VOLUME-8, ISSUE-5, MAY-2019 • PRINT ISSN No. 2277 - 8160

Original Research Paper

Zoology

Analysis of bioactive compounds in Calotropis gigantean and *Curcuma neilgherrensis* leaves using GC-MS, HPLC and FTIR techniques

Mrs. G. Manjula — Research Scholar (Ph.D), Department of Zoology Erode Arts and Science College, Erode

Dr. T. Chitra* — Asso. Prof. & Head, Department of Zoology Erode Arts and Science College, Erode
*Corresponding Author

Mr. S. Uthirasamy — Research Scholar (Ph.D), Department of Zoology Erode Arts and Science College, Erode

ABSTRACT

Herbal plants are effective source of traditional and modern medicines, useful for primary health care. Plants are richest source of bioactive organic chemicals on earth. The active metabolites like Phytochemicals from the medicinal plants were under exploration for the development of novel and biodegradable effective drugs as an alternative to the ineffective contemporary medicine.The bioactive components of *Calotropis gigantean* and *Curcuma neilgherrensis* leaves have been evaluated using GCMS, HPLC and FTIR. The chemical compositions of the extract of *Calotropis gigantean* and *Curcuma neilgherrensis* leaves were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched by the National Institute of Standards and Technology (NIST) library. GC/MS analysis of extract of *Calotropis gigantean* and *Curcuma neilgherrensis* leaves revealed the existence of different compounds from the two plants. The results of FTIR analysis confirmed the presence of various compound. The results of this study offer a platform of using *Calotropis gigantean* and *Curcuma neilgherrensis* leaves as herbal alternative for various diseases

KEYWORDS :

INTRODUCTION

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase in demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as an alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Ashis, 2003).Plant have been traditional medicinal for several thousand year (Abu- Rabia, 2005). The knowledge medicinal plant has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In india, it is reported that traditional healers uses 2500 plants species 100 species plant serves as regular sources of medicine during the last decades there had been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. According to the reports of the world health organization (WHO), as many as 80%of the world's people depend on traditional medicinal for their primary health care needs, due to the considerable economic benefit in their development and use for the treatment of various disease (Igoli*et al*., 2003).

Within a decade, there were a number of dramatic advances in analytical techniques including HPLC, FTIR and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals (Roberts and Xia, 1995). The aim of this study is to determine the bioactive compounds present in the *Calotropis gigantean* and *Curcuma neilgherrensis* leaves extract with the aid of HPLC, FTIR and GC-MS Techniques, which may provide an insight in its use of tradition medicine.FTIR is one of the widely used methods to identify the chemical constituents and pure compounds present at less than 1gm and has been used as requisite method to medicines in pharmacopeia of many countries (Liu *et al*., 2006). GC-MS analysis is a breakthrough in analysis of phyto constituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1ng (Liebler*et al*., 1996).

*C. neilgherrensis*is an indigenous medicinal plant belonging to the family Zingiberaceae. It is an endemic species and a stem less herb with small rootstock, distributed in higher elevations of Western Ghats. *Calotropis* is a small genus of about 6 species of shrubs or small trees,distributed in tropical and subtropical Africa, Asia and central and South America, represented in India by only two species namely *Calotropis procera* and *Calotropis gigantean*linn. Both the species closely resemble each other in structure and find similar uses (Kirtikar*et al,* 1994). *Calotropisgigantea*Linn is a glabrous or hoary, laticiferous shrubs or small trees, about 3-4 m tall commonly known as the swallow-wort or milkweed. Its stems are erect, up to 20 cm in diameter.

Materials and methods

Extraction of the plant material

The fresh plant materials were of *Calotropis gigantean* and *Curcuma neilgherrensis* collected, washed with running tap water and shade dried. The leaveswere crushed to coarsely powdered by grinder. These coarse powders (25g) were then subjected to successive extraction in 250ml of each solvent (methanol, petroleum ether and acetone) by using Soxhlet apparatus. The collected extracts were stored and then taken up for further investigations. The DMSO (Dimethyl sufloxide) is act as dissolved solvents for these extracts.

GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30 m, column thickness 0.50 µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 270 °C; ion-source temperature 200 °C. The oven temperature was programmed from 40 °C (isothermal for 2 min), with an increase of 8 °C/min, to 150 °C, then 8 °C/min to 250 °C, ending with a 20 min isothermal at 280 °C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMassVer 5.2.0 (Srinivasan*et al.,* 2013)

FTIR Spectroscopic analysis

The FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter

paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

HPLC Analysis Sample preparation

The sample was prepared according to the procedure. The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45 °C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50 °C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100 ml mobile phases. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions

Flavonoids were analysed using an RPHPLC method (WeerasakSamee andSuwannaVorarat, 2007), Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated by a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5 µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

Result and Discussion

In the present study, the investigation of phytochemical screening was different solvent extracts likemethanol, petroleum ether and acetone in that methanol extract showed better activity, so the further test was carried out with the methanol extract. The pharmacological activities of any plant sample are due to the presence of metabolites, secondary metabolites and secretory products in it. These usually consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triperinoids, flavonons, carbohydrates and anthraquinones are found distributed throughout the plant kingdom (Harborne, 1973).

