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PHYTOCHEMICAL SCREENING, FUNCTIONAL GROUPS IDENTIFICATION BY FT-IR AND GC-MS ANYALYSIS OF *CLERODENDRUM INFORTUNATUM* L. LEAVES EXTRACT

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Keywords:

Clerodendrum infortunatum L., GC-MS, FTIR, Flavonoids., *etc*

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ABSTRACT: The present investigation focuses on analysis of the active compounds present in the *Clerodendrum infortunatum* L. leaves extract of different solvents as Petroleum ether and methanol with help of the advance tools like GC-MS and FTIR which posses different pharmacological activities like wound healing, anti-inflammatory, anyalgesic, *etc*. The leaves were treated with petroleum ether followed by a 50% methanolic extract. The extractability percentage of the petroleum extract was 5.71%, whereas the methanolic extract had a value of 21.88%. The phytochemical study of extracts indicated the presence of alkaloids, glycosides, carbohydrates, phytosterol, phenolic compounds, tannins, and flavonoids. Fourier Transform Infrared Spectroscopy (FT-IR) analysis revealed functional groups of phenols, alcohols, alkane, carboxylic acids, and their esters, as well as primary amines and proteins in both 50% methanolic and petroleum ether extract. GC-MS analysis revealed presence of 51 compounds in petroleum ether and 27 compounds in 50% methanolic extract.

INTRODUCTION: It is being increasingly recognized that ethnopharmacology, which largely uses natural materials such as herbs and minerals, can contribute as a discovery engine to provide new leads and also offer quality-assured and standardized traditional medicines. There is a clear movement to build a golden triangle between traditional medicine, modern medicine, and modern science [17]. Since, ancient times, people in India have treated different diseases and wounds with natural substances derived from plants. This practice is known as *Ayurveda*, a popular branch of Indian medicine.

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Natural goods have been utilized for generations in many regions of the world; because of their relatively low side effects, natural products are starting to gain the same importance as alternative medicine. For these reasons, scientific research is being done on traditional and natural remedies to improve animal health. For the purpose of treating chronic illnesses, they are used directly as drugs in their crude or raw form [5].

FIG. 1: THE PLANT *CLERODENDRUM INFORTUNATUM*

Pharmacological research on *Clerodendrum infortunatum* L. shows that this plant has enormous promise for treating a wide range of ailments, including diabetes, malaria, coughs, wounds, and inflammation. Additionally, *Clerodendrum infortunatum* L. demonstrates anti-inflammatory, antibacterial, antioxidant, anti-diabetic, wound-healing, anti-venom, and anti-fertility properties [25].

A vast genus of flowering plants, including herbs, shrubs, and small trees, *Clerodendrum* is a member of the Lamiaceae family and is found worldwide in tropical climates. For the first time, Linnaeus described the genus. Butterflies and hummingbirds frequent the plant blooms, while certain insect larvae eat specific types of *Clerodendrum*. Numerous species of the genus have been used in traditional medicine across multiple nations. The pharmacological characteristics and phytochemical content of a select few species have been thoroughly investigated. *Clerodendrum* species have shown pharmacological qualities such as antibacterial, anticancer, antimalarial, antioxidant, antidiabetic, larvicidal, and antidiarrheal activity [26].

The plant *Clerodendrum infortunatum* is known as Bhandir in Sanskrit and an old Sanskrit verse; describe its use in fever, skin diseases, rheumatoid arthritis, worm infestation, diabetes, bleeding disorders, *etc* [1]. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, amino acids, phytosterol, phenolic compounds, tannins, resins, and flavonoids in the 50% ethanolic and 50% methanolic extracts of *C. infortunatum* leaves. In dogs, the 10% concentration of methanolic extract ointment of *C. infortunatum* proved to be more effective than both the ethanolic extract ointment of *C. infortunatum* and the Povidone Iodine ointment [24]. Nowadays, there is a desire to stop the spread of antibiotic resistance and the growing importance of the health economy has a significant impact on the development of topical medications for wound healing. Topical medications need ways to guarantee that the active ingredient is sufficiently bioavailable inside the wound, such as on-site production and/or decreased degradation. In industrialized nations, chronic, non-healing skin lesions account for more than 3% of healthcare spending, and their frequency is on the rise. Two concurrent themes are now influencing advancements in the field of wound healing: the desire to stop the spread of antibiotic resistance and the growing use of value-based and health economics models. The development of new drugs that try to speed up wound healing frequently draws inspiration from the past. For example, plants that were traditionally used in Ayurvedic medicine to treat wounds are now being scientifically evaluated for medicinal ingredients, but those ingredients are now available in clinics [19].

MATERIALS AND METHODS:

Collection and Authentication of Plant Material and Preparation of Extracts: *C. infortunatum* leaves were procured from region of Wardha, Nagpur and Go- Vigyan Anushandhan Kendra, Devlapar, Nagpur. The collected plant material was identified as *Clerodendrum infortunatum* by the expert botanists of Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University (RTMNU), Nagpur. The authenticated herbarium sheet with voucher specimen number (261, 02/12/2023).

