.4	20.0	246	CHO	Gi	T21 1	0.2	Me 1 - 1
44	28.9	346	$C_{17}H_{14}O_8$	Syringetin	Flavonol	0.3	Mass bank
					- Inhibition of the		ID: PR020084
					procoagulant activity		
45	29.2	330	$C_{17}H_{14}O_{7}$	Cirsiliol	-Antiviral Flavone	2.1	Mass bank
43	29.2	330	C ₁₇ 11 ₁₄ O ₇	CHSHIOI	-Sedative and hypnotic	2.1	ID: TY000127
					-sectative and hypnotic		1D. 11000127

46	29.2	462	$C_{22}H_{22}O_{11}$	Scoparin	Flavonoid	5.0	Mass bank
					-Anticancer		ID: TY000252
47	29.2	606	$C_{29}H_{34}O_{14}$	Embinin	Flavone	2.7	Mass bank
					-Antiviral		ID:
					-An influenza		TY000131
48	29.9	224	$C_{11}H_{12}O_5$	Sinapic acid	Phenolic acid	4.6	Mass bank
					-Antinflammatory		ID: PR020014
					-Antioxidant		
49	29.3	610	$C_{27}H_{30}O_{16}$	Rutin	Flavonol	0.6	Mass bank
					-Antioxidant		ID: CE000140
					-Antinflammatory		
					-Hepatoprotective		
50	29.4	538	$C_{30}H_{18}O_{10}$	Amentoflavone	Bi-flavonoid	0.5	Mass bank
					-Antinflammatory		ID: PR030006

RT= Retention time

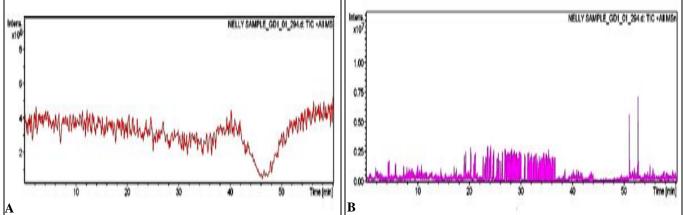


FIG. 1: LC/MS CHROMATOGRAMS OF THE METHANOLIC EXTRACT OF THE AERIAL PARTS OF ANTIGONON LEPTOPUS. A-Total ion chromatogram of the positive ion mode, B-Total ion masses of the identified compounds of the positive ion mode

The first experiment is to select the preference of ion polarity. The selection was based on the abundance of ions determined by the intensity of the peaks. In the second experiment, the preferred ion polarity was used for identification of the compounds. The selection of ion was automatically performed by the system. Compounds were characterized by their retention times, mass spectra and comparison to online mass bank database. Total ion chromatogram of the positive mode and the total masses of the identified compounds are shown in **Fig. 1**. Results of the identified compounds are displayed in **Table 1**.

CONCLUSION: The LC-MS analysis report has shown that the methanolic extract of the aerial parts of *Antigonon leptopus* contains various bio-active compounds. The compounds were flavonoids, flavones, flavonols, flavanones, bi-flavonoids, phenolics, glycosides, anthocyanins and aminoacids. All these diversified phytoconstituents might be responsible for the wide pharmacological actions of the vine.

ACKNOWLEDGEMENT: The author is thankful to the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University for all the facilities provided. Special thanks are owed to the Environmental Studies and Research Institute, El-Sadat City, Egypt for carrying out LC-MS analysis of the sample.

CONFLICT OF INTEREST: Nil

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E- ISSN: 2348-3962, P-ISSN: 2394-5583

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How to cite this article:

Ramadan NA: LC/MS identification of phytoconstituents of the methanolic extract of the aerial parts of *Antigonon leptopus* (Hook & Arn). Int J Pharmacognosy 2018; 5(2): 91-96. doi link: http://dx.doi.org/10.13040/JJPSR.0975-8232.JJP.5(2).91-96.

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