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

Nelly Ashraf Ramadan

Giza Ophthalmology Hospital, Giza, Egypt.

### Keywords:

*Antigonon leptopus* Hook and Arn, vine, Polygonaceae, LC/MS, Flavonoids, Phenolics, Hepatoprotective, Antidiabetic, Antinflammatory

### Correspondence to Author:

**Nelly Ashraf Ramadan**

Pharmacist,  
Giza Ophthalmology Hospital,  
Giza, Egypt.

**Email:** nellyrady@yahoo.com

**ABSTRACT:** *Antigonon leptopus* Hook&Arn (family Polygonaceae), also called: Chain of love, Queen's Wreath and Mexican creeper is a fast growing vine with stems reaching up to 20 feet long. It has heart-shaped, green leaves climbing by tendrils which wrap around many types of the supports. It is native to Mexico and commonly found in tropical Asia, Africa, the Caribbean and the Americas. It possess anticogulant activity, analgesic, antithrombin, antiinflammatory, antidiabetic 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 20<sup>th</sup> 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, Caribbean and the Americas. It is known as coral vine, or Mexican creeper. It is a fast growing vine with the stems reaching to 20 feet long. It climbs by tendrils which wrap around many types of the supports. Long sprays of pink showy 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 anticogulant, analgesic, anti-thrombin, anti-inflammatory and anti-diabetic activities. Also they have been used as hepatoprotective, treating asthma, liver and spleen disorders<sup>1-9</sup>. 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. Terasse 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 air-dried powder of the aerial parts of *A.leptopus* (400 gm) was extracted using methanol 70 % with percolation (cold extraction technique) till exhaustion (4 x 4 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 liquid-liquid fractionation with solvents of increasing polarity; petroleum ether (5 x 500 ml), methylene chloride (4 x 500 ml), ethyl acetate (6 x 500 ml) and *n*-butanol (3 x 500 ml) respectively. The solvents was 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<sup>10-11</sup>.

**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 quaternary pump with an online degasser. A thermo-statted column compartement, photodiode array detector (DAD), an auto sampler 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. Mobile phase was

acetonitrile + 0.1% water (injector temperature set at 38°C). The carrier gas was N<sub>2</sub> with flow rate of 12 ml/min and injection volume was 20 µl. The detector temperature was set at 350 °C .

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

## RESULTS AND DISSCUSSION:

**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, bi-flavonoid, phenolics, glycosides, anthocyanins and amino-acids. Two types of experiment were carried out in order to achieve objectives of this study.

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**.

**TABLE 1: COMPOUNDS IDENTIFIED FROM LC/MS ANALYSIS OF METHANOLIC EXTRACT OF A. LEPTOPUS**

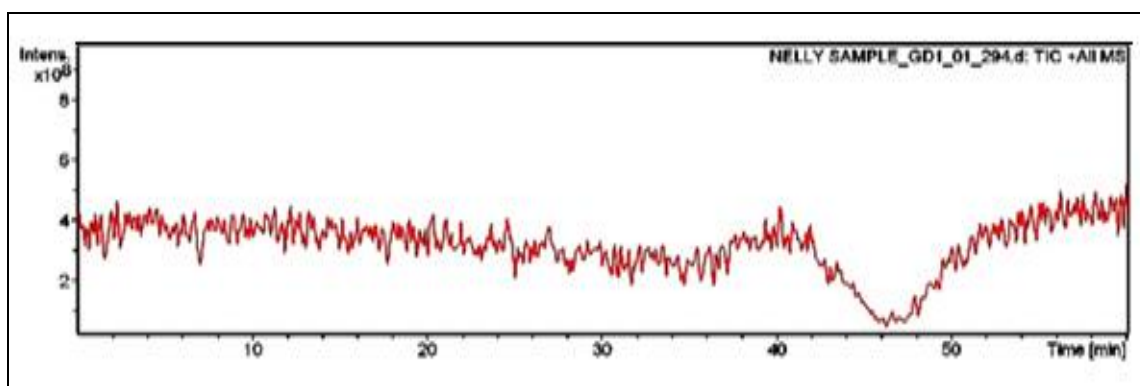
S. no.	RT (min)	Molecular weight	Molecular formula	Compound identification	Class / biological use	Area %	References
1	0.1	316	C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>	Rhamnetin	Flavonol Hepatoprotective -Reduces liver enzymes	0.2	Mass bank ID: PR020009
2	0.2	228	C <sub>14</sub> H <sub>12</sub> O <sub>3</sub>	Resveratrol	Polyphenol “Stilbenoid” -Cardioprotective	0.7	Mass bank ID: CE000097
3	0.4	493	C <sub>23</sub> H <sub>25</sub> O <sub>12</sub>	Malvidin-3-O-beta-D-glucoside	Anthocyanin -Antioxidant	7	Mass bank ID: PR020065