Properly cleaned, dried, and powdered leaves of the *Clerodendrum infortunatum* plant were subjected to extraction as per the method described by Sheel [25]. The accurately weighed powder was first defatted with petroleum ether in jumbo Soxhlet's apparatus at approximately 80°C. The defatted leaf powder of the plant material was air dried and further subjected to 50% methanolic extraction (50 parts methanol with 50 parts distilled water) to obtain the desired fraction or extraction. For later usage, the extract was collected on clean, sterile pre-weighed petri plates and kept in an airtight desiccator for further use.

The percent extractability of extract was determined with following formula:

$$\% \text{ Extractability} = \text{Weight of extract (gm)} / \text{Weight of powder used (gm)} \times 100$$

Qualitative Phytochemical Analysis, GC-MS analysis and (FT-IR) analysis of the Extracts: To determine the presence or absence of various phytoconstituents, the ethanolic and methanolic extracts of *Clerodendrum infortunatum* were subjected to a preliminary qualitative phytochemical examination using the technique outlined by [8, 27, 28]. The characteristics of functional groups in 50% hydromethanolic and petroleum ether extract of the leaf of *C. infortunatum* were identified using an FTIR spectrophotometer. It provides information about the structure of a molecule that could frequently be obtained from its absorption spectrum. 10 mg of plant extracts of the leaf were taken in a KBr vessel and placed in a sample cup of a diffuse reflectance accessory. A small quantity of the *C. infortunatum* extracts was mixed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory BRUKER, Alpha II, FT-IR spectrophotometer was used to identify the functional group available in the extracts. The leaf extracts of *C. infortunatum* were scanned from 400 to 4000 cm^{-1} for 16 times to increase the signal-to-noise ratio. The peak values of the FTIR were recorded. The GC-MS analysis of extracts of leaves of *C. infortunatum* was carried out using SHIMADZU GCMS-QP2020 with a quadra pol 2020 MS detector. The capillary column was GCMS-QP2020 (30 m 250 µm 0.25 µm) composed of 5% phenyl methyl silox. The initial oven temperature was 40ºC for 1 minute which was raised at a rate of 20ºC/min up to 150ºC for 1 minute and then at a rate of 3ºC/min up to 280ºC for a hold time of 10 min. The injector volume was 4 µl. The gas was used as the carrier with a constant flow rate with a split ratio of 25:0. The MS operating conditions were; source temperature of 230ºC (max 250ºC), quad temperature of 150ºC (max 200ºC), solvent delay time of 3 min. Compounds were identified in terms of RT values and mass spectra with those obtained from the NIST search library. The obtained compounds were searched for detailed information.

RESULTS AND DISCUSSION:

Extraction Details: The leaves of *Clerodendrum infortunatum* were shade-dried, crushed, and defatted with petroleum ether and being extracted with Soxhlet's apparatus for 50% methanol. Table 1 shows the extract's physicochemical properties and extractability percentage.

TABLE 1: EXTRACTABILITY PERCENTAGE AND PHYSICAL CHARACTERS OF PETROLEUM ETHER AND 50% METHANOLIC EXTRACT OF *CLERODENDRUM INFORTUNATUM* LEAVES

Sr. no.	Content	Petroleum ether Extract	50% methanolic extract
1	Solvent used	Petroleum ether	50% methanol
2	Quantity of dried leaves of *Clerodendrum infortunatum*	2186 gm	1947 gm
3	Quantity of leaves after extraction	1947 gm	1366 gm
4	Extract prepared	124 gm	426.15 gm
5	Colour	Greenish brown	Brownish
6	Consistency	Semi-solid	Semi-solid
7	Extractability	5.71%	21.88%

In the current investigation, the percentage extractability of petroleum ether and 50% methanolic extract was 5.71% and 21.88%, respectively. They found 4.8% and 5.46% extractability in a petroleum ether extract of *C. infortunatum* leaves [2, 9]. Similarly, Das, Prabhu and Baid showed extractability percentages of 13.50%, 17.07%, 15.55%, and 7.04% for various concentrations of methanolic extracts of *C. infortunatum* leaves [6, 24, 2].

Qualitative Phytochemical Analysis of Petroleum Ether and 50% Methanolic Extract: A preliminary qualitative phytochemical analysis of the petroleum ether and 50% methanolic extracts of *Clerodendrum infortunatum* leaves was carried out. **Table 2** shows the findings obtained when determining the presence of particular phytoconstituents and active principles.

TABLE 2: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF PETROLEUM ETHER AND 50% METHANOLIC EXTRACT OF LEAVES OF *CLERODENDRUM INFORTUNATUM*

Sr. no.	Active principle	Test performed	Observation	Result	
				Petroleum ether	50% methanolic
1	Alkaloids	Mayer's test	No white or creamy precipitate has	Negative	Negative

			ever formed.		
2	Carbohydrates	Fehling's test	Occurrence of red precipitate	Positive	Positive
3	Glycosides	Borntrager's test	Creation of the colour pink	Positive	Positive
4	Saponins	Foam test	Foam Developed	Positive	Positive
5	Proteins and Amino acids	Biuret test	No formation of pink colour in methanolic layer	Negative	Negative
6	Phytosterol	Salkowski's test	Formation of red colour in chloroform layer and greenish yellow colour in lower layer	Positive	Positive
7	Phenolic compounds and tannins	Lead acetate test	Formation of bulky white precipitate	Negative	Positive
8	Fixed oils and fats	Saponification test	Formation/No formation of soap or partial neutralization of alkali	Positive	Negative
9	Resins	Test for resins	Appearance of turbidity	Positive	Positive
10	Flavonoids	Test for flavonoids	Formation of red or pink colour precipitate	Positive	Positive

