***Chapter 1***
Print ISBN: 978-93-90149-88-9, eBook ISBN: 978-93-90149-89-6

# Determining the Bioactive Constituents in *Calotropis gigantea* Leaves by GC-MS, HPLC and FTIR Techniques

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DOI: 10.9734/bpi/nvbs/v1/1788C

## ABSTRACT

Medicinal plants have initiated in many countries because of their contributions to health care. Calotropis gigantea is a biological ingredient with potential therapeutic qualities that belongs to the Asclepiadaceae family.Calotropis gigantea leaves have long been used to treat abdominal pain, tumours, skin problems, wounds, and insect stings.This plant's therapeutic characteristics make it an important source of a medicinal compound. Calotropis gigantean's bioactive components were studied using GC-MS, HPLC, and FTIR. Gas Chromatography-Mass Spectroscopy (GC-MS) study reveals major bioactive chemicals. Only leaves contained the chemical compounds Androstane-11,17-Dione, 3-[(Trimethylsilyl)Oxy]-, 17-[O-(Phenylmethyl)Oxime], 3.Alpha.-(Trimethylsiloxy)Cholest-5-Ene, Urs-12-EN-28-Oic acid, 3-Hydroxy-, Methyl ester, (3.BETA.)-, Pseduosarsasapogenin-5, 20-Dien Methyl Ether, Beta. Carotene, 1.Alpha.,2.Alpha.-Epoxy-1.Beta.-Methylcholesta-4,6-Dien-3-One, 3-O-Acetyl-6-Methoxy-Cycloartenol whereas the rest of the compounds were similar in plants.

*Keywords: Calotropis gigantean; GC-MS analysis; HPLC and FTIR.*

## 1. INTRODUCTION

Due to various their therapeutic diversity, herbal plants are regarded as the most important part of our natural wealth, and so have a unique place. Secondary metabolites, which are implicated in most plants' therapeutic actions, are responsible for the medicinal characteristics of these plants. As it is very effective, inexpensively available, reportedly has no side effects, and is utilised as an alternative to allopathic treatments, there has been an upsurge in demand in international trade. Herbal medications are abundant in nature and have no negative side effects. Medicinal plants have initiated in many countries because of their contributions to health care. The primary benefits of using plant-based medicines are relatively safer than synthetic alternatives, offering marked therapeutic benefits and more affordable treatment. Thus, over 50% of these modern drugs are of natural product origin and these natural products play an important role in the drug development in pharmaceutical industry. The plant has been traditional medicinal for several thousand year [1]. 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 use 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 [2]. *Calotropis gigantea* white commonly known as Mudar Yercum widely distributed in the Eastern and southern parts of India. It is a weed plant commonly known as giant milkweed and scientifically reported for several medicinal properties viz. *Calotropis gigantea* has shown enormous protective properties and

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provides wide treatment opportunities hence it was analyzed for anti-proliferative activity on colorectal cancer [3]. Calotropis gigantea (family Asclepiadaceae) commonly known as 'Sweta Arka' is a highly medicinal drought resistant and relatively high degree salt tolerant wild plant species of the Indian Himalayan region. Plant contain milky latex in the stem which is used as an antidote for snake poison in dried form. Whereas, dried leaves of the Calotropis plant are used as an expectorant and anti- inflammatory for the cure of paralysis and rheumatic pains [4-7]. The flowers have reported to take over analgesic activity, anti-microbial and cytotoxicactivity [8,9]. Leaves and areal parts of the plant have reported for anti-diarrheal activity and anti-bacterial activity, anti-oxidant activity [10]. The α-Amylase inhibition is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes, obesity, dental caries and periodontal diseases [11]. Taking this into consideration, α-Amylase inhibition, antioxidant activity, and phytochemical analysis of Nepalese originated *C. gigantea* (L.) Dry and were evaluated.TLC of fractions showed a compound at RF value at0.45 in toluene: chloroform: methanol with mobile phase ratio 7:2:1 respectively [12]. *Calotropis gigantea* is a widely used plant in the traditional medical system. However, there are no reports on its potential in cancer management. Therefore, we aimed to examine the phytochemical composition and cytotoxic activity of *C. Gigantean* methanolic leaf extract against three different cancer cell lines: HeLa (cervical), MCF7 (breast), and A549 (lung) [13]. Roots have reported to contain anti-pyretic activity [14]. Chitme HR et al. [15] 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 [16]. Plant biochemistry is epitomized the considerable diversity of organic substances that are intricate and accumulated through plants, the chemical composition of these substances, their biosynthesis turn over and metabolism in plants, their Innate circulation and their biological medicinal plants are supportive for therapeutic as well as for remedial of human diseases, since of the occurrence of phytochemical constituents [17]. The aim of this study is to determine the bioactive compounds present into the *Calotropis gigantean* leaves extract with the aid of HPLC, FTIR and GC-MS Techniques, which may provide an insight in its use of traditional medicine.

