**International Journal of Botany Studies**
**www.botanyjournals.com**
**ISSN: 2455-541X**
**Received: 13-11-2021, Accepted: 28-11-2021, Published: 14-12-2021**
**Volume 6, Issue 6, 2021, Page No. 1055-1061**

# Gas chromatography-mass spectrometry (GC-MS) analysis of bioactive compounds of ethanolic extract of *Acmella Calva* (DC.) R.K Jansen

**Priyadharshni K[1], P Shanthi[2*]**
[1] Scholar, Departments of Botany, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India
[2] Assistant Professor and Dean of Extension, Departments, of Botany, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India

**Abstract**
Thousands of different constituents are present in plant extracts. To study the pharmacological efficacy in plant extracts there is a need of separation of the active components present in the plants. The present study was aimed to screen the bioactive compounds of one of the important traditional medicinal herb *Acmella calva* (DC.) R. K. Jansen (Asteracecae). The ethanolic whole plant extract was analyzed using GC-MS. The compounds found in the extract was related with NIST library. The Gas chromatography-Mass spectrometry study revealed the presence of 25 compounds. The compounds are 2(5h)-Furanone, 3-Hydroxy-4,5-Dimethyl-, Butane, 1,1-Diethoxy-3-Methyl-, 3,3-Diethoxy-2-Butanone, Cyclopentane, 1,1,3-Trimethyl-, 1-Tetradecanol, Dodecanoic Acid, Diethyl Phthalate, 1-Pentadecene, Phenol, 4-Octyl-, Hept-2-Ene, 2,4,4,6-Tetramethyl-, 1-Tridecene, Neophytadiene, 6-Octen-1-Ol, 3,7-Dimethyl-, Propanoate, 2-[Ethyl(Methyl)Amino]-1-Phenyl-1-Propanol, N-Hexadecanoic Acid, Hexadecanoic Acid, Ethyl Ester, Phytol, 9,12-Octadecadienoic Acid (Z,Z), 8,11,14-Eicosatrienoic Acid, (Z,Z,Z), (2e,4e)-N-Isobutyldodeca-2,4-Dienamide, Bicyclo [10.1.0], Tridec-1-Ene, Octadecanoic Acid, N-Isobutyl-(2e,4z,8z,10e)-Dodecatetraenamide, 1,2-Benzenedicarboxylic Acid, Dicyclohexyl Ester, Pentadecanal. The compounds were identified by comparing their retention time and peak area with that of literature data. The compounds identified in the present study are potential phytocompounds, which are used in industries for various purpose like as antioxidant, antifungal agent, pesticide, flavoring agent and to prevent cancer.

**Keywords:** antioxidant, active components, antifungal, pharmacological, cancer, microbial

## Introduction
Plants are the key source of effective medicine for thousands of years. Ample of secondary metabolites and volatile matter discharged by plants play a very important role in Drug discovery [1]. For understanding and evaluating the herbs therapeutic value, isolation and the structure elucidation of their compounds are necessary. All over the world, medicinal plants are used for the treatment and prevention of various ailments [2].
Analysis of components enduring in medicinal plants and traditional medicines is achieved by "Mass spectrometry coupled with chromatographic separations such as GC-MS [3]. Chromatography is a separation technique in which mobile phase carrying a mixture is allowed to move in contact with the absorbent stationary phase. It plays as an analytical technique to control the quality and phytotherapeutical standardization. The combination of sensitivity, speed and high resolving power in gas chromatography enables adequate technique for the separation of complex samples. For the direct identification of unknown compounds is very simple to establish with the help of combined spectrometric methods such as mass spectrometry [4]. Acmella calva (DC.) R.K. Jansen. (Local name-Toothache plant) is a rare perennial herb belongs to the Aster family, Asteraceae. It is native to America, Pacific islands, Asia and common in Tropical regions. It is used medicine. It is also act as ornamental plant and has insecticidal properties. It acts as an herbal remedy for toothache and oral infections [5]. Various parts of Acmella is used to cure wounds, paralysis of tongue, psoriasis, cardiovascular disease, dysentery, tincture, tumour, thyroid disorder and stimulate secretion of saliva [6, 7].

## Materials and Methods
### Authentication of plant material
The plant was identified and authenticated by Dr. S. Soosairaj, Assiatant Professor, St. Joseph's College, Tiruchirappalli-02 in accordance with the "Flora of Central and Northern Tamil Nadu" by John Britto S (2019) and the specimen accessed as 3002.

