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EXPLORING THE BIOACTIVE COMPOUNDS OF ACALYPHA INDICA THROUGH GAS CHROMATOGRAPHY-MASS SPECTROMETRY: SHEDDING LIGHT ON ITS MEDICINAL POTENTIAL

Dr. Sethuramani A.[1*], Sasikumar A.[2], Raxshiya Smily J.[2], Chandrasamy M.[2], Dr. Venkata Rathina Kumar T.[3], Dr. Abdul Hassan Sathali A.[4]

[1]Assistant professor, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India.

[2]PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India.

[3]Head of the Department, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India

[4]Principle, Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India.

***Corresponding Author: Dr. Sethuramani A.**

Assistant professor, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India.

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ABSTRACT

Copper leaf, scientifically known as *Acalypha indica*, a member of the Euphorbiaceae family, has long been used in Indian medicine, particularly in Ayurveda. This herb exhibits antiinflammation, analgesic, antipyretic, and antispasmodic properties. Historical uses of *Acalypha indica* include treating fever, inflammation, pain, and gastrointestinal conditions. As a traditional healing method, its leaves are crushed and applied topically to wounds to reduce inflammation and prevent infection. Furthermore, it regulates menstrual cycles and relieves menstrual cramps. GC-MS analysis of ethanolic extracts of *Acalypha indica* leaves was performed in the present study in order to identify bioactive compounds. Several different compounds were found in the substance, including phenols, terpenoids, alkaloids, carboxylic acids, aromatics, nitro compounds, and esters. In general, terpenoids, fatty acids, and phenols were found to be the most prevalent compound classes, followed by other cyclic compounds. There was a higher retention time for DL-alpha-tocopherol (37.8324), Cholesterol (37.6496), Gamma tocopherol (36.6378) and Lupeol (35.2932). The extract also contains triterpenoids, such as beta-amyrin, obtisifolid, and lupeol, which may be beneficial for the management of migraines. In addition to providing significant implications for holistic wellness across a wide range of health domains, *Acalypha indica* demonstrates its versatility as a herbal remedy.

KEYWORDS: GC-MS bioactive compounds, *Acalypha indica*, Terpenoids, Cyclic compounds, Herbal remedy, Traditional medicines.

INTRODUCTION

Indian copperleaf, scientifically known as *Acalypha indica* and belonging to the Euphorbiaceae family, holds a significant place in traditional Indian medicine, particularly within Ayurveda. With a history steeped in medicinal use, it offers a spectrum of therapeutic benefits, including anti-inflammatory, analgesic, antipyretic, and antispasmodic properties.[1] Historically, *Acalypha indica* has been employed to address various ailments such as fever, inflammation, pain, and gastrointestinal issues. Traditionally, its leaves are crushed and applied topically to wounds to facilitate healing by reducing inflammation and preventing infections. Moreover, it plays a role in regulating menstrual cycles and easing menstrual cramps.[2] In a recent research endeavor, scientists aimed to unveil the bioactive compounds present in the ethanolic extract of *Acalypha indica* leaves through Gas Chromatography-Mass Spectroscopy (GC-MS).

MATERIAL AND METHODS

GC –MS ANALYSIS

EEGG was analysed by GC-MS

Make: Perkin Elmer

GC model: Clarus 680

Mass Spectrometer: Clarus 600 (EI)

Software: Turbo Mass ver 5.4.2

Instrumentation Acquisition Parameters

Oven: Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 minutes

Total Run Time: 32 minutes
Injected Temperature: 260°C
Volume: 1 µL
Split: 10:1
Flow Rate: 1 mL/minute
Carrier Gas: Helium
Solvent Delay: 1min
Column: Fused silica column, Elite-5MS (30.0m, 0.25mmID, 250µm df)
Transfer line temperature: 210 °C
Ion source temperature: 210 °C
Scan time:0.2 sec, 40 to 600Da
Scan interval:0.1 sec

PROCEDURE

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min), followed by 300 °C at the rate of 10 °C min−1, and 300 °C, where it was held for minutes. The mass detector conditions were: transfer line temperature 240 °C, ion source temperature 240 °C and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Gas Chromatography-Mass Spectrometry (GC-MS) is a versatile analytical instrument utilized in various fields, including chemistry, biochemistry, pharmacology, and forensic science. By combining the separation capabilities of gas chromatography with the detection and identification abilities of mass spectrometry, GC-MS allows for the accurate detection and identification of a wide range of compounds present in complex mixtures. Its remarkable sensitivity and selectivity make it indispensable for both qualitative and quantitative analyses of organic substances, spanning drugs, environmental pollutants, natural products, and metabolites.