Preliminary phytochemical analysis of a petroleum ether extract of leaves revealed the presence of carbohydrates, flavonoids, glycosides, phytosterol, resins, fixed oils, and fats, while 50% methanolic extracts of *C. infortunatum* leaves revealed the presence of alkaloids, carbohydrates, glycosides, amino acids, phytosterol, phenolic compounds, tannins, resins, and flavonoids. Sheel, and Ram found alkaloids, steroids, flavonoids, carbohydrates, and tannins in a methanolic extract of *C. infortunatum*. In a broader sense, phytochemistry is concerned with the vast array of organic compounds that plants create and accumulate [24, 25]. Medicinal herbs have therapeutic qualities because they contain various complex chemical compounds with diverse compositions that occur as secondary plant metabolites in one or more parts of these plants [20]. Preliminary phytochemical investigation of different extracts of leaves of *C. infortunatum* L. shows the presence of sterols, carbohydrates, tannins, terpenoids, flavonoids, and saponin indicating the plant is a prominent model for various types of pharmacological activity [31].

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis of Extracts: FTIR is a powerful versatile and non-destructive analytical technique used for the chemical characterization of different compounds and can provide fundamental information on the molecular structure of organic compounds in plant extracts. The infrared spectrum of gallic acid, quercetin, rutin, and tannic acid in the frequency range of 4000–400 cm^{-1} was obtained to identify the characteristic absorption peaks corresponding to stretching vibrations of different functional groups are shown in Fig. and Fig. The IR spectrum petroleum ether and 50% methanolic extract displayed different peaks corresponding to different functional groups present in *C. infortunatum* in **Table 3** and **4.**