4	0.4	610	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	Luteolin-3',7-di-O-glucoside	Flavone -Antioxidant	0.2	Mass bank ID: PR020059
5	1.2	286	C <sub>16</sub> H <sub>14</sub> O <sub>5</sub>	Sakuranetin	Flavanone -Antimicrobial -Antimutagenic	0.1	Mass bank ID: PR020021
6	1.3	655	C <sub>29</sub> H <sub>35</sub> O <sub>17</sub>	Malvin	Anthocyanin -Antioxidant	0.3	Mass bank ID: PR020064
7	1.5	331	C <sub>17</sub> H <sub>15</sub> O <sub>7</sub>	Neohesperidin dihydrochalcone	Flavanone -Decreases gastric mucus and ulcers	0.1	Mass bank ID: PR020039
8	1.8	302	C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>	Quercetin	Flavonoid -Antioxidant -Prevents lipid peroxidation	4.2	Mass bank ID: CE000171
9	1.8	300	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	Kaempferide	Flavonol -Antioxidant	0.2	Mass bank ID: PR020013
10	1.9	450	C <sub>21</sub> H <sub>22</sub> O <sub>11</sub>	Marein	Phenol "Chalconoid" -Antioxidant	1.8	Mass bank ID: PR020071
11	1.9	464	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	Isoquercitrin	Flavonoid -Antioxidant -Antiinflammatory -Antimicrobial	4	Mass bank ID: PR040188
12	2.1	331	C <sub>17</sub> H <sub>15</sub> O <sub>7</sub>	Malvidin	Anthocyanin -Antioxidant	0.7	Mass bank ID: PR020010
13	2.8	448	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	Quercetrin	Flavonoid -Antiviral -Antioxidant -Hypoglycaemic -Hypolipideamic	2.1	Mass bank ID: PR020079
14	2.9	594	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	Saponarin	Flavone -Hypoglycaemic -Hepatoprotective -Antioxidant	0.8	Mass bank ID: PRO030006
15	3.1	165	C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>	L-Phenyl alanine	Amino-acid Essential amino acid -Treats depression, arthritis	0.2	Mass bank ID: KO003668
16	3.3	204	C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	L-tryptophan	Amino-acid -Essential amino acid -Treats anexiety, depression and premenstrual syndrome	0.5	Mass bank ID: KNA001015
17	3.3	448	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	Luteolin-7-O-glucoside	Flavone -Antioxidant -Antiinflammatory -Antibacterial	0.2	Mass bank ID: PR040140
18	12.1	318	C <sub>15</sub> H <sub>10</sub> O <sub>8</sub>	Myricetin	Flavonoid -Antioxidant -Protects against DNA damage	0.2	Mass bank ID: PR020006
19	15.9	862	C <sub>42</sub> H <sub>38</sub> O <sub>20</sub>	Senoside A	Senna Glycoside -Purgative	3	Mass bank ID: TY000200
20	16.4	286	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	Luteolin	Flavonoid -Antiallergic -Antiinflammatory	0.5	Mass bank ID: TY000143
21	19.5	608	C <sub>28</sub> H <sub>32</sub> O <sub>15</sub>	Flavocommelin	Flavone -Antioxidant	0.5	Mass bank ID: TY000133
22	19.7	286	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	Flavanone	Flavanone -Antimicrobial	0.1	Mass bank ID: PR020003