## 2. MATERIALS AND METHODS

### Extraction of the Plant Material

The plant *Calotropis gigantean* leaves were collected washed with running tap water and shade dried. The leaves were crushed to coarsely powdered by a grinder. These coarse powders (25 g) were then subjected to successive extraction in 250 ml of each solvent (methanol, petroleum ether and acetone) by using Soxhlet apparatus. The collected extracts were preserved in airtight bottles at 4°C in refrigerator and then taken up for further investigations. The DMSO (Dimethyl sulfoxide) acts as dissolved solvents for these extracts.

### GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising an AOC-20i auto sampler and gas chromatography interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32 mm, column length is 30 m, column thickness 0.50 µm), operating in electron impact mode at70 eV; 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 from40ºC (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 70 eV; 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 adapted to handle mass spectra and chromatograms was aTurboMassVer 5.2.0 [18].

### FTIR Spectroscopic Analysis

In 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 A Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using the 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 conformation.

### HPLC Analysis Sample Preparation

In 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 an 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 RP HPLC methods [12]. 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 CLASS VP 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, the 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 a 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. The detection wavelength was 280 nm.

## 3. RESULT AND DISCUSSION

In the present study, the investigation of phytochemicals screening was different solvent extracts like methanol, 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 phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triterpenoids, flavonoids, carbohydrates and anthraquinones are found distributed throughout the plant kingdom [19].

**GCMS Analysis:** The studies on the active principles in the *Calotropis gigantea* 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 names are presented in (Tables 1 and 2).

**FTIR Analysis:** The FT-IR spectrum was used to identify the functional groups of the active components present in the extract based on the peak values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on the ratio of its peak. The results of FTIR analysis confirmed the presence of Intermolecular 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*.