### Collection and preparation of plant material
The whole Plant of *Acmella calva* (DC.) R.K. Jansen were collected in the month of October from the Nanjikottai road, Tanjore, Tamil Nadu, India. The whole plant was washed and shade dried under room temperature. The dried plant was powdered coarsely.

### Preparation of ethanol extracts
The extract was prepared using soxhlet apparatus. The extract was concentrated and dried by using a rotary evaporator under reduced pressure. The final condensed dried sampl was stored in labeled sterile bottle and kept at -20ºC for further analysis [8].

### Identification of compounds
The extract was concentrated to 1 ml by bubbling nitrogen into the solution. The extract contained both polar and non-

polar phytocomponents. 2µl of the ethanolic extract of Acmella calva was employed for GC-MS analysis. The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 and the components were separated using Helium as carrier gas at constant flow of 1 ml/min. The 2µl of sample was injected into the column and was detected by the Turbo gold mass detector with the aid of the Turbo mass 5.1 software. During the 36th minute of GC extraction process, the oven was maintained at a temperature of 110°C with 2 minutes. The injector temperature was set at 250°C (mass analyser). The different parameters involved in the operation of the Clarus 500 MS, were also standardized. Mass spectra were taken at 70 electron volt with the scan interval of 0.5s and fragments from 45 to 450 Da were detected. The MS detection was completed in 36 minutes [9].

## Results

GC-MS is one of the best technique to evaluate the components with long chain, alcohol, esters, volatile matter, branched hydrocarbons and acids etc. The Gas chromatography-Mass spectroscopy analysis of *A. calva* ethanolic extract of whole plant revealed the presence of 25 compounds (Fig-1). The identified compounds were 2(5h)-Furanone, 3-Hydroxy-4,5-Dimethyl, Butane, 1,1-Diethoxy-3-Methyl-, 3,3-Diethoxy-2-Butanone, Cyclopentane, 1,1,3-Trimethyl-, 1-Tetradecanol, Dodecanoic Acid, Diethyl Phthalate, 1-Pentadecene, Phenol, 4-Octyl-, Hept-2-Ene, 2,4,4,6-Tetramethyl-, 1-Tridecene, Neophytadiene, 6-Octen-1-Ol, 3,7-Dimethyl-, Propanoate, 2-[Ethyl(Methyl)Amino]-1-Phenyl-1-Propanol, N-Hexadecanoic Acid, Hexadecanoic Acid, Ethyl Ester, Phytol, 9,12-Octadecadienoic Acid (Z,Z), 8,11,14-Eicosatrienoic Acid, (Z,Z,Z)-, (2e,4e)-N-Isobutyldodeca-2,4-Dienamide, Bicyclo[10.1.0], Tridec-1-Ene, Octadecanoic Acid, N-Isobutyl-(2e,4z,8z,10e)-Dodecatetraenamide, 1,2-Benzenedicarboxylic Acid, Dicyclohexyl Ester, Pentadecanal. These phytochemical compounds constitute the medicinal quality of the plant. The phytochemical compounds identified were confirmed based on the retention time, peak area and molecular formula. The active principles with their molecular formula, peak area in percentage, height and retention time were calculated and presented in Table-1 (Fig-2a-2y).