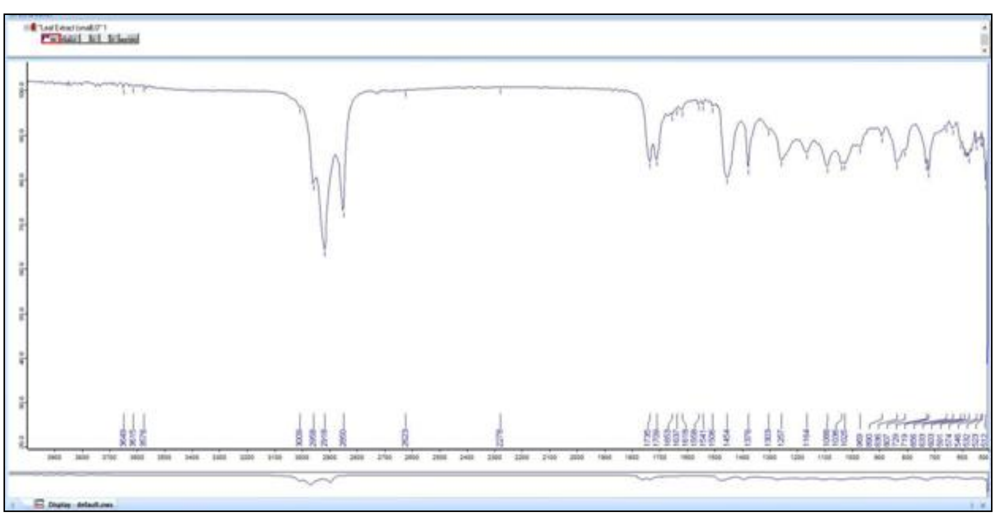

FIG. 2: FT-IR SPECTRA OF PETROLEUM EXTRACT OF *CLERODENDRUM INFORTUNATUM*

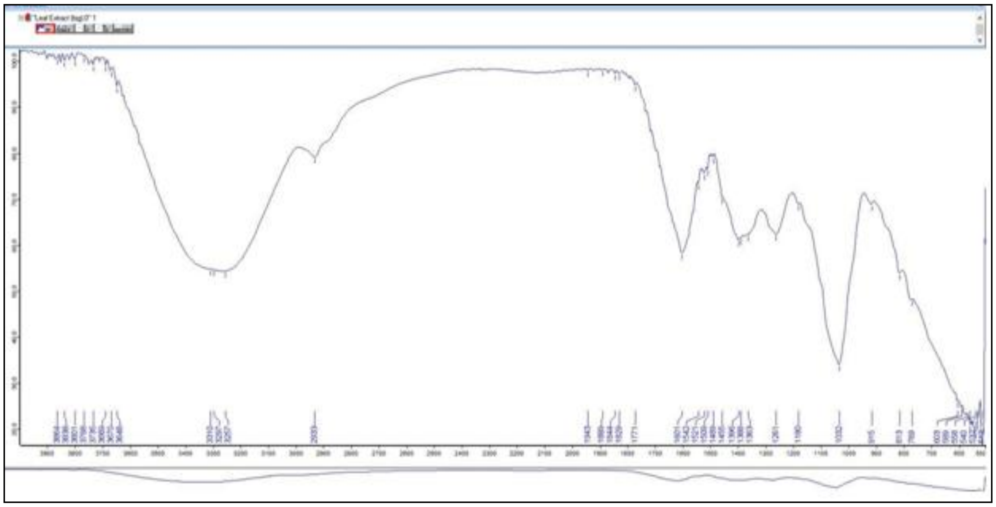

FIG. 3: FT-IR SPECTRA OF 50% METHANOLIC EXTRACT OF *CLERODENDRUM INFORTUNATUM*

TABLE 3: ALL POTENTIAL BANDS, CORRESPONDING FUNCTIONAL GROUPS, AND POSSIBLE COMPOUNDS IDENTIFIED IN THE PETROLEUM ETHER EXTRACT OF THE FORMULATION USING FT-IR SPECTROSCOPY

Sr. no.	Characteristic absorptions (cm^{-1})	Type of bonds	Functional group	Type of vibration	Intensity	Possible Compounds
1	3649-3009	O-H	Alcohol	Stretch, free	Strong, sharp	Phenols, Alcohols
2	2958-2623	C-H	Alkane	Stretch	Strong	
3	2278	C=O	Aliphatic ketone	Stretching		
4	1735-1653	C=O	Carbonyl	Stretch	Strong	Carboxylic acids and their esters
5	1637-1558	N-H	Amide	Bending	-	
6	1541-1454	N-O	Nitro	Stretch	Strong, two band	Primary amines and proteins
7	1376-729	-C-H	Alkane	Bending	Variable	
8	719-523	=C-H	Alkene	Bending	Strong	

TABLE 4: ALL POTENTIAL BANDS, CORRESPONDING FUNCTIONAL GROUPS, AND POSSIBLE COMPOUNDS IDENTIFIED IN THE 50% METHANOLIC EXTRACT OF THE FORMULATION USING FT-IR SPECTROSCOPY

Sr. no.	Characteristic absorptions (cm^{-1})	Type of bonds	Functional group	Type of vibration	Intensity	Possible Compounds
1	3864-3668	O-H	Alcohol	Stretch, free	Strong, sharp	Phenols, Alcohols
2	2933	C-H	Alkane	Stretch	Strong	
3	2278	C=O	Aliphatic ketone	Stretching		
4	1771	C=O	Carbonyl	Stretch	Strong	Carboxylic acids and their esters
5	1601	N-H	Amide	Bending	-	Primary amines
6	1509	C=C	Aromatic	Stretch	Medium- weak, multiple bands	Aromatic amines
7	1541-1454	N-O	Nitro	Stretch	Strong, two band	Primary amines and proteins
8	1388-1180	-C-H	Alkane	Bending	Variable	
9	769 -512	=C-H	Alkene	Bending	Strong	

The current FT-IR investigation petroleum ether and 50% methanolic extracts revealed presence of functional groups like Alcohol (O-H), Alkane (C-H), Aliphatic ketone (C=O), Carbonyl (C=O), Amide (N-H), Nitro (N-O), Alkene (=C-H) and Alcohol (O-H), Alkane (C-H), Carbonyl (C=O), Amide (R (C = O) N R 1 R), Nitro (N-O), Aromatic (C=C), Alkene (=C-H) resp. At different frequencies. FTIR bands in the 4000-1500 cm^{-1} range identified functional groups, whereas high absorption bands in the 1500-500 cm^{-1} area identified fingerprints. The O-H and C-H stretching frequencies were identified in the 3700-2500 cm^{-1} range, whereas the C-H stretching vibration occurred in the 2900-2800 cm^{-1} region, which corresponded to the fingerprint region [7]. 21 O-H and C-H stretching frequencies occur between 3700 and 2500 cm^{-1}, [21] whereas C-H stretching vibrations occur between 2900 and 2800 cm^{-1}. Thus, FTIR was utilized to determine the functional groups contained in various phytochemicals in plant samples.

The peak at 1734-1745 cm^{-1} was identified as C=O ester and may be connected to pheophytin and chlorophyll. In this investigation, the peak was at 1743 cm^{-1}. Carotenoids were anticipated to be present in *E. acoroides'* dried leaf powder. The FTIR spectrum at 1654 cm^{-1} indicates the C=O conjugate. The conjugated double bond in carotenoids has been identified as the structure responsible for light absorption. Prior research found that a peak at 1654 cm^{-1} indicates chlorophyll and protein content [22].

The FTIR spectra of *Clitoria ternatea* leaf extract indicated the existence of phenols and alcohols, with peaks at 3389.57 cm^{-1} representing the hydroxyl and O-H bonding frequencies, respectively. The peaks at 2925.41 cm^{-1} and 2856.66 cm^{-1} are ascribed to C-H stretching, indicating the presence of certain alkene compounds. The peak value of 1632.33 cm^{-1} indicates primary amines. The peak value of 1409.06 cm^{-1} indicates aromatic amines. The peak value at 1057.61 cm^{-1} verifies aliphatic amines, 926.50 cm^{-1} confirms carboxylic acids, and 869.00 cm^{-1} confirms primary and secondary amines [14].

