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GC-MS PROFILING AND ANTHELMINTIC ACTIVITY OF *ANTIGONON LEPTOPUS* LEAVES

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ABSTRACT: The study was designed to determine the phytocomponents and in-vitro anthelmintic activity of chloroform fraction of methanolic extract of *Antigonon leptopus* leaves. GC-MS analysis of chloroform fraction of the methanolic extract of *Antigonon leptopus* was performed on a GC–MS equipment (Thermo Scientific Co. Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II). The extract under study has been screened for anthelmintic activity on *Pheretima posthuma.* The chloroform fraction was analysed by GC-MS which revealed the presence of many diversified compounds including phenols, hydrocarbons, quinazolines, coumarins, steroids and terpenes like cadinene, juniper camphor etc. All the compounds were identified from Wiley spectral library. Mass spectral data of 16 compounds is presented. The results obtained from the study indicate significant anthelmintic activity, supporting folk use of the plant when compared with the standard. From the results, it is evident that *Antigonon leptopus* contains various bioactive compounds and biological activity which is recommended as a plant of phytopharmaceutical importance.

INTRODUCTION: Plants have been an important source of medicine for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [1]. Most traditional medicines are developed from nature. They have not yet fulfilled the scientific requirements so as to be classified as modern medicines [2].

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Extract of plants and other natural products have also been utilized for the protection of metals against corrosion [3-5]. Usually, most components that are useful for medicinal purposes are secondary metabolites. Helminthiasis or worm infestation is one of the most prevalent diseases and one of the most serious public health problems in the world. Hundreds of millions if not billions of human infections by helminths exist worldwide with increased world travel and immigration from the developing countries [6].

Antigonon leptopus, commonly known as Mexican Creeper or coral vine, is a species of flowering plant in the buckwheat family, Polygonaceae. It can easily grow to 30-40 ft. in length and the coral vine

has attractive green heart shaped leaves. The coral vine is found in coastal areas of Andhra Pradesh, India. Traditionally the leaves of *A. leptopus* have been used to reduce swelling, and a tea from the leaves can be made to treat diabetes and the blossoms are used to treat high blood pressure. It possesses anti-coagulant activity, analgesic, anti-thrombin, anti-inflammatory activity, anti-diabetic and lipid peroxidation inhibitory activities, anti-helmintic activity and anti-convulsant activity [7]. To the best of our knowledge, there is no such previous study on the exploration of bioactive compounds using GC-MS (Gas chromatography mass spectrometry) and *in-vitro* anthelmintic activity of methanolic extract of *Antigonon leptopus* leaves.

MATERIALS AND METHODS:

Plant material: The fresh leaves of plant *Antigonon leptopus* were collected on November 2012 from local areas of Korangi, Kakinada, Andhra Pradesh. The plant was identified and authenticated (specimen no. BSI/DRC/2012-13/TEH/513) by Mr.P.V.Prasanna, Scientist-'E'-incharge Botanical Survey of India, Deccan regional centre, Hyderabad-500048 where a voucher specimen has been deposited.

Extraction of plant material: 300g fresh leaves of the plant *Antigonon leptopus* were washed with distilled water to remove dust particles. The Shade dried leaves were powdered. The ground fine powder (125g) of the leaves was extracted with absolute methanol (1 litre) at room temperature (30^0c) for three days. The extract was filtered through Whatman no.1 filter paper and then concentrated at 45^0c using a rotary vacuum evaporator. This process was repeated thrice to obtain a sufficient quantity of absolute methanol extract. The methanol extract (13g) was dissolved in distilled water and then fractionation was performed by using different polarity based solvents and n-hexane (3.2g), Chloroform (3.5g), Ethyl acetate (2.3g) and n-butanol (4.1g) fractions were obtained. The remaining residue was further extracted with 95 % methanol (95:05, methanol: water, v/v) (1.2g) and 90 % methanol (90:10, methanol: water, v/v) (1.1g). All these obtained extracts and fractions were stored at -4^{o}C till further analysis. In pilot bioactivity testing, chloroform fraction had shown the positive effect and hence was chosen for further analysis.

Preliminary phytochemical screening: The methanol extract was tested for carbohydrates, proteins & amino acids, fixed oils, alkaloids, glycosides, flavonoids, tannins, steroids, saponins, phenols [8, 9].

Gas chromatography/mass spectrometry (GC/MS) analysis: The GC/MS analysis of chloroform fraction was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1 µl. Samples dissolved in chloroform were run fully at a range of 50–650 m/z and the results were compared by using Wiley Spectral library search programme.

