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GC-MS ANALYSIS OF THE METHYLENE CHLORIDE AND *n*-BUTANOL FRACTIONS OF THE METHANOLIC EXTRACT OF *ANTIGONON LEPTOPUS* (HOOK & ARN.)

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Keywords:

Antigonon leptopus Hook & Arn., Vine, Polygonaceae, GC-MS, Methylene chloride, *n*-butanol, Flavonoids, Phenolics, Hepatoprotective, Antidiabetic, 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 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 supports. It is native to Mexico and commonly found in tropical Asia, Africa, the Caribbean, and the Americas. It possesses anticoagulant activity, analgesic, antithrombin, anti-inflammatory, antidiabetic, and anti-depressant activities. Aerial parts of the vine have been used as hepatoprotective and for spleen disorders. During the current study, the GC-MS experiment was conducted on both the methylene chloride and *n*-butanol fractions of the methanolic extract of the aerial parts of the vine. Results revealed the identification of total thirty-five (35) and thirty-seven (37) compounds respectively in both fractions examined. Compounds were characterized by their retention times, mass spectra with comparison to online mass bank database. The presence of flavonoids, phenolic acids, coumarins, and organic acids in both fractions examined may be responsible for its wide pharmacological actions.

INTRODUCTION: *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. The tea prepared from the aerial portion of *A. leptopus* is used for the prevention and treatment of cough, sore throat, flu, and menstrual pains.

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Flowers are used to treating high blood pressure. Leaves possess anti-coagulant, analgesic, anti-thrombin, anti-inflammatory, and antidiabetic activities. Also, they have been used as hepato-protective, treating asthma, liver and spleen disorders [1-7]. There is no such detailed study on the exploration of bioactive compounds using GC-MS (Gas chromatography/Mass spectrometry) of the methylene chloride and *n*-butanol fractions 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".

Extraction of Plant Material and Preparing the Fractions: 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 × 4 L). The solvent was evaporated under reduced pressure at 60 ^{o}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 (12 g), methylene chloride (0.7 g), ethyl acetate (2.8 g) and *n*-butanol (1.7 g) respectively. The obtained solvents were evaporated in each case under reduced pressure at 45 °C using rotary evaporator.

Preliminary Phytochemical Screening: The methanolic extract was tested for carbohydrates, tannins, flavonoids, saponins, sterols, alkaloids, anthraquinones and cardiac glycosides [8-9].

Sample Preparation: One-half gram (0.5 gm) of the methylene chloride and *n*-butanol fractions of the methanolic extract of the aerial parts of the methanolic extract prepared, each separately was dissolved in 1 ml methanol 70 %. The sample was then filtered along with sodium sulfate to remove traces of water. The filtrate is then concentrated to 1 ml by bubbling nitrogen into the solution. Each sample 1 µl was then employed into the GC-MS unit, to carry on the GC-MS analysis.

Gas Chromatography/Mass Spectrometry (GC-MS) Analysis: The analysis of the both fractions was performed on a GC-MS equipment (Trace GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG, dimensions (30 m), ID: 0.25 mm, film thickness: 0.25µm and equipped with elite 5 MS (5% di-phenyl / 95% dimethyl polysiloxane). The flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, injector temperature programme was 50 °C and raised to 300 °C by 10 °C /min to 150 °C withhold 2 min, then increased to 300 °C with 5 °C /min and hold for 5 min. The solvent delay was 3 min, and diluted samples of 1 µl were injected automatically using an autosampler "AS3000" coupled with GC in the split mode. Detector temperature was set at 250 °C and EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50-650 in full scan mode. The ion source and transfer line temperatures were set at 200 and 300 °C respectively. The components were identified by comparison of their retention times and mass spectra with those of online mass bank database. The GC-MS chromatograms and total compounds identified of both fractions are illustrated in **Fig. 1** and **2**.