**Table 1. GCMS Analytical results of *Calotropis gigantea***

| Sl. no | RT | Compound name | Molecular weight | Molecular formula |
|---|---|---|---|---|
| **1** | 695 | ANDROSTANE-11,17-DIONE, 3-[(TRIMETHYLSILYL)OXY]-, 17-[O-(PHENYLMETHYL)O | 481 | C29H43O3NSi |
| **2** | 695 | 3.ALPHA.-(TRIMETHYLSILOXY)CHOLEST-5-ENE | 458 | C30H54OSi |
| **3** | 690 | 1-MONOLINOLEOYLGLYCEROL TRIMETHYLSILYL ETHER | 498 | C27H54O4Si2 |
| **4** | 682 | 9,10-SECOCHOLESTA-5,7,10(19)-TRIENE-1,3-DIOL, 25-[(TRIMETHYLSILYL)OXY]-, (3. | 488 | C30H52O3Si |
| **5** | 670 | 9,12-OCTADECADIENOIC ACID, (2-PHENYL-1,3-DIOXOLAN-4-YL)METHYL ESTER, CI | 442 | C28H42O4 |
| **6** | 659 | 3.BETA.-HYDROXYGUAIA-4(15),10(14),11(13)-TRIEN-6,12-OLIDE 8-(.ALPHA.,.BETA.- | 364 | C19H24O7 |
| **7** | 644 | PSEDUOSARSASAPOGENIN-5,20-DIEN METHYL ETHER | 428 | C28H44O3 |
| **8** | 638 | DODECANEDIOIC ACID, BIS(TRIMETHYLSILYL) ESTER | 374 | C18H38O4Si2 |
| **9** | 636 | 9,12-OCTADECADIENOIC ACID, (2-PHENYL-1,3-DIOXOLAN-4-YL)METHYL ESTER, T | 442 | C28H42O4 |
| **10** | 631 | 2-TRIMETHYLSILOXY-6-HEXADECENOIC ACID, METHYL ESTER | 356 | C20H40O3Si |
| **11** | 623 | PENTACOSENOIC ACID, 2-[(TRIMETHYLSILYL)OXY]-, METHYL ESTER | 482 | C29H58O3Si |
| **12** | 622 | PENTACOSANOIC ACID, 2-[(TRIMETHYLSILYL)OXY]-, METHYL ESTER | 484 | C29H60O3Si |
| **13** | 620 | 9,12,15-OCTADECATRIENOIC ACID, 2-PHENYL-1,3-DIOXAN-5-YL ESTER | 440 | C28H40O4 |
| **14** | 611 | 9,12-OCTADECADIENOIC ACID, 2-PHENYL-1,3-DIOXAN-5-YL ESTER, CIS- | 442 | C28H42O4 |
| **15** | 605 | Z,Z,Z-1,4,6,9-NONADECATETRAENE | 260 | C19H32 |
| **16** | 594 | CIS-5,8,11-EICOSATRIENOIC ACID, TRIMETHYLSILYL ESTER | 378 | C23H42O2Si |
| **17** | 592 | BICYCLO[3.1.1]HEPTAN-ENDO-6-OL, SYN-7-BROMO- | 190 | C7H11OBr |
| **18** | 695 | URS-12-EN-28-OL | 426 | C30H50O |
| **19** | 660 | URS-12-EN-28-OIC ACID, 3-HYDROXY-, METHYL ESTER, (3.BETA.)- | 470 | C31H50O3 |
| **20** | 655 | STIGMASTEROL TRIMETHYLSILYL ETHER | 484 | C32H56OSi |
| **21** | 618 | DOCOSA-8,14-DIYN-CIS-1,22-DIOL, BIS(TRIMETHYLSILYL) ETHER | 478 | C28H54O2Si2 |
| **22** | 588 | BICYCLO[10.1.0]TRIDECA-4,8-DIENE-13-CARBOXYLIC ACID (2-HYDROXY-4-NITROP | 356 | C20H24O4N2 |
| **23** | 575 | 3.BETA.-ACETOXY-BISNOR-5-CHOLENAMIDE | 387 | C24H37O3N |
| **24** | 570 | URS-12-EN-28-AL | 424 | C30H48O |
| **25** | 570 | 1-PROPYL-3-(PROPEN-1-YL)ADAMANTANE | 218 | C16H26 |
| **26** | 547 | A-NORCHOLESTAN-2-ONE, (5.ALPHA.)- | 372 | C26H44O |
| **27** | 546 | .ALPHA.-AMYRIN | 426 | C30H50O |
| **28** | 544 | OLEANOLIC ACID | 456 | C30H48O3 |
| **29** | **700** | PSEDUOSARSASAPOGENIN-5,20-DIEN METHYL ETHER | 428 | C28H44O3 |
| **30** | **692** | BETA. CAROTENE | 536 | C40H56 |
| **31** | **689** | STIGMASTERYL TOSYLATE | 566 | C36H54O3S |
| **32** | **678** | STIGMASTAN-6,22-DIEN, 3,5-DEDIHYDRO- | 394 | C29H46 |
| **33** | **677** | VITAMIN A ALDEHYDE | 284 | C20H28O |