Gas Chromatography- Mass Spectrophotometry (GC-MS) Analysis: GC-MS is one of the most reliable biophysical methods for phytochemical profiling and the plant *C. infortunatum* showed the presence of several bioactive phytoconstituents like Octadecane, hexadecanoic acid, ecosinane, *etc*. When different parts of the plant are analyzed with GC-MS technique [3, 11].

In the present study, the GC-MS analysis of 50% methanolic extract of *C. infortunatum* leaves revealed the presence of a total of 27 active phytocostitutes. The details of the compounds along with their synonyms, chemical formula, molecular weight, retention time, Peak Height, and area percentage (%) are listed in **Table 5** and **6**, its chromatogram is depicted in **Fig. 4** and **5.**

TABLE 5: DETAILS OF THE ACTIVE PRINCIPLES FOUND IN GC-MS ANALYSIS OF THE 50% METHANOLIC EXTRACT OF *C. INFORTUNATUM* LEAVES

Sr. no.	RT (Min.)	Compound Identified	Formula	Molecular weight	Peak Height	% Area
1	4.065	Nonane, 5-(1-methylpropyl)-	$C_{13}H_{28}$	184	1681386	0.09
2	4.065	Oxalic acid, 2-ethylhexyl hexyl ester	$C_{16}H_{30}O_4$	286		
3	8.763	Hexadecane	$C_{16}H_{34}$	226	1782240	0.21
4		Decane, 3,7-dimethyl	$C_{12}H_{26}$	170		
5		Undecane, 2,3-dimethyl-	$C_{13}H_{28}$	184		
6		Dodecane, 1-iodo	$C_{12}H_{25}I$	286		
7		Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212		
8	6.073	Tridecane, 1-iodo	$C_{13}H_{27}I$	310	2053940	0.20
9		Tetradecane	$C_{14}H_{30}$	198		
10		2-Bromotetradecane	$C_{14}H_{29}Br$	276		
11		Heptadecane, 8-methyl-	$C_{18}H_{38}$	254		
			$C_{17}H_{36}$	240		
12		Decane, 1-iodo	$C_{10}H_{21}$	268		
			$C_{13}H_{28}$	240		
13	4.905	Octadecane, 1-iodo-	$C_{18}H_{37}I$	380	2974787	0.26
14	4.436	Octane, 2-methyl	C_9H_{20}	128	1047240	0.08
15	4.323	Sulfurous acid, pentyl undecyl ester	$C_{16}H_{34}O_3S$	306	1625543	0.15
16	4.516	Naphthalene	$C_{10}H_8$	128	3797304	0.50
13		Disulfide, di-tert-dodecyl	$C_{24}H_{50}S_2$	402		
14	6.873	Eicosane	$C_{20}H_{44}$	282	3975321	0.39
15	6.994	Eicosane, 1-iodo-	$C_{20}H_{41}I$	408	3068789	0.31

16		Docosane, 1-iodo-	$C_{22}H_{45}I$	436		
17	8.554	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	9868886	1.33
18	40.22	Methyl nitrate	CH_3NO_3	77	960950	0.15
19	46.034	1,2-Propanediol, 3,3'-oxydi-, tetranitrate	$C_6H_{10}N_4O_{13}$	346	1097312	0.30
20	42.540	Nitrogen dioxide	NO_2	46	831450	0.08
21	42.540	Ethane, 1,1,1-trinitro	$C_2F_4N_2O_4$	192	1137602	0.08
22	43.286	Methane, fluorotrinitro-	CFN_3O_6	169	594462	0.18
23	46.084	Nitroglycerin	$C_3H_5N_3O_9$	227	1178183	0.38
24	57.260	Ethylene glycol, dinitrate	$C_2H_4N_2O_6$	152	627755	0.13
25	46.035	Nitroisobutanetriol trinitrate	$C_4H_6N_4O_{11}$	286		
26	47.611	Acetone-D6	C_3D_6O	64	895715	0.22
27	59.650	Nitroacetonitrile	$C_2H_2N_2O_2$	86		

TABLE 6: DETAILS OF THE ACTIVE PRINCIPLES FOUND IN GC-MS ANALYSIS OF THE PETROLEUM ETHER EXTRACT OF *C. INFORTUNATUM* LEAVES

