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GC-MS ANALYSIS AND ANTHELMINTIC ACTIVITY OF CHLOROFORM EXTRACT OF *LANTANA CAMARA L.*

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ABSTRACT

The aim of the present study was to evaluate the phytochemical investigation of bio-active compounds by using GC-MS and anthelmintic activity of whole plant of chloroform extract of *Lantana camara L.* GC-MS analysis of chloroform extract of *Lantana camara L.* was performed on a GC–MS equipment (Thermo Scientific Co. Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II). The chloroform extract of *Lantana camara L.* was screened for anthelmintic activity on *Pheritima posthuma*. The preliminary phytochemical study revealed that chloroform extract of *Lantana camara L.* contains carbohydrates, alkaloids, glycosides, Flavonoids, tannins, saponins, steroids, phenols. The extracts found significant anthelmintic activity. Based on the result in the study, it was concluded that the GC MS analysis report has shown that *Lantana camara L.* contain various bio-active compounds like ketones, alkaloids, esters, alcohol, alkenes etc. The phytochemical screening reports revealed the presence of Flavonoids and tannins may be responsible for the anthelmintic activity.

INTRODUCTION

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (Fahn, 1989). Or say, traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy (Cutter, 1978). Natural products are a source of synthetic and traditional herbal medicine. They are the primary health care system in some parts of the world (Badgujar et al., 2016). The past decade has seen considerable change in opinion regarding ethno pharmacological therapeutic applications (Kumar et al., 2007; Kumar et al., 2007). Recently, a number of studies have been reported on the phytochemistry of plants.

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Many of the important medicinal plants in India have pharmacognostically characterized and they have been enumerated in standard literature on pharmacognosy (Mitra, 1985). Like a good marriage, both gas chromatography and mass spectrometry (GC-MS) bring something to their union. GC can separate volatile and semi volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. Depending on the pumping speed of the mass spectrometer, about 1 to 5% of the GC effluent was split off into the mass spectrometer, venting the remaining 95 to 99% of the analytes into the atmosphere. It was soon recognized that this was not the best way to maintain the high sensitivity of the two techniques, and improved GC-MS interfaces were designed (Watson and Biemann, 1965). These interfaces were no longer just GC carrier gas splitters, but carrier gas separators; that is, they separated the carrier gas from the organic analytes and actually increased the concentration of the organic compounds in the carrier gas stream. The most important commercial GC carrier gas separator is called the jet separator (Ryhage, 1964). GC-MS is used both for the qualitative identification and for the quantitative measurement of individual components in complex mixtures (Hites and

Biemann, 1970). GC-MS can also be used to measure the concentration of one or more analytes in a complex mixture. Quantitation can be based on peak areas from mass chromatograms or from selected ion monitoring (Sweely, 1966). Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anemia, eosinophilia and pneumonia (Bundy, 1994). The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases (Sondhi *et al*., 1994). The development of anthelmintic resistance and the high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants as an alternative source of anthelmintic (Eguale and Giday, 2009). Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries due to poor management Helminthiasis practices (Dhar *et al*., 1982). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al*., 1956; Said, 1969; Akhtar *et al*., 2000). A detailed literature review on the whole plants in investigation has shown that so far there are no published reports worldwide, related to our research work and also related to chloroform extract. Hence, in the present study, we were interested in carrying out GC-MS analysis and anthelmintic activity of chloroform extract of *Lantana camara L.*

MATERIALS AND METHODS

Collection of plant materials

The whole plant of *Lantana camara L.* was collected from local areas of Korangi, Kakinada, and Andhra Pradesh. The plant was identified and authenticated by Mr. P. V. Prasanna, Scientist-'E'-incharge Botanical Survey of India, Deccan regional centre, Hyderabad-500048 where a voucher specimen has been deposited. The whole plant of *Lantana camara L.* was washed thoroughly 2-3 times with running water and once with sterile distilled water. The whole plant material was then air-dried on sterile blotter under shade.