Anthelmintic activity: The anthelmintic activity was performed on the adult Indian earthworm *Pheritima posthuma* [10]. Albendazole, the standard drug, was diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations and was poured into Petri dishes. Chloroform fraction of plant is diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations. Normal saline (0.9% NaCl) alone served as the negative control. All these dilutions were poured into the Petri dishes accordingly. Ten petridishes of equal size were taken & numbered. Six earthworms (n=6) of similar sizes (about 8 cm) were placed in each petridish at room temperature. Time for paralysis was noted down when no movement of any sort could be observed, except when the worms were shaken vigorously. Time of death for worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50^{o}C). The paralysis time and lethal time were recorded in terms of minutes.

RESULTS AND DISCUSSION:

Preliminary phytochemical screening: The preliminary phytochemical study revealed that chloroform fraction of *Antigonon leptopus* contains carbohydrates, fixed oils, alkaloids, glycosides, flavonoids, tannins, steroids, phenols.

GC-MS analysis: The results pertaining to GC-MS analysis of the n-hexane fraction from methanolic leaf extract of *A. leptopus* lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC. The 16 components present in the chloroform fraction of *A. leptopus* that were detected by the GC-MS are shown in **Table 1**. 1-Tetradecene, 2-tert-Butyl-4-isopropyl-5-methylphenol, Spiro[3.6]deca-5,7-diene-1-one, ë-Cadinene , Juniper camphor , 1-Nonadecene ,10-azatricyclo[4.3.1.0(1,6)]deca-2,4-diene, 6-Hydroxy-4,7-dimethylcoumarin, Cholest-2-eno[2,3-b]naphthalene, Cholesta-5,7,9(11)-trien-3-ol, 4,4dimethyl-, (3á)-, 6-Chloro-2-[2-(4-methylphenyl)-1,1-diphenylethyl]-4H -3,1-benzoxazine-4-one, 4,5-Bis(p-bromophenoxy)-1,2-dicyanobenzene, 4,4'-Isopropylidene-Bis-(2-Cyclo hexylphenol), 2-Thioxo-2,3-dihydro-3,5-dimethyl-1,3,4-oxadiazole, 9,19-Cyclolanostane-3,7-diol, 2-Chloro-1,4-bis(dibromomethyl) benzene were present in the chloroform fraction of methanolic leaf extract of *A. leptopus*. The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in **Fig. 1.** The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library (Wiley Spectral library).

TABLE 1: COMPOUNDS IDENTIFIED IN THE CHLOROFORM FRACTION OF *ANTIGONON LEPTOPUS* BY GC-MS

S. No.	RT	Compound Name	Compound Nature	Molecular Formula	Molecular Weight	Area%
1.	14.61	1-tetradecene	Hydrocarbon	$C_{14}H_{28}$	196	0.70
2.	17.11	2-tert-butyl-4-isopropyl-5-methylphenol	Alcohol	$C_{14}H_{22}o$	206	0.93
3.	17.45	spiro[3.6]deca-5,7-diene-1-one	Spiro Ketone	$C_{10}H_{12}o$	148	0.19
4.	19.72	ë-cadinene	Bicyclic sesqui terpene	$C_{15}H_{24}$	204	0.22
5.	20.12	Juniper camphor	Terpene	$C_{15}H_{26}O$	222	0.09
6.	22.72	1-nonadecene	Alkene	$C_{19}H_{38}$	266	1.02
7.	23.01	10-azatricyclo[4.3.1.0(1,6)]deca-2,4-diene	Heterocyclic	$C_9H_{11}N$	133	0.32
8.	24.21	6-Hydroxy-4,7-dimethylcoumarin	Benzopyrone	$C_{11}H_{10}O_3$	190	0.08
9.	33.43	cholest-2-eno[2,3-b]naphthalene	Steroid	$C_{35}H_{50}$	470	0.07
10.	34.43	cholesta-5,7,9(11)-trien-3-ol, 4,4-dimethyl-, (3á)-	Sterol	$C_{29}H_{46}O$	410	0.08
11.	36.02	6-chloro-2-[2-(4-methylphenyl)-1,1-diphenylethyl]-4h -3,1-benzoxazine-4-one	Aromatic	$C_{29}H_{22}ClNO_2$	451	0.09
12.	36.53	4,5-bis(p-bromophenoxy)-1,2-dicyanobenzene	Aromatic	$C_{20}H_{10}Br_2N_2O_2$	468	0.16
13.	36.86	4,4'-isopropylidene-bis-(2-cyclohexylphenol)	Phenol	$C_{27}H_{36}O_2$	392	0.10
14.	37.98	2-thioxo-2,3-dihydro-3,5-dimethyl-1,3,4-oxadiazole	Heterocyclic	$C_4H_6N_2OS$	130	0.13
15.	38.63	9,19-cyclolanostane-3,7-diol	Triterpene	$C_{30}H_{52}O_2$	444	1.07
16.	45.13	2-chloro-1,4-bis(dibromomethyl)benzene	Aromatic	$C_8H_5Br_4Cl$	452	3.29

RT= Retention time (min).