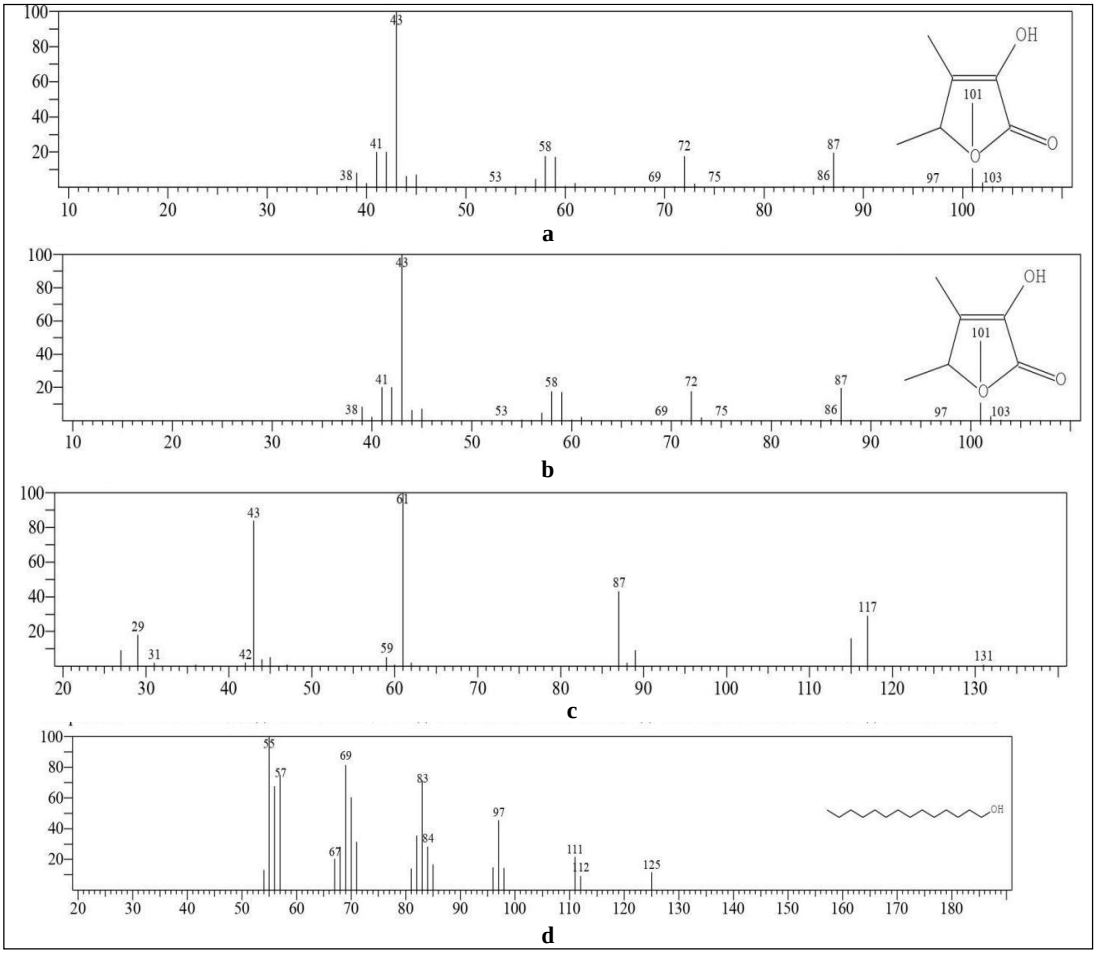
In the present study, a maximum of 14 compounds such as 2(5h)-Furanone, 3-Hydroxy-4,5-Dimethyl-, 3,3-Diethoxy-2-Butanone, Cyclopentane, 1,1,3-Trimethyl-, 1-Pentadecene, Hept-2-Ene, 2,4,4,6-Tetramethyl-, Neophytadiene, Phytol, 9,12-Octadecadienoic Acid (Z,Z)-, 8,11,14-Eicosatrienoic Acid, (Z,Z,Z)-, Octadecanoic Acid, N-Isobutyl-(2e,4z,8z,10e)-Dodecatetraenamide, 1,2 Benzenedicarboxylic Acid, Dicyclohexyl Ester, Pentadecanal were noticed which are potential compounds possess antimicrobial and anticancer activity. Among the different compounds identified, the high retention time (34.86 min.) was shown by pentadecanal, a long chain fatty acid which has the antimicrobial property. It was followed by an anticancer compounds Octadecanoic Acid and N-Isobutyl-(2e,4z,8z,10e)-Dodecatetraenamide with the retention time of 34.59 min. Low retention time (4.708 min.) was shown by 2(5h)-Furanone, 3-Hydroxy-4, 5-Dimethy which are the potential anticancer and antibacterial compounds. From the present investigation, it is concluded that the identified phytochemicals through GC-MS showed many biological activities like antifungal, antimicrobial, antifoaming, antioxidant, antimicrobial agent, anticancer, cosmetics, plasticizer, surfactant and flavouring agent (Table-2). The biological activities listed are based on Dr. Duke's phytochemical and ethanomedicinal database created by Dr. Jim Duke of Agricultural Research, USDA.