| Sl. no | RT | Compound name | Molecular weight | Molecular formula |
|---|---|---|---|---|
| **34** | **674** | 26-HYDROXYCHOLESTEROL | 402 | C27H46O2 |
| **35** | **674** | CARYOPHYLLENE | 204 | C15H24 |
| **36** | **674** | KAUREN-18-OL, ACETATE, (4.BETA.)- | 330 | C22H34O2 |
| **37** | **673** | BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYLENE-,[1R-(1R*,4Z,9S*)] | 204 | C15H24 |
| **38** | **668** | ERGOSTA-7,22-DIEN-3-OL, ACETATE, (3.BETA.,5.ALPHA.)- | 440 | C30H48O2 |
| **39** | **664** | ERGOSTA-7,22-DIEN-3-OL, (3.BETA.,22E)- | 398 | C28H46O |
| **40** | **663** | (E,E,E)-3,7,11,15-TETRAMETHYLHEXADECA-1,3,6,10,14-PENTAENE | 272 | C20H32 |
| **41** | **660** | ARACHIDONIC ACID, TRIMETHYLSILYL ESTER | 376 | C23H40O2Si |
| **42** | **660** | 26,26-DIMETHYL-5,23-ERGOSTADIEN-3.BETA.-OL | 426 | C30H50O |
| **43** | **658** | ALPHA.-FARNESENE | 204 | C15H24 |
| **44** | **656** | 8(14),22-ERGOSTADIENOL ACETATE | 440 | C30H48O2 |
| **45** | **653** | 7,22-ERGOSTADIENOL | 398 | C28H46O |
| **46** | **670** | 1.ALPHA.,2.ALPHA.-EPOXY-1.BETA.-METHYLCHOLESTA-4,6-DIEN-3-ONE | 410 | C28H42O2 |
| **47** | **665** | TRICYCLO[4.3.0.0(7,9)]NON-3-ENE, 2,2,5,5,8,8-HEXAMETHYL-, (1.ALPHA.,6.BETA.,7. | 204 | C15H24 |
| **48** | **650** | URS-12-EN-28-OIC ACID, 3-HYDROXY-, METHYL ESTER, (3.BETA.)- | 470 | C31H50O3 |
| **49** | **640** | AZULENO[5,6-C]FURAN-3(1H)-ONE, 4,4A,5,6,7,7A,8,9-OCTAHYDRO-1,4,8-TRIHYDRO | 282 | C15H22O5 |
| **50** | **636** | METHANOL, [6,8,9-TRIMETHYL-4-(1-PROPENYL)-3-OXABICYCLO[3.3.1]NON-6-EN-1- | 236 | C15H24O2 |
| **51** | **630** | BICYCLO[5.1.0]OCTAN-2-ONE, 4,6-DIISOPROPYLIDENE-8,8-DIMETHYL- | 232 | C16H24O |
| **52** | **623** | DIHYDROTACHYSTEROL | 398 | C28H46O |
| **53** | **623** | 8(14),22-ERGOSTADIENOL ACETATE | 440 | C30H48O2 |
| **54** | **618** | TETRACYCLO[6.1.0.0(2,4).0(5,7)]NONANE, 3,3,6,6,9,9-HEXAMETHYL-, CIS,CIS,TRAN | 204 | C15H24 |
| **55** | **616** | METHANOL, [6,8,9-TRIMETHYL-4-(2-FURYL)-3-OXABICYCLO[3.3.1]NON-6-EN-1-YL]- | 262 | C16H22O3 |
| **56** | **613** | BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYLENE-,[1R-(1R*,4Z,9S*)] | 204 | C15H24 |
| **57** | **611** | DOCOSA-8,14-DIYN-CIS-1,22-DIOL, BIS(TRIMETHYLSILYL) ETHER | 478 | C28H54O2Si2 |
| **58** | **606** | CARYOPHYLLENE | 204 | C15H24 |
| **59** | **604** | 1-(3,3-DIMETHYL-BUT-1-YNYL)-2,2,3,3-TETRAMETHYLCYCLOPROPANECARBOXYLI | 222 | C14H22O2 |
| **60** | **598** | TRICYCLO[4.1.0.0(2,4)]HEPTANE, 3,3,7,7-TETRAMETHYL-5-(2-METHYL-1-PROPENY | 204 | C15H24 |
| **61** | **598** | ARACHIDONIC AMIDE, N-[5-HYDROXY-N-PENTYL]- | 389 | C25H43O2N |
| **62** | **597** | D-MANNITOL, 1-DECYLSULFONYL- | 370 | C16H34O7S |
| **63** | **719** | 3-O-ACETYL-6-METHOXY-CYCLOARTENOL | 498 | C33H54O3 |
| **64** | **685** | PSEDUOSARSASAPOGENIN-5,20-DIEN METHYL ETHER | 428 | C28H44O3 |
| **65** | **661** | LUPEOL | 426 | C30H50O |
| **66** | **650** | PSEDUOSARSASAPOGENIN-5-EN METHYL ETHER | 460 | C29H48O4 |
| **67** | **649** | CEDRAN-DIOL, 8S,14- | 238 | C15H26O2 |
| **68** | **642** | 3-ISOPROPYL-6A,10B-DIMETHYL-8-(2-OXO-2-PHENYL-ETHYL)-DODECAHYDRO-BE | 396 | C26H36O3 |