Sr. no.	RT (Min.)	Compound Identified	Formula	Molecular weight	Peak Height	% Area
1		5,15-Dimethylnonadecane	$C_{21}H_{44}$	296		
2	4.175	2-Ethylbutyl isobutyl carbonate	$C_{11}H_{22}O_3$	202	32083825	1.59
3		3-Ethyl-3-methylheptadecane	$C_{20}H_{42}$	282		
4		Sulfurous acid, decyl hexyl ester	$C_{16}H_{34}O_3S$	306		
5	4.437	Cyclopentane, 1-pentyl-2-propyl	$C_{13}H_{26}$	182	27066827	0.45
6	4.470	Decyl octyl ether	$C_{18}H_{38}O$	270	27084804	0.84
7		9-Oxabicyclo[3.3.1]nonan-2-one, 6-hydroxy	$C_8H_{12}O_3$	156		
8	4.772	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	212	2824999	0.14
9		Undecane, 2-cyclohexyl	$C_{17}H_3$	238		
10		Decane, 2-cyclohexyl	$C_{16}H_{32}$	224		
10	4.534	Cyclohexane, undecyl	$C_{17}H_{34}$	238	18908580	0.25
11	4.633	Cyclohexane, 1,1'-(1-methyl-1,3-propanediyl	$C_{16}H_{30}$	222	8241137	0.43
12		Octane, 2-cyclohexyl	$C_{14}H_{28}$	196		
13	4.679	Trichloroacetic acid, decyl ester	$C_{12}H_{21}Cl_3O_2$	302	6282848	0.09
14	4.724	2-Nonanone	$C_9H_{18}O$	142	15762458	0.44
15		Oxetane, 2,2-dimethyl	$C_5H_{10}O$	86		
16		Pentane, 1-nitro	$C_5H_{11}NO_2$	117		
17		Pentanal, 2-methyl	$C_6H_{12}O$	100		
18		Eicosane, 2-cyclohexyl	$C_{26}H_{52}$	364		
19	4.856	3-n-Propyl-5-methylhexan-2-one	$C_{10}H_{20}O$	156	14592801	0.42
20		3,5-Heptanedione, 2,2,4,6-tetramethyl	$C_{11}H_{20}O_2$	184		
21	4.900	Oxirane, hexyl-	$C_8H_{16}O$	128	10328129	0.18
23		Butanoic acid, 5-hexenyl ester	$C_{10}H_{18}O_2$	170		
24	4.934	Tricosane-2,4-dione	$C_{23}H_{44}O_2$	352	34153155	0.50
25		Nonadecane-2,4-dione	$C_{19}H_{36}O_2$	296		
26	7.647	Decane, 5-ethyl-5-methyl	$C_{13}H_{28}$	184	4118577	0.12
27	6.098	Tetradecane	$C_{14}H_{30}$	198	15763469	0.37
28	7.178	Heptadecane, 2,6,10,15-tetramethyl	$C_{21}H_{44}$	296	11980218	0.30
29	4.982	Dodecane, 4-methyl	$C_{13}H_{28}$	184	14072236	0.20
30	5.063	2,4-Octanedione	$C_8H_{14}O_2$	182	33308771	0.62
31		Tricosane-2,4-dione	$C_{23}H_{44}O_2$	352		
32	7.032	Nonadecane-2,4-dione	$C_{19}H_{36}O_2$	296	5400936	0.16
33	5.187	Pentanal, 2,3-dimethyl	$C_7H_{14}O$	114	27754573	0.56
34	5.185	Morpholin-3-one, 2-hydroxy-2,5,5-trimethyl	$C_7H_{13}NO_3$	159		
35		Pentane, 1-(2-propenyloxy)	$C_8H_{16}O$	128		
36	5.240	2-Ethylbutyl 2-methylbutanoate	$C_{11}H_{22}O_2$	186	19695064	0.76
37	5.391	Dihydro citronellyl angelate	$C_{15}H_{28}O_2$	240	21629281	1.00
38		Dodecyl nonyl ether	$C_{21}H_{44}O$	312		
39		Carbonic acid, decyl nonyl ester	$C_{20}H_{40}O_3$	328		
40		5-Octadecanone	$C_{18}H_{36}O$	268		
41		3-Methyl-2-butenoic acid, pentadecyl ester	$C_{20}H_{38}O_2$	310		
42	5.469	4-Methyldocosane	$C_{23}H_{48}$		2835025	0.05

43		Behenyl chloride	$C_{22}H_{45}Cl$	344		
44	6.944	Eicosane	$C_{20}H_{42}$	282	9889704	0.31
45		Triacontane, 1-iodo	$C_{30}H_{61}I$	548		
46	8.771	Heneicosane	$C_{21}H_{44}$	296	10978822	0.36
47		Octacosane, 1-iodo	$C_{28}H_{57}I$	520		
48		Tetracosane, 1-iodo	$C_{24}H_{49}I$	464		
49	7.587	11-Methyltricosane	$C_{24}H_{50}$	338	5873144	0.22
50		13-Methylheptacosane	$C_{28}H_{58}$	394		
51	8.556	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	8147062	0.34

Previous GC-MS investigations of the methanolic extracts of the leaves of *Clerodendrum* spp. reported many similar phytoconstituents in the plant, such as Octacosane, 2-methy, Eicosane, Neophytadiene, Hexadecane, 2,4-Di-tert-butylphen, Tridecane, 1-iodo, 3Oxatricyclo [3.2.1.0(2,4)] Octane. The GC-MS analysis revealed 16 peaks of several phytoconstituents, including acetamide, N, N-carbonyl bis-, 4Pyranone, 2,3-dihydro-, alpha-D-Galactofuranoside, methyl 2,3,5,6-tetra-Omethyl-, glycerin, xylitol, N, N-Dimethylglycine, 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl-, Benzofuran,2,3-dihydro-,5-Hydroxymethylfurfural, 2(1H) Pyrimidinone, 1-methyl-,2, 4-Dihydroxy-5,6-dimethylpyrimidine, 3-Deoxyd-mannoic lactone, 1, 3-Methylene-d-arabitol, Orcinol, n-Hexadecanoic acid, Phenol,4,4'-(1-methyl ethylidene), *etc* [8, 18].