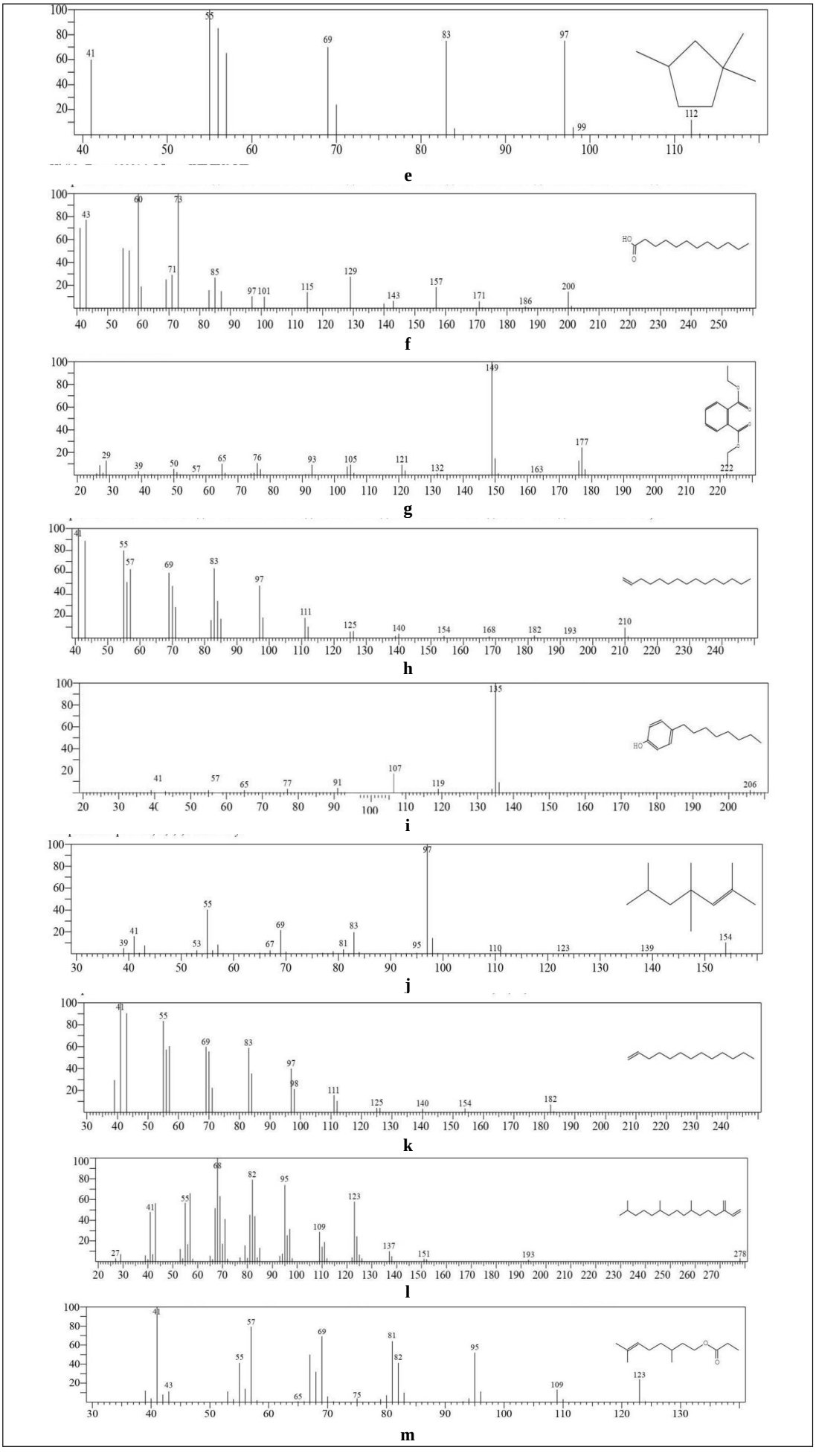
**Table 1:** Compounds present in ethanolic extract of whole plant of *Acmella calva.*