| Sl. no | RT | Compound name | Molecular weight | Molecular formula |
|---|---|---|---|---|
| **69** | **633** | 1-MONOLINOLEOYLGLYCEROL TRIMETHYLSILYL ETHER | 498 | C27H54O4Si2 |
| **70** | **609** | URS-12-EN-28-OIC ACID, 3-HYDROXY-, METHYL ESTER, (3.BETA.)- | 470 | C31H50O3 |
| **71** | **595** | 2-PHENANTHRENOL, 1,2,3,4,4A,4B,5,6,8A,9,10,10A-DODECAHYDRO-4A,7-DIMETHY | 431 | C25H41O3NSi |
| **72** | **591** | 1,3-BIS-T-BUTYLPEROXY-PHTHALAN | 296 | C16H24O5 |
| **73** | **583** | 3.BETA.-ACETOXY-BISNOR-5-CHOLENAMIDE | 387 | C24H37O3N |
| **74** | **576** | 9-OCTADECENOIC ACID, (2-PHENYL-1,3-DIOXOLAN-4-YL)METHYL ESTER, CIS- | 444 | C28H44O4 |
| **75** | **575** | ALPHA.-AMYRIN | 426 | C30H50O |
| **76** | **571** | TRICYCLO[4.1.0.0(2,4)]HEPTANE, 3,3,7,7-TETRAMETHYL-5-(2-METHYL-1-PROPENY | 204 | C15H24 |
| **77** | **555** | 2,3-O-BENZAL-D-MANNOSAN | 250 | C13H14O5 |
| **78** | **540** | 1-HYDROXY-1,7-DIMETHYL-4-ISOPROPYL-2,7-CYCLODECADIENE | 222 | C15H26O |
| **79** | **527** | 5-PREGNEN-3.BETA.,9.ALPHA.-DIOL-20-ONE 3-ACETATE | 374 | C23H34O4 |

**Table 2. HPLC analysis of *Calotropis gigantean***

| PEAK | RT | AREA | HEIGHT |
|---|---|---|---|
| 1 | 3.162 | 69862612 | 587132 |
| 2 | 9.683 | 6483142 | 24885 |
| 3 | 14.61 | 4473815 | 14367 |
| 4 | 24.19 | 5576357 | 260022 |
| 5 | 27.11 | 86721642 | 33834 |