Extraction of plant material

425g fresh whole plant of *Lantana camara L.* was washed with distilled water to remove dust particles. The Shade dried whole plant materials were powdered. The whole plant parts of *Lantana camara L.* were shade dried at room temperature, powdered and passed through 60 mesh size sieves. 200 gms of plant powdered were weighed accurately and extracted with 1450 ml Chloroform solvent using cold maceration method. Thus obtained extract were filtered through Whatman No.1 filter paper and the filtrate was concentrated. The extract (2.3 g) were transferred to sterile screw cap bottles, labeled and stored in refrigerator until use.

Phytochemical evaluation

The chloroform extract was tested for carbohydrates, proteins & amino acids, fixed oils, alkaloids, glycosides, Flavonoids, tannins, steroids, saponins, phenols (Sathyaprabha *et al*., 2010; Evans, 2002).

GC-MS analysis

The GC-MS analysis of chloroform extract 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 ^{o}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 Pheretima posthuma (Ghosh *et al*., 2005). 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 extract 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. Seven 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 ^{0}C). The paralysis time and lethal time were recorded in terms of minutes.

RESULTS AND DISCUSSIONS

Phytochemical evaluation

The preliminary phytochemical study revealed that chloroform extract of *Lantana camara L.* contains carbohydrates, alkaloids, glycosides, Flavonoids, tannins, saponins, steroids, phenols.

GC-MS analysis

The results pertaining to GC-MS analysis of the chloroform extract of *Lantana camara L.* lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC. The 12 components present in the chloroform extract of *Lantana camara L.* that were detected by the GC-MS are shown in Table-1. Irgacure 184, 2-Pentadecanone, 6,10,14-trimethyl-, Hexadecanoic acid, methyl ester, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-, Benzeneacetic acid,à,4bis[(trimethylsilyl)oxy]-,trimethylsilyl ester, 1,2-Benzenedicarboxylic acid, dioctyl ester, Docosane, Nonadecane, Heptacosane, Octacosane, Heptadecane,9-hexyl- were present in the chloroform extract of *Lantana camara L.* The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Figure-1.

Table 1. Compounds identified in the chloroform extract of *Lantana camara L.* by GC-MS

S.No	RT	Compound Name	Molecular Formula	Molecular Weight	Area%
1.	20.60	Irgacure 184	$C_{13}H_{16}O_2$	204	0.18
2.	23.92	2-Pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.25
3.	25.72	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.52
4.	29.33	9,12,15-Octadecatrienoicacid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	0.38
5.	29.57	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	296	0.43
6.	33.55	Benzeneaceticacid,à,4bis[(trimethylsilyl)oxy]-,trimethylsilyl ester	$C_{17}H_{32}O_4Si_3$	384	0.24
7.	38.00	1,2-Benzenedicarboxylicacid, dioctyl ester	$C_{24}H_{38}O_4$	390	0.51
8.	41.10	Docosane	$C_{22}H_{46}$	310	0.78
9.	43.32	Nonadecane	$C_{19}H_{40}$	268	0.89
10..	44.67	Heptacosane	$C_{27}H_{56}$	380	1.68
11.	46.14	Octacosane	$C_{28}H_{58}$	394	1.43
12.	47.89	Heptadecane,9-hexyl-	$C_{23}H_{48}$	324	1.88

RT= Retention time.

Table 2. *in-vitro* anthelmintic effect of *Lantana camara L.* against *Pheritima posthuma*

Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Albendazole	25	65.6±3.0	77.0±2.0
(standard)	50	48.6±1.5	63.0±2.0
	100	35.0±2.0	48.0±1.0
Chloroform extract of Lantana camara L.	25	77.0±2.0	95.0±2.0
	50	69.0±2.0	79.0±2.0
	100	50.0±1.0	69.3±1.5
Control	-	-	-
(Saline solution 0.9 % NaCl)			