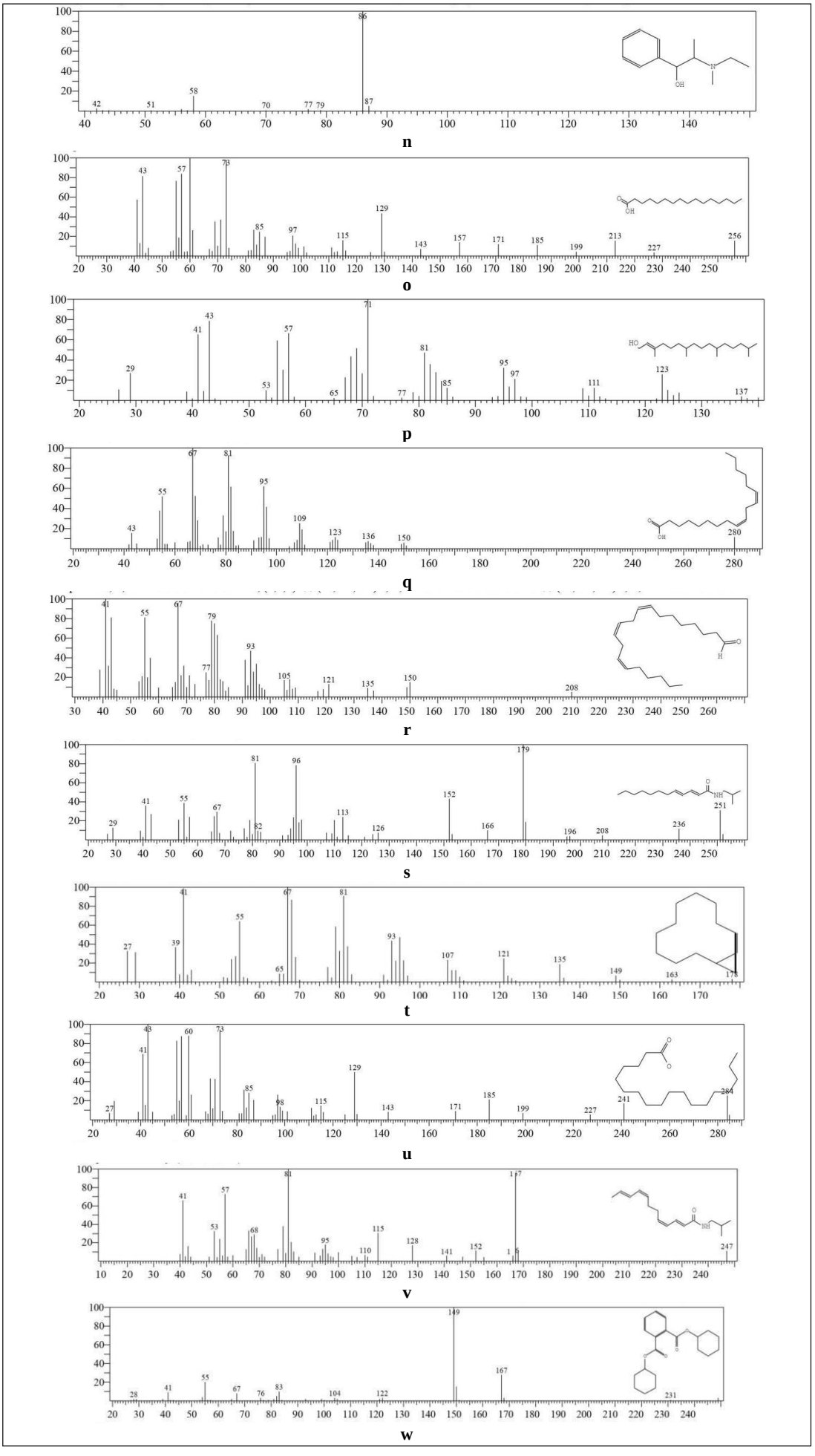
| Peak | Retention time | Area | Area% | Height | Molecular formula | Name |
|---|---|---|---|---|---|---|
| 1 | 4.708 | 24935 | 0.3 | 17623 | C6H803 | 2(5h)-Furanone, 3-Hydroxy-4,5-Dimethyl- |
| 2 | 5.769 | 102130 | 1.21 | 53971 | C9H20O2 | Butane, 1,1-Diethoxy-3-Methyl- |
| 3 | 5.942 | 27677 | 0.33 | 18486 | C8H16O3 | 3,3-Diethoxy-2-Butanone |
| 4 | 15.889 | 45562 | 0.54 | 21087 | C8H16 | Cyclopentane, 1,1,3-Trimethyl- |
| 5 | 18.016 | 166816 | 1.97 | 80248 | C14H30O | 1-Tetradecanol |
| 6 | 19.443 | 66087 | 0.78 | 26303 | C12H24O2 | Dodecanoic Acid |
| 7 | 19.932 | 995573 | 11.78 | 397797 | C12H14O4 | Diethyl Phthalate |
| 8 | 20.031 | 67300 | 0.8 | 32800 | C15H30 | 1-Pentadecene |
| 9 | 20.26 | 87056 | 1.03 | 40224 | C14H22O | Phenol, 4-Octyl- |
| 10 | 21.987 | 31149 | 0.37 | 17354 | C11H22 | Hept-2-Ene, 2,4,4,6-Tetramethyl- |
| 11 | 23.759 | 40192 | 0.48 | 21571 | C13H26 | 1-Tridecene |
| 12 | 24.51 | 163855 | 1.94 | 77355 | C20H38 | Neophytadiene |
| 13 | 25.244 | 39313 | 0.47 | 21191 | C13H24O2 | 6-Octen-1-Ol, 3,7-Dimethyl-, Propanoate |
| 14 | 25.458 | 90948 | 1.08 | 26384 | C12H19NO | 2-[Ethyl(Methyl)Amino]-1-Phenyl-1-Propanol |
| 15 | 26.663 | 2815885 | 33.31 | 784276 | C16H32O2 | N-Hexadecanoic Acid |
| 16 | 27.118 | 120949 | 1.43 | 49062 | C18H36O2 | Hexadecanoic Acid, Ethyl Ester |
| 17 | 28.987 | 132484 | 1.57 | 41532 | C20H40O | Phytol |
| 18 | 29.314 | 1137932 | 13.46 | 346202 | C18H3202 | 9,12-Octadecadienoic Acid (Z,Z)- |
| 19 | 29.403 | 1221287 | 14.45 | 301552 | C20H3402 | 8,11,14-Eicosatrienoic Acid, (Z,Z,Z)- |
| 20 | 29.563 | 199923 | 2.37 | 49850 | C16H29O2 | (2E,4E)-N-Isobutyldodeca-2,4-Dienamide |
| 21 | 29.693 | 148782 | 1.76 | 60776 | C13H22 | Bicyclo[10.1.0]Tridec-1-Ene |
| 22 | 29.761 | 269817 | 3.19 | 71191 | C18H36O2 | Octadecanoic Acid |
| 23 | 30.183 | 248730 | 2.94 | 78373 | C16H25NO | N-Isobutyl-(2E,4Z,8Z,10E)-Dodecatetraenamide |
| 24 | 34.599 | 117549 | 1.39 | 38113 | C20H26O4 | 1,2-Benzenedicarboxylic Acid, Dicyclohexyl Ester |
| 25 | 34.864 | 90584 | 1.07 | 28168 | C15H30O | Pentadecanal |