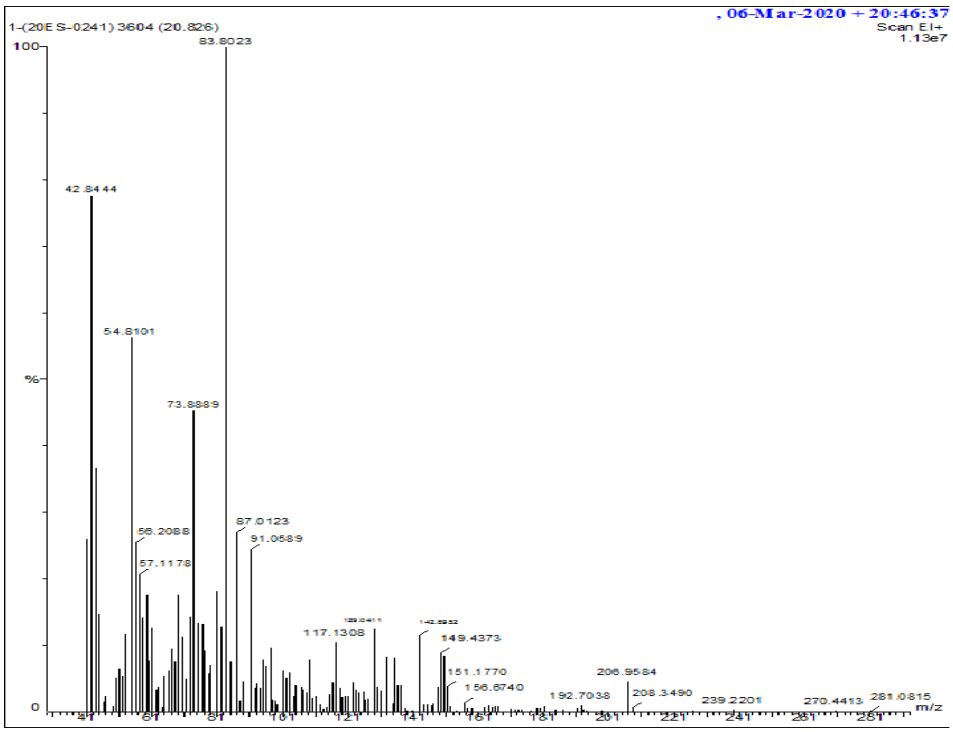


**Fig. 1. GCMS Analysis of *Calotropis gigantea***

**Table 3. FTIR analysis of *Calotropis gigantea***

| NO | PEAK | INTENSITY | CORR. INTENSITY | BASE(H) | BASE(L) | AREA | CORR. AREA |
|---|---|---|---|---|---|---|---|
| 1 | 474.49 | 70.11 | 7.405 | 551.64 | 432.05 | 15.18 | 2.134 |
| 2 | 615.29 | 70.796 | 11.949 | 823.6 | 551.64 | 22.43 | 5.806 |
| 3 | 1099.43 | 62.058 | 2.908 | 1138 | 1083.99 | 10.178 | 0.465 |
| 4 | 1423.47 | 68.859 | 1.453 | 1431.18 | 1408.04 | 3.617 | 0.12 |
| 5 | 1620.21 | 47.907 | 5.717 | 1627.92 | 1570.06 | 13.894 | 0.846 |
| 6 | 3415.93 | 20.943 | 6.071 | 3444.87 | 2999.31 | 178.39 | -10.195 |