In our research findings speculate nearly 203 compounds are identified. These compounds are separated based on its class as follows;

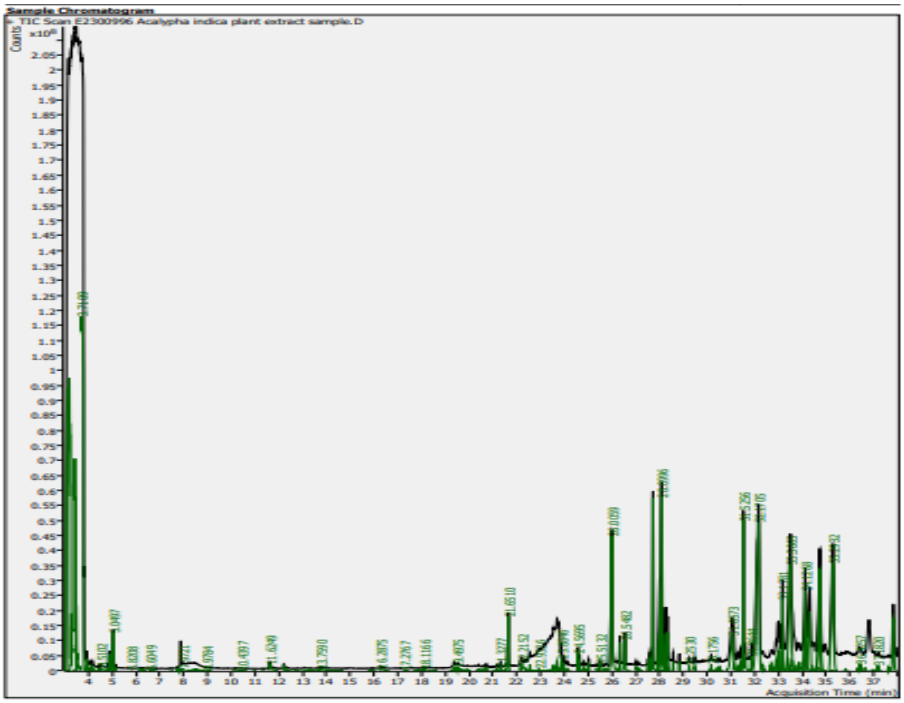

Figure no. 1: Graphical representation of GCMS Compounds.

Table 1: Phenol Class of Compound.

SI.NO	COMPOUND	TYPE	MOL. FORMULA	M.W	R.T	AREA
1.	Phenol	Phenol	C6H6O	94	7.8218	603878.7
2.	1-Pentanone, 1-(4-methylphenyl)-	Phenol	C12H16O	176	10.9890	203842.2
3.	t-Butyldiphenyl(prop-2-ynyloxy)silane	Phenol	C19H22OSi	294	15.9122	212290.1

4.	Phenol, 5-ethenyl-2-methoxy-	Phenol	C9H10O2	150	16.2875	5277246.6
5.	Phenol, 2,6-dimethoxy-, acetate	Phenol	C10H12O4	196	17.3505	557972.7
6.	Phenol, 3,5-bis(1,1-dimethylethyl)-	Phenol	C14H22O	206	20.3960	1327918.8
7.	Butylated Hydroxytoluene	Phenol	C15H24O	220	20.4114	922762.8
8.	Phenol, 4-ethenyl-2,6-dimethoxy-	Phenol	C10H12O3	180	21.2446	462104.8
9.	4-Cyanophenol, TBDMS derivative	Phenol	C13H19NOSi	233	31.7672	893654.0

Table 2: Terpenoid Class of Compound.

SI.NO	Compound	Type	Mol. Formula	M.W	R.T	Area
1.	Limonene	Terpenoid, Monoterpene.	C10H16	136	8.7681	275379.7
2.	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	Terpenoid	C18H30O	262	25.4734	2166125.9
3.	Phytol	Diterpene alcohol	C20H40O	296	27.7322	114915265.5
4.	Phytol	Diterpene alcohol	C20H40O	296	29.2530	3226224.5
5.	Squalene	Triterpenoid	C30H50	410	30.4512	1942221.2
6.	Obtusifoliol	Triterpenoid	C30H50O	426	32.9032	15851928.6
7.	beta.-Amyrin	Triterpenoid	C30H50O	426	34.1268	111362484.3
8.	Squalene	Triterpenoid	C30H50	410	34.1284	56028950.2
9.	Lup-20(29)-en-3-one	Triterpenoid	C30H48O	424	34.7430	207805909.2
10.	Lupeol	Triterpenoid	C30H50O	426	35.2932	252845763.2
11.	Campesterol	Phytosterol	C28H48O	400	31.0573	96577922.7
12.	Stigmasterol	Phytosterol	C29H48O	412	32.1705	410937850.5
13.	gamma.-Sitosterol	Phytosterol	C29H50O	414	33.5065	253661396.5
14.	Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-	Sterol	C29H48O	412	33.8842	15598908.8
15.	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, (3.beta.,4.alpha.,5.alpha.)-	Sterol	C30H50O	426	34.3097	127650748.6
16.	.alpha.-Tocospiro A	Vitamin-E	C29H50O4	462	34.4233	6914063.2
17.	.alpha.-Tocospiro A	Vitamin-E	C29H50O4	462	34.6337	5770057.3
18.	gamma.-Tocopherol	Vitamin-E	C28H48O2	416	36.6378	3303392.4
19.	Cholesterol	Sterol	C27H46O	386	37.6496	3204293.9
20.	dl-.alpha.-Tocopherol	Vitamin-E	C29H50O2	430	37.8324	71847273.6