GCMS Analysis

The studies on the active principles in the *Calotropis gigantean* and *Curcuma neilgherrensis* leaves of Methanolic extract by GC MS analysis clearly showed the presence of various compounds. The active principles with their Retention Time (RT), Molecular Formula (MF), Molecular Weight (MW), and compound name are presented in (Table 1 and 2).

Figure 1: GCMS Analysis of *Calotropis gigantean*

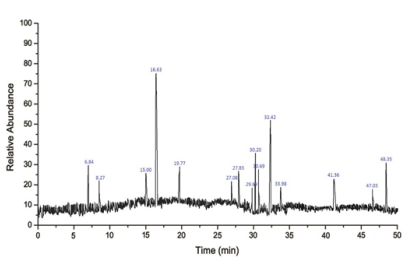


Table 1: GCMS Analysis of *Calotropis gigantean*

S.No	RT	Compound Name	Molecular Formula	Molecular Weight
1	6.84	Decane	$CH_3(CH_2)_8CH_3$	142.28168
2	8.27	2 – H Benzofuranone 5,6,7,7A, tetrahydro 4,4,7A trimethyl	$C_{11}H_{16}O_2$	180.24354
3	15.00	6,10,14 – trimethylpentadecanone	$C_{18}H_{36}O$	268.47784
4	16.63	2,6, dimethyl tetra – 1,5 - decane	$C_{10}H_{18}$	198.182
5	19.77	2 – Methyl - 4, 6 quinolinediol	$C_9H_7NO_2$	161.16
6	27.08	9 – Octadecenoic acid (z) -	$C_{18}H_{34}O_2$	282.46136
7	27.83	9,12,15 – Octadecatrienoic acid, Methyl ester, (z,z,z,)	$C_{19}H_{32}O_2$	292.4562
8	29.89	2 – Hexadecen – 1 - 01	$C_{20}H_{40}O$	296.531
9	30.20	Farnesol isomer	$C_{15}H_{26}O$	222.36634
10	30.69	Tetratetracontane	$C_{44}H_{90}$	619.1854
11	32.42	1,2 Dimethyl benzene	C_8H_{10}	106.17
12	33.98	Betulin	$C_{30}H_{50}O_2$	442.7168
13	41.36	Oxirane	C_2H_4O	44.05256
14	47.03	2- Methyl benzoic acid	$C_8H_8O_2$	136.14792
15	48.35	11 – Tridecen -1-01	$C_{13}H_{26}O$	198.3449

The *Calotropis gigantean* 15 compounds were present and in *Curcuma neilgherrensis* leaves Methanolic extract totally 8 compounds were present by GC MS analysis

Figure 2:GCMS Analysis of *Curcuma neilgherrensis*

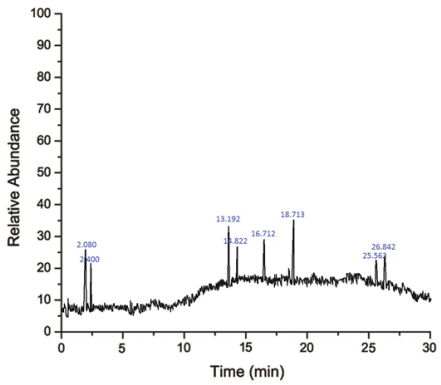


Table 2: GCMS Analysis of *Curcuma neilgherrensis*

S.No	RT	Compound Name	Molecular Formula	Molecular Weight
1	2.080	Benzene – nonyl	$C_{15}H_{24}$	204
2	2.400	Fenadone	$C_{11}H_{14}ONCl$	184
3	13.192	IR*, 3R*, 8S*, 11S*, 14S* S,14 dimethyl -2, 10 – dioxa – 13 - methyl	$C_{15}H_{18}O_3$	246
4	14.822	Vitamin A aldehyde	$C_{20}H_{28}O$	284
5	16.712	Phytol acetate	$C_{22}H_{42}O_2$	338
6	18.713	9 – Octcadecane, 1, 1, Dimethoxy	$C_{20}H_{40}O$	312
7	25.562	1 – formyl – 2, 2, dimethyl - 3-trans – (3 methyl -2- butn - 1- yl)	$C_{15}H_{24}O$	246
8	26.842	3€ -12- epiobtusenyne	$C_{15}H_{20}OclBr$	330

FTIR Analysis

The FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FT-

IR, the functional groups of the components were separated based on its peaks ratio.The results of FTIR analysis confirmed the presence ofIntermolecular hydrogen bonded OH strong with tertiary alcohol, Methylamino alkanes (Strong), Alkanes, Aromatic methane (weak) and aliphatic aldehyde which is very strong which are present in *Calotropis gigantean*(Figure 3).