**Table 2:** Biological activity of compounds identified in ethanolic extract of whole plant of *Acmella calva*.

| S.no | Compound name | Molecular formula | Nature of compound | Biological activity |
|---|---|---|---|---|
| 1. | 2(5h)-Furanone, 3-Hydroxy-4,5-Dimethyl- | C6H803 | Sotolone (Aromatic compound) | Aticancer, antiprotozoal, antibacterial |
| 2. | Butane, 1,1-Diethoxy-3-Methyl- | C9H20O2 | Aldehyde | Flavouring agent |
| 3. | 3,3-Diethoxy-2-Butanone | C8H16O3 | Butanone | Anticancer, antioxidant |
| 4. | Cyclopentane, 1,1,3-Trimethyl- | C8H16 | Alkanes | Antimicrobial |
| 5. | 1-Tetradecanol | C14H30O | Myristyl alcohol | Comsmetics, plasticizer, antifoaming agent |
| 6. | Dodecanoic Acid | C12H24O2 | Saturated fatty acid | Antioxidant |
| 7. | Diethyl Phthalate | C12H14O4 | Phthalate ester | Cosmetics, plasticizer |
| 8. | 1-Pentadecene | C15H30 | Alkene | Antibacterial |
| 9. | Phenol, 4-Octyl- | C14H22O | Acyclic diterpene alcohol | Fragrance industry, cosmetics, shampoos, soaps |
| 10. | Hept-2-Ene, 2,4,4,6-Tetramethyl- | C11H22 | Alkene | Antimicrobial |
| 11. | 1-Tridecene | C13H26 | Acryclic olefir | Antifoaming agent, coagulating agent |
| 12. | Neophytadiene | C20H38 | Diterpene | Antibacterial, antifungal |
| 13. | 6-Octen-1-Ol, 3,7-Dimethyl-, Propanoate | C13H24O2 | Fatty alcohol esters | Emulsifer, surfactant, flavouring agent |
| 14. | 2-[Ethyl(Methyl)Amino]-1-Phenyl-1-Propanol # | C12H19NO | Alcohol | Decongestants |
| 15. | N-Hexadecanoic Acid | C16H32O2 | Saturated fatty acid | Antioxidant |
| 16. | Hexadecanoic Acid, Ethyl Ester | C18H36O2 | Ethyl ester | Cosmetics, Antimicrobial, antioxidant |
| 17. | Phytol | C20H40O | Diterpene | Antimicrobial, antifungal, diuretic, anticancer, cholesterol lowering agent |
| 18. | 9,12-Octadecadienoic Acid (Z,Z)- | C18H3202 | Polysaturated omega 6 fatty acid | Anticancer, dermatology |
| 19. | 8,11,14-Eicosatrienoic Acid, (Z,Z,Z)- | C20H3402 | Unsaturated fatty acid | Anticancer |
| 20. | (2E,4E)-N-Isobutyldodeca-2,4-Dienamide | C16H29O2 | Icosatrienoic acid | Nutraceuticals, fungal metabolites |
| 21. | Bicyclo[10.1.0]Tridec-1-Ene | C13H22 | Saturated aliphatic hydrocarbon | Cosmetics, lubricants, petrochemical industry |
| 22. | Octadecanoic Acid | C18H36O2 | Saturated fatty acid | Anticancer |
| 23. | N-Isobutyl-(2E,4Z,8Z,10E)-Dodecatetraenamide | C16H25NO | Alcohol | Cosmetics, antifungal, antimicrobial |
| 24. | 1,2-Benzenedicarboxylic Acid, Dicyclohexyl Ester | C20H26O4 | Aromatic dicarboxylic acid | Antioxidant, antimicrobial |
| 25. | Pentadecanal- | C15H30O | Long chain fatty aldehyde | Antimicrobial agent, volatile oil |