Table 3: Alkaloid Class of Compound.

SI.NO	COMPOUND	TYPE	MOL. FORMULA	M.W	R.T	AREA
1.	1-(1'-pyrrolidinyl)-2-butanone	Psychoactive Alkaloid	C8H15NO	141	13.0619	2341174.8

Table 4: Fatty Acid Class of Compounds.

SI.NO	COMPOUND	TYPE	MOL. FORMULA	M.W	R.T	AREA
1.	Tetradecanoic acid	Saturated Fatty acids.	C14H28O2	228	23.6946	13350071.6
2.	n-Hexadecanoic acid	Saturated Fatty acids (palmitic acid).	C16H32O2	256	26.0059	191060256.2
3.	Hexadecanoic acid, ethyl ester	Saturated Fatty acids.	C18H36O2	284	26.3391	18877153.9
4.	Heptadecanoic acid	Saturated Fatty acids (margaric acid).	C17H34O2	270	27.1284	2145628.7
5.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Mono unsaturated Fatty acids.	C19H34O2	294	27.5254	4503730.0
6.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Mono unsaturated Fatty acids.	C19H32O2	292	27.6023	8300320.4
7.	Methyl stearate	Fatty acids.	C19H38O2	298	27.8629	679051.4
8.	9-Octadecenoic acid, (E)-	Mono unsaturated	C18H34O2	282	28.0825	119711326.7

		Fatty acids.				
9.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Mono unsaturated Fatty acids.	C18H30O2	278	28.0996	268986995.0
10.	Octadecanoic acid	Mono unsaturated Fatty acids.	C18H36O2	284	28.2666	30167555.4
11.	(E)-9-Octadecenoic acid ethyl ester	Mono unsaturated Fatty acids.	C20H38O2	310	28.3227	13283796.2
12.	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	Mono unsaturated Fatty acids.	C20H34O2	306	28.3352	29627977.7
13.	Octadecanoic acid, ethyl ester	Mono unsaturated Fatty acids.	C20H40O2	312	28.5767	5578146.7
14.	9-Octadecenoic acid (Z)-, pentyl ester	Mono unsaturated Fatty acids.	C23H44O2	352	32.9987	17728234.8
15.	Octadecanoic acid, 2,3-dihydroxypropyl ester	Mono unsaturated Fatty acids.	C21H42O4	358	33.1781	49161500.3

Table 5: Biological Activities of The Selected Compounds.

COMPOUNDS	BIOLOGICAL ACITIVITY
Phytol	Anti- oxidant and Anti- Inflammatory properties
Beta amyrin	Anti- oxidant and Anti- Inflammatory properties
Campesterol	Cholesterol lowering agent
Stigmasterol	Cholesterol lowering agent
.gamma.-Sitosterol	Cholesterol lowering agent
Squalene	Used in skincare and in the production of antibodies.
.alpha.-Tocospiro A (Vit-E)	Anti oxidant
Lupeol	Anti mirobial, anti oxidant Anti flammatory and anti cancer properties.
Gamma tocoperol	Anti oxidant
Cholesterol	Essential components of cell membrane
dl-.alpha.-Tocopherol	Anti oxidant
Obtusifolid	Anti- oxidant and Anti- Inflammatory properties
9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl-, (3.beta.,4.alpha.,5.alpha.)-	Anti- oxidant, Anti- Inflammatory properties
Neophytadiene	Analgesic, Antipyretic, Antioxidant and Anti inflammatory properties.