Figure 3:FTIR Analysis of *Calotropis gigantean*

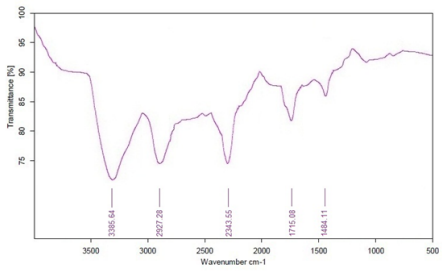
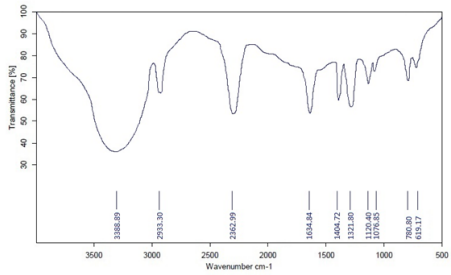


Figure 4: FTIR Analysis of *Curcuma neilgherrensis*



The FTIR results of *Curcuma neilgherrensis*Intermolecular hydrogen bonded OH strong with tertiary alcohol, Cycloalkanes (medium), alkanes, secondary amines (weak), Quaternary compounds (strong), primary amines (strong), secondary amines (weak to medium) aliphatic esters (very strong), Mono-substituted benzenes (very strong) and primary amines (medium)(Figure 4).

Determination of HPLC retention times

HPLC profiles of *Calotropis gigantean* and *Curcuma neilgherrensis* were analysed and various compounds were present, having different elution times could be obtained (Figure 5 and Table 3) when each compound was analysed individually using the mobile gradient phase consisting of methanol and 1% acetic acid in water during 30 minutes run time.

Table3: HPLC analysis of *Calotropis gigantean*

Peak	RT	Area	Height
1	3.153	69853709	579143
2	9.672	6383053	24877
3	14.59	4384926	13479
4	24.03	5498468	250013
5	26.14	80631834	32940

Figure5: HPLC analysis of *Calotropis gigantean*

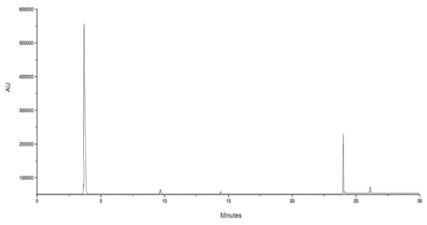
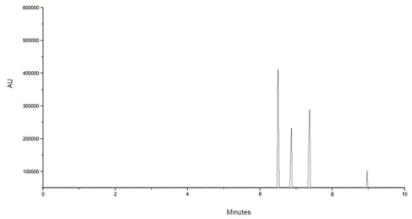


Table4: HPLC analysis of *Curcuma neilgherrensis*

Peak	RT	Area	Height
1	6.48	87432114	431078
2	6.89	68219937	249861
3	7.30	73418046	303490
4	8.98	5013790	117621

Figure 6: HPLC analysis *of Curcuma neilgherrensis*



Conclusion

The presence of various bioactive compounds justifies the use of the leaves for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Calotropis gigantean* and *Curcuma neilgherrensis*contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and agricultural products.

REFERENCES

1. Abu-rabia. A. Urinary diseases and ethnobotny among postoralnamedsin the Middle East. Journal of Ethnobiology and Ethnomedicine. (2005).
2. Ashis G. Herbal folk remedies of Bankura and Medinipur districts, West Bengal. Indian Journal of Traditional Knowledge 2003; 2:393-396.
3. Harborne JB. Phytochemical Methods, A Guide to modern Techniques of Plant Analysis. Chapman and Hall London, 1973, 33-41
4. Igoli, J.O., I.C. Igwue and N.P.Ioli, Traditional medicinal practices amongst the Igede People of Nigeria, J. Herb, Species and Medicinal Plants, (2003). 10 (4): 1-10.
5. Liebler D.C., Burr J.A., Philips L. and Ham A.J.L., Gas chromatography-mass spectrometry analysis of vitamin E and its oxidation products, Anal. Biochem., 1996; 236: 27-34.
6. Liu HX, Sun SQ, Lv GH and Chen KK. Study on Angelica and its different extracts by Fourier Transform Infrared Spectroscopy and two dimensional correlation IR Spectroscopy. Mol.Biomol. Spec. 2006; 64:321-326.