e
f
g
h
i
j
k
l
m



**n**

**o**

**p**

**q**

**r**

**s**

**t**

**u**

**v**

**w**

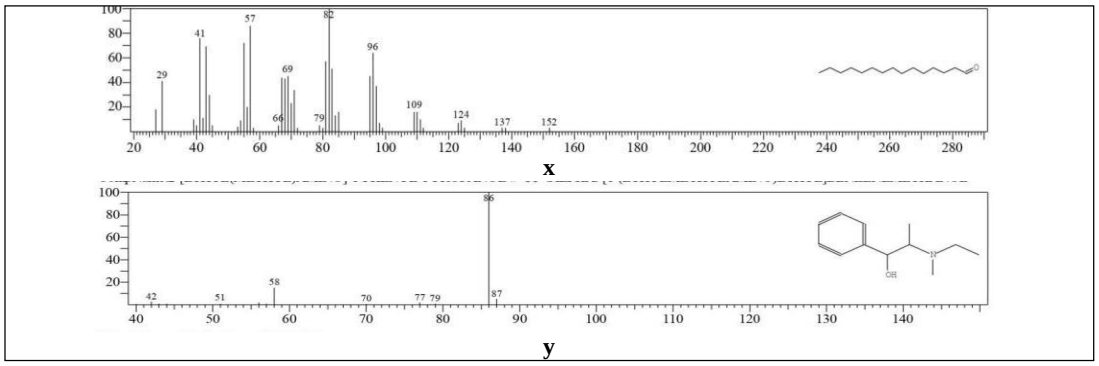



**Fig 1:** (a-y): Phytochemical compounds identified in the ethanolic extract of *Acmella calva* (DC.) R.K. Jansen using Gas chromatography – Mass spectrometry.

## Discussion

Evaluation of the ethanolic extract of *A. calva* whole plant showed positive results for the mass of 25 phytochemical compounds. Bestowed to the results, identified compounds are medicinally very potent to cure many infectious diseases. For instance, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Linolenic acid (R/T 29.34) possesses insectifuge, anti-inflammatory, cancer preventive, hypocholesterolemic, hepatoprotective, nematicide, antihistaminic, antiacne, antieczemic, 5-alpha reductase inhibitor, antiarthritic, antiandrogenic and anticoronary properties. n-Hexadecanoic acid-palmitic acid (R/T 26.6) can be an hypocholesterolemic, antioxidant, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Similar findings were reported by Patil and Wanjare (2017) [10] in acetone and ethanol stem extract of *F. hispida.* They inferred that the presence of 15 different phytochemical compound in the mass spectra.

Phytol is one among the twenty five compounds obsereved in the present study, which possess antibacterial activities. Our results finds support from Maria Jancy Rani *et al.,* (2011) [11] and Sridharan *et al*., (2011) [12]. They stated that, the compound phytol is a key acyclic diterpene alcohol that is a precursor for vitamins K1 and E. Phytol- Diterpene (R/T 28.9) is an anticancer, antimicrobial, anti-inflammatory, antifungal and diuretic agent (Praveen Kumar *et al*., 2010 and Sarumathy *et al*., 2011) [13, 14]. Hexadenoic acid is a staturatted fatty acid which has an antimicrobial activity has been identified in the peresnt investigation. Similar compound was reported in alcohol leaves extract of *Melissa officinalis (*Sharafzadeh *et al.,* 2011) [15] and *Kigelia pinnatai* (Grace *et al*., 2002) [16].