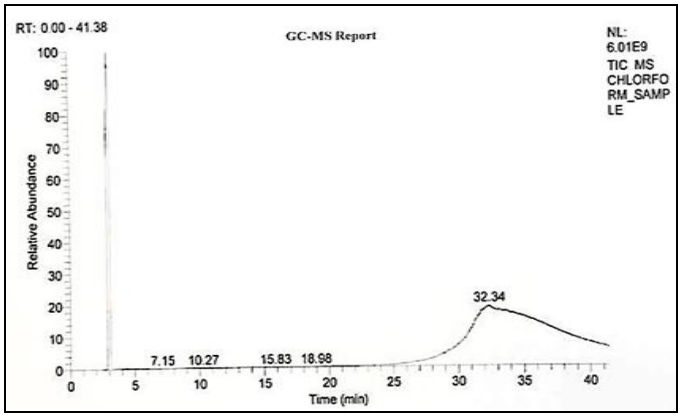

FIG. 1: GC-MS CHROMATOGRAM OF THE METHYLENE CHLORIDE FRACTION OF THE METHANOLIC EXTRACT OF *ANTIGONON LEPTOPUS*

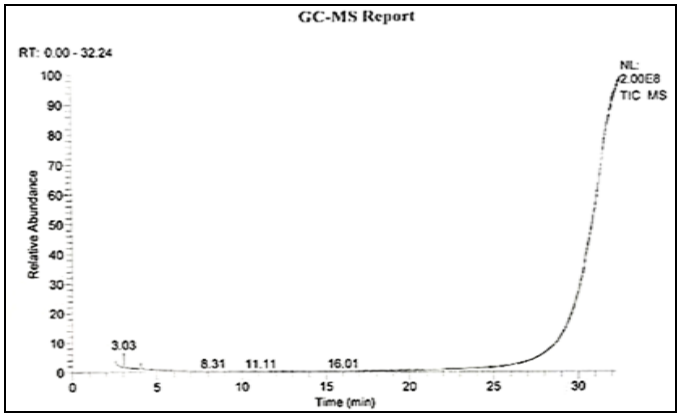

FIG. 2: GC-MS CHROMATOGRAM OF THE *n*-BUTANOL FRACTION OF THE METHANOLIC EXTRACT OF *ANTIGONON LEPTOPUS*

RESULTS AND DISCUSSION:

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

GC-MS Analysis: The GC-MS analysis study of both the methylene chloride and *n*-butanol fractions of the methanolic extract of the aerial parts of *Antigonon leptopus*, comprises various bio-active compounds (35 and 37 compounds respectively). These compounds were identified through mass spectrometry attached with the GC. The GC-MS chromatograms and total compounds identified of both fractions are illustrated in **Fig. 1** and **2** and displayed in **Table 1** and **2** respectively. The mass spectrometer analyzes the compounds eluted at different times to identify the nature of the compounds. Mass spectra of the identified compounds were compared to that of the online mass bank database.