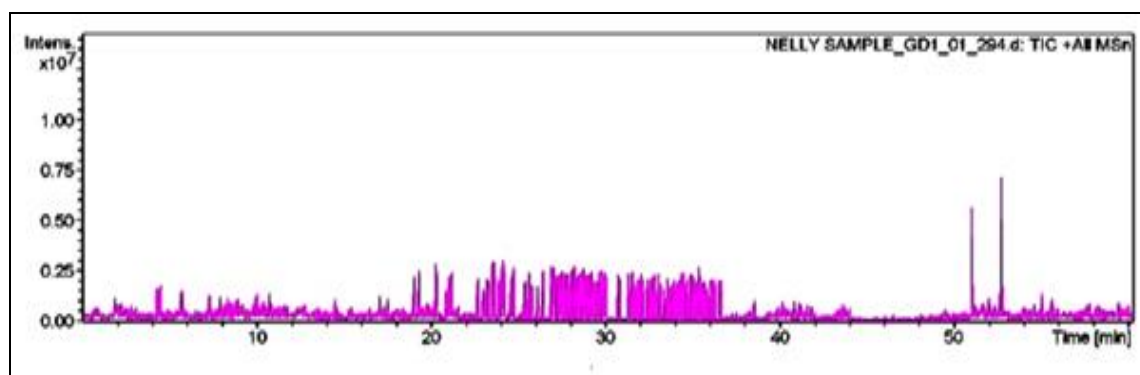
23	20.9	462	C <sub>22</sub> H <sub>22</sub> O <sub>11</sub>	Diosmetin-7-O-beta-D-glucopyranoside	Flavone -Antioxidant -Anticancer -Phytoestrogen fights breast cancer	0.8	Mass bank ID: TY000189
24	21.3	300	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	Chrysoeriol	Flavone -Antioxidant -Antiinflammatory	1.1	Mass bank ID: TY000126
25	21.4	284	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	Acacetin	Flavone -Dietary estrogen -Antidiabetic “α-glucosidase inhibitor”	3.1	Mass bank ID: TY000176
26	21.4	558	C <sub>30</sub> H <sub>22</sub> O <sub>11</sub>	Fukugetin	Flavonoid -Antibacterial -Hypoglycaemic	0.7	Mass bank ID: TY000181
27	21.5	270	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	Apigenin	Flavonoid -Inhibits skin and thyroid carcinogenesis	3.7	Mass bank ID: PR020018
28	21.7	181	C <sub>9</sub> H <sub>11</sub> NO <sub>3</sub>	L-tyrosine	Amino-acid -Non essential amino acid -Precursor to neurotransmitters and hormones	0.8	Mass bank ID: KNA00264
29	22.1	464	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	Quercetin-3-beta-O-galactoside	Flavonoid -Antiinflammatory -Antiproliferative	1.2	Mass bank ID: PR020075
30	22.2	314	C <sub>17</sub> H <sub>14</sub> O <sub>6</sub>	Velutin	Flavonoid -Antiinflammatory -Antifungal	1.5	Mass bank ID: FIO00265
31	22.4	284	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	Biochanin A	Flavone -Phytoestrogen Inhibits prostate cancer	2.3	Mass bank ID: PR0000261
32	22.8	448	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	Maritimein	Phenol -Antioxidant	0.4	Mass bank ID: PR020067
33	25.3	290	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	Epicatechin	Flavonol -Antioxidant	0.5	Mass bank ID : CE000092
34	26.4	418	C <sub>20</sub> H <sub>18</sub> O <sub>10</sub>	Juglanin	Flavonoid -Inhibits hepatic diseases and hepatitis viruses	0.1	Mass bank ID: Ty000222
35	26.5	286	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	Kaempferol	Flavonol -Antioxidant -Antiinflammatory	2.6	Mass bank ID : CE000175
36	26.6	578	C <sub>27</sub> H <sub>30</sub> O <sub>14</sub>	Kaempferitrin	Flavonol -Insulinomimetic	1.0	Mass bank ID: TY000224
37	27.0	328	C <sub>18</sub> H <sub>16</sub> O <sub>6</sub>	Salvigenin	Flavonoid -Immunomodulatory -Antitumor	1.7	Mass bank ID : Ty000249
38	27.3	286	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	Fisetin	Flavonol -Antioxidant -Enhances memory	0.4	Mass bank ID : FIO00086
39	27.5	374	C <sub>16</sub> H <sub>22</sub> O <sub>10</sub>	Swertiamarin	Glycoside “Secoiridoid” -Hepatoprotective -Antioxidant	0.3	Mass bank ID : Ty000077
40	27.5	300	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	Diosmetin	Flavonoid -Antioxidant -Anticancer -Cytoprotective	8.5	Mass bank ID: Ty000129
41	27.5	272	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	Naringenin	Flavonoid -Antioxidant	2.8	Mass bank ID: PR020017

42	28.0	300	$C_{16}H_{12}O_6$	Hispidulin	-Neuroprotective -Regulate HepG2 Flavone	0.1	Mass bank ID: Ty000233
43	28.2	592	$C_{28}H_{32}O_{14}$	Fortunellin	-Fights pancreatic cancer Flavonoid	0.1	Mass bank ID: PR020026
44	28.9	346	$C_{17}H_{14}O_8$	Syringetin	-Antioxidant Flavonol	0.3	Mass bank ID: PR020084
45	29.2	330	$C_{17}H_{14}O_7$	Cirsiliol	- Inhibition of procoagulant activity -Antiviral Flavone	2.1	Mass bank ID : TY000127
46	29.2	462	$C_{22}H_{22}O_{11}$	Scoparin	-Sedative and hypnotic Flavonoid	5.0	Mass bank ID: TY000252
47	29.2	606	$C_{29}H_{34}O_{14}$	Embinin	-Anticancer Flavone	2.7	Mass bank ID: TY000131
48	29.9	224	$C_{11}H_{12}O_5$	Sinapic acid	-Antiviral Phenolic acid	4.6	Mass bank ID : PR020014
49	29.3	610	$C_{27}H_{30}O_{16}$	Rutin	-Antinflammatory -Antioxidant Flavonol	0.6	Mass bank ID : CE000140
50	29.4	538	$C_{30}H_{18}O_{10}$	Amentoflavone	-Antioxidant -Antinflammatory -Hepatoprotective Bi-flavonoid	0.5	Mass bank ID : PR030006

RT= Retention time



A



B

**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

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

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**CONFLICT OF INTEREST:** Nil

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