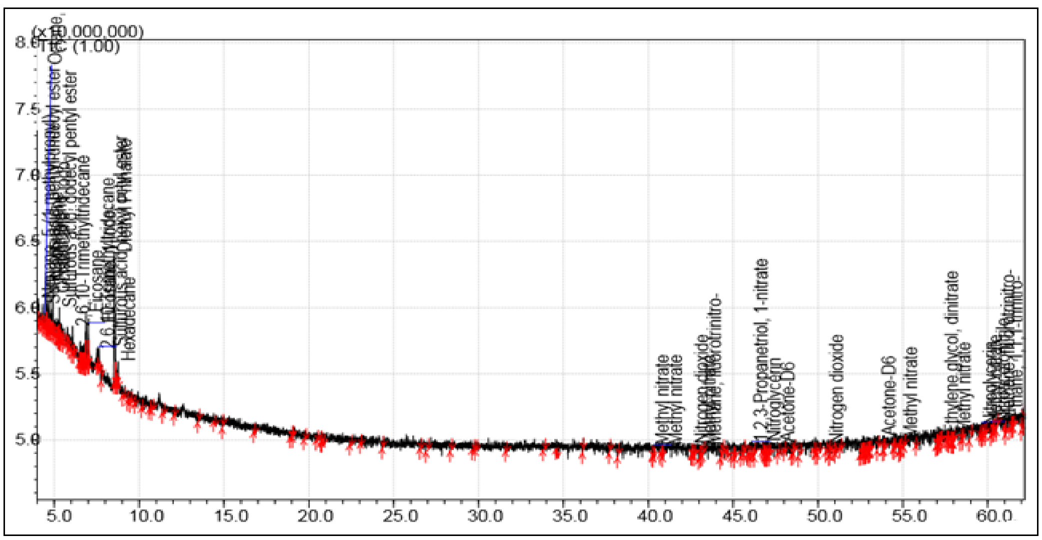

FIG. 4: CHROMATOGRAM OF GC-MS SPECTRA OF 50% METHANOLIC EXTRACT OF *CLERODENDRUM INFORTUNATUM*

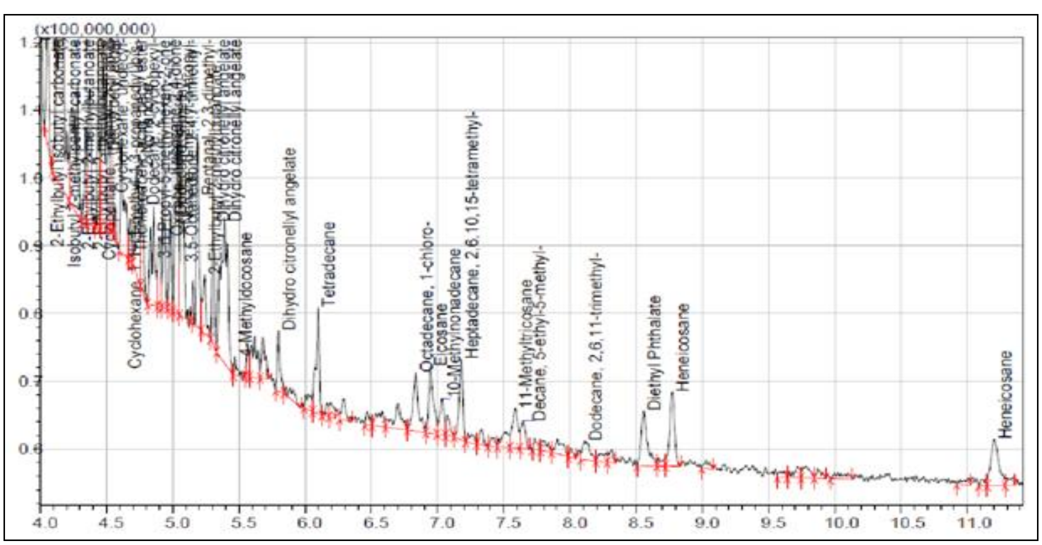

FIG. 5: CHROMATOGRAM OF GC-MS SPECTRA OF PETEROLEUM EXTRACT OF *CLERODENDRUM INFORTUNATUM*

Earlier phytochemical studies of *Clerodendrum infortunatum* revealed the presence of Terpenoid compounds (Clerodolone, Clerodone, Clerodol [16]. In the *Clerodendrum* genus, the main chemical elements are phenolic compounds (acetone, methyl, and ethyl ester of caffeic acid), steroids (clerodolone, clerodole, sterol Clerosterol), and fixed oil glycerides of linolenic, oleic, stearic, and lignoceric acid [23].