RESULTS

A total of 203 compounds were identified in the GC-MS analysis of the ethanolic extract of *Acalypha indica*. Based on their peak areas, the molecular formulae, molecular weights, and retention times of these compounds were determined. WEILY and NIST library's GC-MS software was used to analyze spectral data of these bioactive compounds.

In the analysis, terpenoid, fatty acid, and phenol compounds made up the majority of the compounds, with smaller proportions of other cyclic compounds detected. There was a range of retention times (RTs) between 8.7681 and 37.8324 observed for terpenoids, while a range of RTs between 7.8218 and 31.7672 was observed for phenols. The elution of fatty acids occurred at RT values between 23.6946 and 33.1781.

According to the data, the major compound classes were predominantly distributed within the retention time ranges of 20-37, including terpenoids, phenols, and fatty acids. The highest retention time was registered by dl-.alpha.-Tocopherol at 37.8324, followed by phospholipids at 37.6496, gamma tocopherol at 36.6378, and luteol at 35.2932.

This study demonstrates that ethanolic extracts of *Acalypha indica* contain a diverse composition of bioactive compounds, including terpenoids, fatty acids, and phenols. Lupeol, gamma tocopherol, and dl-alpha.-Tocopherol, along with dl-.alpha.-Tocopherol, support the potential pharmacological significance of this plant extract.

DISCUSSION

Almost 203 phytobioactive compounds were identified by our analysis, which was matched against findings from previously cited literature.[3-6] It should be noted that 1-H-Pyrrole-2,5-dione-ethyl corresponds with previous findings, while Hexadecane, as detected, also appears in our study.[3,5,6,7] The findings of our investigation, however, revealed the presence of additional compounds not previously reported in these books. Among them are a large amount of terpenoids and

phenols.[8] There was no mention of terpenoids or phenols in previous reports, which contrasts with the current study. In order to explain the presence of fatty acids in EEAI, in addition to the alkaloids, these studies infer that there is also the presence of fatty acids. Based on our own research, we demonstrated how GC-MS is capable of elucidating chemical relationships within complex mixtures of plant metabolites.

CONCLUSION

In the present study, Gas Chromatography and Mass Spectrometry analyses revealed a multitude of compounds with a variety of chemical structures, suggesting that these compounds may have potential pharmacological benefits. According to the results of these studies, ethanolic extracts of *Acalypha indica* (EEAI) can be used as a treatment for migraine headaches, particularly in the context of leveraging sesquiterpene compounds recognized for their efficacy in addressing neurodegenerative diseases. In addition, the extract of this plant contains a number of triterpenoids including beta-amyrin, obtisifolid, and lupeol, which have anti-inflammatory and antioxidant properties, which contribute to the management of migraine attacks. Consequently, *Acalypha indica* leaves give the opportunity for the creation of a variety of traditional medicinal products. It is vital to continue exploring in order to discover novel active compounds that may revolutionize treatment for various ailments that are currently incurable.

REFERENCES

1. Mohana Vamsi N, Venkata Sunil Kumar M, Kodandaram N, Padmanabha Reddy Y. Evaluation of Anti-inflammatory activity of *Acalypha indica.* Ind Pharm., 2008; 7: 89-91.
2. Badani *et al.* Antimicrobial, antioxidant activity and phytochemical screening of *Acalypha indica* crude leaf extract. Int. J. Pharm. Clin. Res., 1999; 8(6): 583-588.
3. Hussain, A.Z., Kumaresan, S., GC-MS analysis and antibacterial evaluation of *Acalypha indica*, Asian journal of chemistry, 2013; 25(17): 9701-9704.
4. Beena Joy, Molly Mathew, Anti-oxidant studies and chemical investigation of ethanol extract of *Acalypha indica* Linn. Recent Progress in Medicinal Plants, July 2010; 27(4): 165-198.
5. Chandra Mohan S, Dinakar S, Anand T, Elayaraja R, SathiyaPriya B Phytochemical, GC-MS analysis and Antibacterial activity of a Medicinal Plant *Acalypha indica* Int. J. PharmTech Res., 2012; 4(3).
6. Jagatheeswari D, Deepa J, Sheik Jahabar Ali H et al., *Acalypha indica* L- an important medicinal plant: a review of its important traditional uses, and pharmacological properties. Int. J Bot., 2013; 3: 19–22.
7. M Aminuar Rahman, Sitesh C Bachar and Mohammed Rahmatullah, Analgesic and Anti inflammoraty activity of methanolic extract of *Acalypha indica* L., Pak. J. Pharm. Sci., July 2010;p 23(3): 256-258.
8. Parastoo Karimi Alavijeh, Parisa Karimi Alavijeh and Devindra Sharma. Asian J Plant Sci. Res., 2012; 2(4): 496 – 502.