TABLE 1: GC-MS ANALYSIS OF THE METHYLENE CHLORIDE FRACTION OF THE METHANOLIC EXTRACT OF *ANTIGONON LEPTOPUS*

S. no.	R_t (min)	Compound identified	Molecular formula	Molecular weight	Area	Mass bank ID
1	3.62	Naringenin	$C_{15}H_{12}O_5$	272	1436847.90	OUF00380
2	3.85	4'hydroxy 6,8 di methyl flavones	$C_{17}H_{14}O_3$	266	65607564.78	JP000654
3	3.85	5,7 di methyl- 3',4' methylene dioxy flavones	$C_{18}H_{14}O_4$	294	65607564.78	JP000657
4	4.18	Epicatechin	$C_{15}H_{14}O_6$	291	1670477.55	OUF00223
5	4.18	5,7 di hydroxyl 6,8 di methyl 4'methoxy flavone	$C_{18}H_{18}O_5$	314	1670477.55	JP000719
6	5.51	Coumarin	$C_9H_6O_2$	146	1492115.73	JP0007406
7	6.62	Unknown	$C_{30}H_{50}$	410	939116.58	JP009552
8	7.05	Tetrahydroquinoline	$C_9H_{11}N$	133	1972434.82	JP007403
9	7.05	Unknown	$C_{27}H_{46}O$	386	1972434.82	JP003431
10	7.05	Catechol	$C_6H_6O_2$	110	1972434.82	OUF00134
11	7.40	Sakuranetin	$C_{16}H_{14}O_5$	286	1757776.61	OUF00449
12	7.48	Benzylacetophenone	$C_{15}H_{14}O$	210	6106199.87	JP008611
13	7.48	Benzophenone	$C_{13}H_{10}O$	182	6106199.87	JP003592
14	8.48	Benzaldehyde	C_7H_6O	106	1703074.15	JP003547
15	9.03	Unknown	$C_{28}H_{58}$	394	1144059.02	JP002659
16	9.03	Unknown	$C_{29}H_{60}$	408	1144059.02	JP008141
17	10.27	6 benzylamino-flavanone	$C_{22}H_7NO_3$	333	1413709.86	JP000679
18	11.00	Unknown	$C_{29}H_{50}O$	414	1245674.70	JP005753
19	11.00	Ferulic acid	$C_{10}H_{10}O_4$	194	1245674.70	JP011308
20	13.50	Chlorogenic acid	$C_{16}H_{18}O_9$	354	613654.99	OUF00136
21	13.50	Unknown	$C_{28}H_{48}O$	400	613654.99	JP002703
22	18.18	7 methoxy flavonol	$C_7H_{12}O_6$	192	1726091.58	JP000643
23	18.57	Quinic acid	$C_{21}H_{28}N_2O_3$	356	3554443.57	OUF00440
24	18.97	Gallic acid	$C_7H_6O_5$	170	5254260.60	OUF00237
25	18.97	Citric acid	$C_6H_8O_7$	192	5254260.60	KZ000114
26	19.53	Apigenin	$C_{15}H_{10}O_5$	270	1148973.72	OUF00117
27	20.83	4'bromo-6-carboxy flavonol	$C_{16}H_9BrO_5$	359	4633453.34	JP000696
28	20.91	Unknown	$C_{24}H_{40}O_5$	408	3447901.27	JP011901
29	21.84	3,4 di hydroxyl phenyl acetic acid	$C_8H_8O_4$	168	1592946.13	KZ000174
30	22.03	Kaempferol	$C_{15}H_{10}O_6$	286	7420363.93	OUF00285
31	25.34	2'hydroxy-5,7 di methyl flavanone	$C_{17}H_{16}O_3$	268	1243871.39	JP000660
32	25.34	4'methoxy flavones	$C_{16}H_{12}O_3$	252	1243871.39	JP000702
33	28.81	Homovanillic acid	$C_9H_{10}O_4$	182	66007394.29	GLS00077
34	29.41	Pyrogallol	$C_6H_6O_3$	126	14962387.37	OUF00437
35	32.68	Ascorbic acid	$C_6H_8O_6$	176	34145816.92	OUF00120

TABLE 2: GC-MS ANALYSIS OF THE OF THE *n*-BUTANOL FRACTION OF THE METHANOLIC EXTRACT OF *ANTIGONON LEPTOPUS*

S. no.	R_t (min)	Compound identified	Molecular formula	Molecular weight	Area	Mass bank ID
1	2.51	Kaempferol	$C_{15}H_{10}O_6$	286	83656.28	OUF00285
2	2.62	Apigenin	$C_{15}H_{10}O_5$	270	67081.27	OUF00117
3	2.67	Sakuranetin	$C_{16}H_{14}O_5$	286	412455.76	OUF00449
4	2.79	Gallic acid	$C_7H_6O_5$	170	387833.54	OUF00237
5	3.04	Coumarin	$C_9H_6O_2$	146	32713842.29	JP007406
6	3.29	Naringenin	$C_{15}H_{12}O_5$	272	382392.12	JP006237
7	3.36	Lutein	$C_{40}H_{56}O_2$	568	125326.57	CA000113
8	3.36	Unknown	$C_{13}H_{40}O_2$	228	248081.47	JP006237
9	4.29	Oxalic acid	$C_2H_2O_4$	89	135589.37	JP003891
10	4.57	Alpha tocopherol	$C_{29}H_{50}O_2$	430	119203.68	PR010089