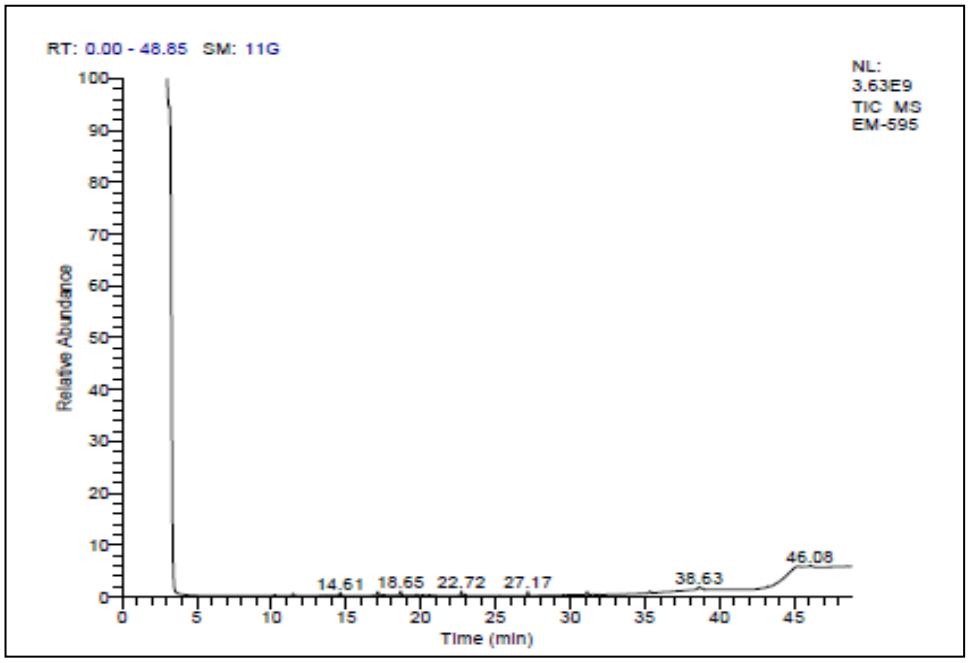

FIG. 1: GC-MS CHROMATOGRAM OF *ANTIGONON LEPTOPUS* LEAVES

Anthelmintic activity: The result show that for the 25mg/ml concentration, albendazole showed the best activity for death time (103.83±6.99min) and the chloroform fraction of *Antigonon leptopus* showed a death time of 127±2.82min. Also, for the 50mg/ml concentration, albendazole showed the highest activity against the worms (84.5±4.84min) and the chloroform fraction of *Antigonon leptopus* showed a death time of 118.0±9.75min. For the 100mg/ml concentration, albendazole showed the least death time 62.83±4.16min and the chloroform fraction of *Antigonon leptopus* showed a death time of 77.6±3.26min. The paralysis and death times of the plant along with the standard is given in **Table 2**. The study revealed that the chloroform fraction of *Antigonon leptopus* had significant activity (moderate) at the higher concentration (100mg/ml).

TABLE 2: IN-VITRO ANTHELMINTIC EFFECT OF *ANTIGONON LEPTOPUS* AGAINST *PHERITIMA POSTHUMA*

Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Albendazole (Standard)	25	51.66±2.59	103±6.99
	50	41.33±1.32	84.5±4.84
	100	30.66±0.88	62.83±4.16
Antigonon leptopus fraction	25	69.16±3.65	127.0±2.82
	50	53.33±4.13	118.0±9.75
	100	39.0±4.38	77.63±3.26
Control	-	-	-

±SD value, n=6, P <0.01.

CONCLUSION: The GC MS analysis report has shown that *A.leptopus* leaves contain various bio-active compounds like phenols, hydrocarbons, quinazolines, coumarins, steroids and terpenes like cadinene, juniper camphor etc. Quinazolines are having antimalarial and anticancer activities. Coumarines exhibit anticoagulant properties. Juniper camphor and cadinene are commonly found terpenes in the plant oils. All these diversified phytoconstituents are responsible for many pharmacological actions. The results of the present study revealed that *Antigonon leptopus* plant leaves contained considerable potential of anthelmintic activity. *A. leptopus* leaves could be used as a potential source for folk medicine, to preserve foods, for the exploration of new compounds as anthelmintic agents.

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