NaCl=Sodium chloride

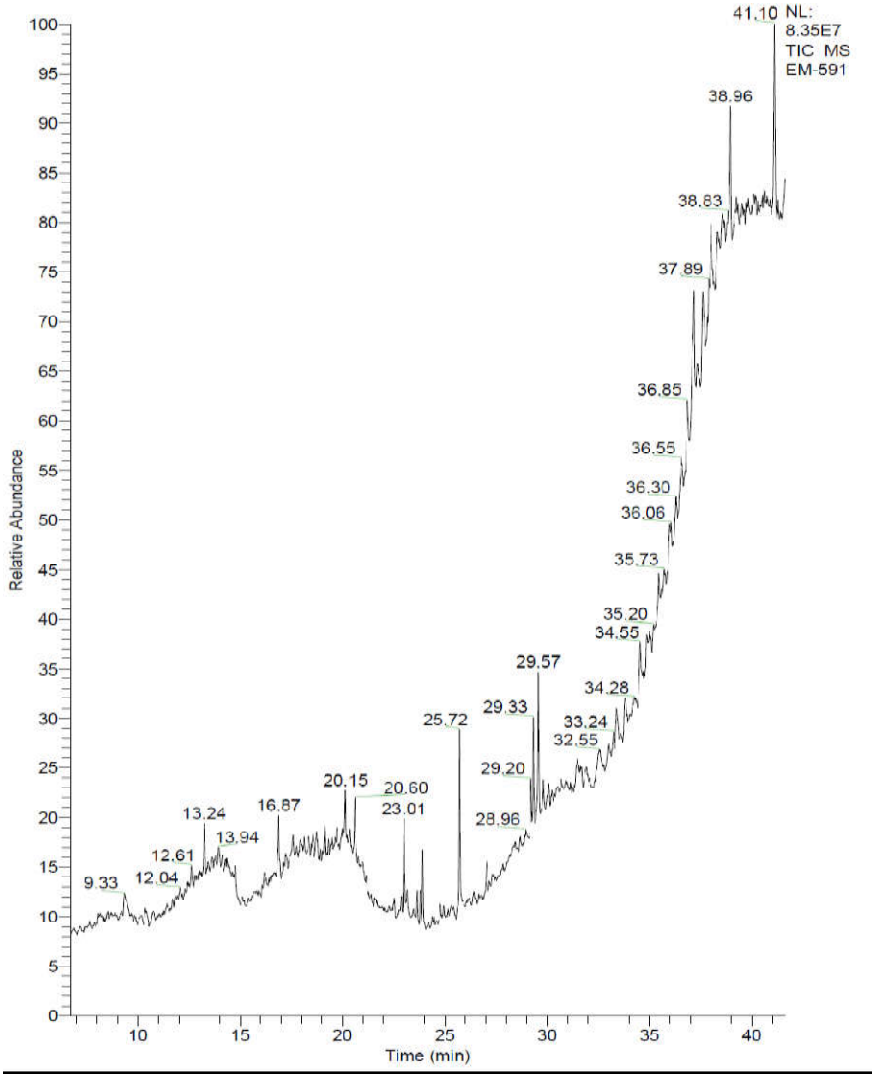

Figure 1. GC-MS Chromatogram of *Lantana camara. L*

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 gave rise to appearance of peaks at different m/z ratios.

Anthelmintic activity

The result showed that for the 25mg/ml concentration, albendazole showed the best activity for death time (77±2.0 min) and the chloroform extract of *Lantana camara L.* showed a death time of 95±2.0 min. Also, for the 50mg/ml concentration, albendazole showed the highest activity against the worms (63±2.0 min) and the chloroform extract of *Lantana camara L.* showed a death time of 79±2.0 min. For the 100mg/ml concentration, albendazole showed the least death time (48±1.0) min and the chloroform extract of *Lantana camara L.* showed a death time of 769.3±1.52 min. The paralysis and death times of the plant along with the standard is given in Table-2. The study revealed that the chloroform extract of *Lantana camara L.* had significant activity (moderate) at the higher concentration (100 mg/ml).

Conclusion

Based on the result in the study, it was concluded that the GC-MS analysis report has shown that *Lantana camara L.* contain various bio-active compounds. All these diversified phytoconstituents are responsible for many pharmacological actions. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will be definitely giving fruitful results and will open a new area of investigation of individual components and their pharmacological potency. The results of the present study revealed that *Lantana camara L.* whole plant contained considerable potential of anthelmintic activity. *Lantana camara L.* 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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