## Conclusion

The present investigation demonstrated the presence of 25 phytochemicals from the ethanolic extract of whole plant of *A. calva*. The presence of those phytochemicals may be liable to display various biological activities in the ethnopharmacological uses of the plant for the treatment of assorted diseases. Further, isolation and biological evaluation of these compounds are assured for the drug discovery as well as to authorize the traditional use of this plant. From this result, it is concluded that, the ethanolic extract of *A. calva* having the effective potential compounds, which may leads to the formation of effective drug for cariogenic disease.

## Reference

1. Sathya B, Indu H, Seenivasan R, Geetha S. Influence of seaweed liquid fertilizer on the growth and biochemical composition of legume crop, Cajanus cajan (L.) Mill sp. Journal of Phytology, 2010, 2(5).
2. Balaji V, Man B, Surya N, Prasad VM. Effect of dried powder of Tulsi leaf, ginger and cardamom addition on Aonla jam with granulated particles of cashew. Environment and Ecology,2012:30(3A):635-640.
3. Panda A, Parida AK, Rangani J. Advancement of metabolomics techniques and their applications in plant science: Current scenario and future prospective. In Plant Metabolites and Regulation Under Environmental Stress, 2018, 1-36.
4. Andrew RL, Wallis IR, Harwood CE, Henson M, Foley WJ. Heritable variation in the foliar secondary metabolite sideroxylonal in Eucalyptus confers cross-resistance to herbivores. Oecologia,2007:153(4):891-901.
5. Priyadharshni K, Dr. P Shanthi. In-Silico Modelling And Molecular Docking Studies Of A Novel Compound Isolated From Acmella calva (DC.) R.K. Jansen,2019:9(1):3776-3781.
6. Farooqui AH, Jain SP, Shukla YN, Ansari SR, Kumar S. Medicinal plants in oral health care in India. J Med Arom Pl Sci,1998:20:441-50.
7. Ganesan CM, Paulsamy S. Mass propagation of a threatened medicinal plant, Artemisia nilagirica (CB Clarke) Pampan inhabiting high hills of Nilgiris, the Western Ghats. Ind J Fund Appl Life Sci,2011:1(3):14-21.
8. Rajput AP, Rajput TA. Isolation of Stigmasterol and β-Sitosterol from chloroform extract of leaves of Corchorus fascicularis Lam. International Journal of biological chemistry,2012:6(4):130-5.
9. Janakiraman N, Johnson M, Sahaya SS. GC-MS analysis of bioactive constituents of Peristrophe bicalyculata (Retz.) Nees. (Acanthaceae). Asian Pacific Journal of Tropical Biomedicine,2012:2(1):S46-S49.
10. Patil US, Wanjare PD. Antimicrobial activity in fruit extract of Psoralea corylifolia L. on pathogenic organisms. Int. J. Appl. Res,2017:3(7):275-260
11. Maria Jancy Rani P, Kannan PSM, Kumaravel S. GC-MS Analysis of *Lantana camara* L. Leaves. JPRD,2011:2(11):63-66.
12. Sridharan S, Meenaa V, Kavitha V, Agnel Arul John Nayagam. GC-MS study and phytochemical profiling of *Mimosa pudica* Linn. J.Pharm. Res,2011:4(3):741-742.
13. Praveen Kumar P, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS

study of Vitex negundo. Afr. J. Biochemistry Res,2010:4(7):191-195.
14. Sarumathy K, Vijayayakanthia T, Dhana Rajan MS. A Protective effect of *Caesalpinia sappan (CS)* on acetaminophen induced Nephrotoxicity and oxidative stress in male *albino* rats. J. Pharmacology and Toxilcology,2011:1(2):11-21.
15. Sharafzadeh S, Morteza Khosh-Khui, Javidnia K. Aroma Profile of Leaf and Stem of Lemon Balm (*Melissa Officinalis* L.) Grown under Greenhouse Conditions. Advan. Environmental Biol,2011:5(4):547-550.
16. Grace OM, Light ME, Lindsey KL, Moholland DA, Staden JV, Jader AK. Antibacterial activity and isolation of antibacterial compounds from fruit of the traditional African medicinal plant, *Kigelia africana*. S. Afr. J. Bot,2002:68:220-222.
17. Hussain SZ, Maqbool K, Naseer B. High performance thin layer chromatography: Principle, working and applications. Int J Res Pharm Pharm Sci. 2019;4:83-8.