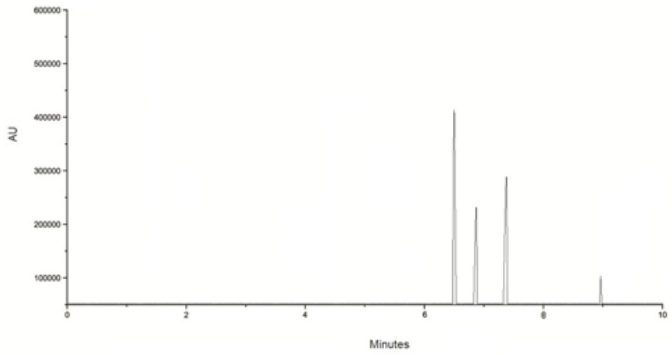


**Fig. 2. HPLC Analysis of *Calotropis gigantea***

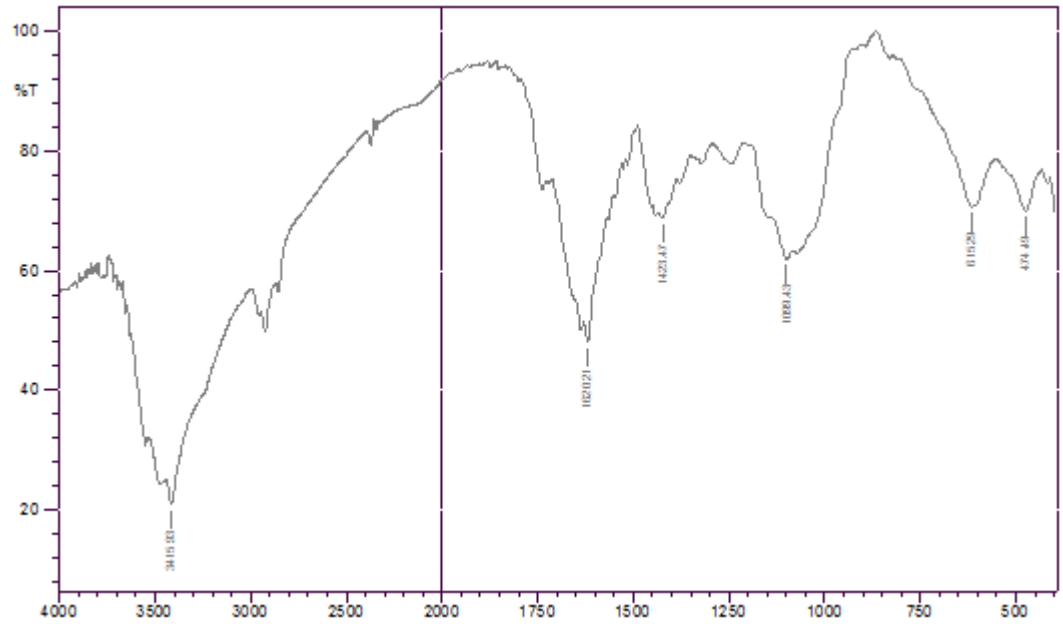


**Fig. 3. FTIR analysis of *Calotropis gigantea***

## 4. CONCLUSION

The phytochemicals present in the methanolic extract of *Calotropis gigantea* leaves GC-MS chromatogram of methanolic extract of leaves showed nearly 79 compounds. Most of the compounds which were reported from leaves were contains Androstane-11,17-Dione, 3-[(Trimethylsilyl)Oxy]-, 17-

[O-(Phenylmethyl)Oxime], 3.Alpha.-(Trimethylsiloxy) Cholest-5-Ene, Urs-12-EN-28-Oic acid, 3-Hydroxy-, Methyl ester, (3.BETA.)-, Pseduosarsasapogenin-5, 20-Dien Methyl Ether, Beta. Carotene, 1.Alpha.,2.Alpha.-Epoxy-1.Beta.-Methylcholesta-4,6-Dien-3-One, 3-O-Acetyl-6-Methoxy-Cycloartenol. High- Performance Liquid Chromatography (HPLC) This peaks with retention times in methanolic extract is found to be 3.1, 9.6, 14.6,24.1 and 27.1 respectively Table 2. The peak impurities and peak purity is shown in Fig. 2. As per the data analysis of FTIR from using infrared spectroscopy correlation table for *Calotropis gigantea* methanolic extract, it was found that amines groups at the frequency 474.49 to 3415.93 frequency gave maximum peaks. Hence, we can conclude that the Caloptropis methanolic extract is rich in amines and C = O groups.

**Suggestion for further research:** The present study on *Calotropis gigantea* leaves proved *C. gigantea* leaves confirm the existence of various biologically active molecules with their possible functional groups. Moreover, these leaves are already in use for a wide range of treatments traditionally such as fever, indigestion, cough, cold, asthma, nausea, vomiting, diarrhea, etc. *The Calotropis gigantea* has various medicinal applications, but it is the need of the hour to explore its medicinal values at a molecular level with the help of various biotechnological tools and techniques. The present study may be an initiative for further and pharmacological investigations required to separate the novel active compounds from the leaves to formulate new drugs in order to treat incurable diseases.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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This chapter is an extended version of the article published by the same author(s) in the following journal.
Asian Journal of Advances in Research, 7(1): 1-8, 2021..