11	5.28	Epigallo-catechin	$C_{15}H_{14}O_7$	306	202790.28	OUF00224
12	6.11	6-Iodo-flavanone	$C_{15}H_{11}IO_2$	349	187381.48	JP000711
13	6.35	Homogenentistic acid	$C_8H_8O_4$	168	122382.32	GLS00105
14	7.10	Gentisic acid	$C_7H_6O_4$	154	74646.38	OUF00239
15	8.22	Genistein	$C_{15}H_{10}O_4$	254	103595.20	OUF00238
16	17.43	4-hydroxybenzoic acid	$C_7H_6O_3$	138	846978.56	GLS00140
17	17.83	L-Ascorbic acid	$C_8H_8O_6$	200	237456.41	PR010208
18	17.89	5-dehydroquinic acid	$C_7H_{10}O_6$	190	129568.06	OUF00073
19	17.89	Citric acid	$C_6H_8O_7$	200	129568.06	KZ000114
20	18.84	L-Ornithine	$C_5H_{12}N_2O_2$	132	1112382.69	KZ000236
21	19.43	Chlorogenic acid	$C_{16}H_{18}O_9$	354	390955.97	OUF00135
22	19.53	Quinic acid	$C_7H_{12}O_6$	192	94640.57	OUF00440
23	19.53	6-benzyl-amino flavanone	$C_{22}H_{17}NO_3$	343	94640.57	JP000679
24	20.42	Pyrogallol	$C_6H_6O_3$	126	212690.19	OUF00437
25	21.36	4-nitrophenol	$C_6H_5NO_3$	139	223179.90	JP010794
26	24.04	Myricetin	$C_{15}H_{10}O_8$	318	412803.62	OUF00360
27	24.08	Ferulic acid	$C_{10}H_{10}O_4$	194	57010.97	KZ000133
28	24.46	16-hydroxy decanoic acid	$C_{16}H_{32}O_3$	272	669207.53	KZ000168
29	25.01	6-carboxy flavonol	$C_{16}H_{10}O_5$	282	224489.36	JP000671
30	25.01	4',7 di methoxy flavones	$C_{17}H_{14}O_4$	282	224489.36	JP000655
31	25.23	Homovanillic acid	$C_9H_{10}O_4$	182	173267.35	GLS00077
32	25.23	Protocatechuic acid	$C_7H_6O_4$	106	173267.35	KZ000068
33	25.67	6-carboxy-3-'nitro flavonol	$C_{16}H_9NO_7$	327	922092.64	JP000691
34	26.18	4-coumaric acid	$C_9H_8O_4$	180	438685.78	OUF00416
35	27.47	4'methoxy-6,8 di methyl flavonol	$C_{18}H_{16}O_4$	296	333337.37	JP000645
36	29.68	4',5,7-tri hydroxyl flavonol	$C_{15}H_{10}O_6$	286	699828.54	JP000687
37	30.41	Cinnamyl alcohol	$C_9H_{10}O$	134	13364833.77	GLS00145

CONCLUSION: The GC-MS analysis report of both methylene chloride and *n*-butanol examined fractions of the methanolic extract of the aerial parts of *Antigonon leptopus* vine, showed the presence of flavonoids mainly: flavones, flavonols, flavanones, phenolic acids, coumarins, and organic acids. All these various bio-active compounds might be responsible for its wide pharmacological actions. Further research should be done to isolate and purify these compounds for their valuable uses.

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CONFLICT OF INTEREST: Nil

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