Plant furans have been linked to a variety of medicinal qualities, including anticancer, analgesic, anti-inflammatory, antibacterial, and anti-histaminic effects. Furans are heterocyclic chemicals found in many therapeutic medicines. Furan containing compounds are pharmacologically active and hence appear in a wide range of medicinal products [5]. The plant *P. zeyplanica* (Chitra moalam) has several medicinal qualities, including anti-inflammatory, antiatherosclerotic, and antimicrobial action. The phytoconstituent heneicosane was isolated from the plant and suspected of having antibacterial and antifungal properties. Heneicosane displayed excellent antibacterial and antifungal efficacy at all doses [29].

Medicinal herbs have therapeutic capabilities because they include a variety of chemical substances of varying compositions that are found as secondary plant metabolites in one or more portions of the plant. In a larger sense, phytochemistry is concerned with the great variety of organic substances produced and stored by plants [15].

Many phytoconstituents discovered in GC-MS analysis of extracts of *C. infortunatum* in this work were previously reported for varied pharmacological activities, such as 1,2 cyclop-nentanedione, which exhibits high antioxidant action [12]. 2-Methoxy-4-Vinylphenol reduces inflammation in wound healing by inhibiting NF-B and MAPK (Mitogen-Activated Protein Kinase) activation [10]. Earlier literature clearly reveals that flavonoids are the major class of compounds that are mainly present in *Clerodendrum* species. Some of the major flavonoids present in the genus are cynaroside, 5 – hydroxyl – 4 – 7 – dimethoxy - methylflavone, kaempferol, salvigenin, 4-methyl scutellarein, 5,7,4-O-trihydroxyflavone, apigenin, luteolin, acacetin-7-O-glucuronide, hispidulin, 2-4-4trihydroxy 6-methyl chalcone, 7-hydroxy flavone, luteolin, naringin-4-O-glucopyranoside, pectolin-arigenin, cirsimaritin, cirsimaritin-4glucoside, quercetin-3-methyl ether which were isolated from *C. inerme, C. phlomidis, C. petasites, C. trichotomum, C. mandarinorum* and *C. infortunatum* [26]. The petroleum ether extract of *Clerodendrum phlomidis* had shown in GC-MS spectral studies the presence of three compounds are as follows: (1) Isopropyl Linoneate, (2) Hexadecanoic Acid, 2Hydroxyl-1-[Hydroxymethyl]Ethyl Ester, (3) 9-Octadecenoic Acid [Z]-, 2-Hydroxy-1-[Hydroxymethyl] EthylEster [3].

As reviewed more than 300 chemical constituents that had been isolated and identified from the genus of *Clerodendrum,* cataloged as 58 diterpenoids, 43 flavonoid and flavonoid glycosides, 40 phenylethanoid glycosides, 43 steroids, and steroid glycosides, 31 triterpenoids, 27 monoterpene and its derivatives, 13 cyclohexylethanoids, 4 anthraquinones, 3 sesquiterpene, 2 cyanogenic glycosides, and pharmacological studies indicated that the crude extracts and some special monomer compounds of the genus *Clerodendrum* exert various biological activities, such as anti-inflammatory and anti-nociceptive, antioxidant, anticancer, antimicrobial, antihypertensive, anti-obesity, anti-diarrheal, hepatoprotective, memory enhancing, and neuroprotective activities.

Terpenes, including monoterpene and its derivatives, sesquiterpene, diterpenoids, and triterpenoids, as the major characteristic constituents with significant biological activities, have great potential to be developed into new drugs, especially for anti-inflammatory, antioxidant, anticancer, and antimicrobial agents. In addition, important activities, such as anti-hypertensive, anti-obesity, and hepatoprotective activities indicated that *the Clerodendrum* genus can be a promising source of biologically active compounds for these diseases [31]. Morpholine Derivatives gives a general overview of the various synthetic leads that contain the morpholine ring. It also covers the most effective molecules in each group and uses structure-activity relationship (SAR) to identify the active pharmacophores responsible for the antitubercular, antihyperlipidemic, antiviral, anticancer, antioxidant, antimicrobial, and antileishmanial properties. The presence of morpholine nucleus as an active pharmacophore in this sizable pool of marketed medications illustrates the importance of morpholine in drug development [13]. As GC-MS analysis of petroleum ether extract has detected Morpholin-3-one, 2-hydroxy-2,5,5-trimethyl at retention time 5.185 min contains Morpholine nucleus with N-H and -O- as functional group which possess anti-inflammatory and analgesic activity. FT-IR analysis shows the presence of phenols, carboxyl acids, alcohols, alkenes, and alkynes supports the qualitative analysis of extract as well as GC-MS analysis and their compounds also support the wound healing potential of the *C. infortunatum* extract formulations in cattle.

CONCULSION: The petroleum ether and 50% methanolic extracts of *C. infortunatum* abundantly possess several pharmacologically active phytoconstituents like flavonoids, tannins, etc. as supported by the preliminary qualitative phytochemical investigation and FT-IR and GC-MS analysis. As a result, the present study revealed the presence of bioactive phytoconstituents found in plant extracts when subjected to preliminary phytochemistry, detailed GC-MS, and FT-IR analysis.

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REFERENCES:

1. Prashant BK: Bhandira *Clerodendrum infortunatum*: Uses, Reasearch, Remedies. https://www.easyayurveda.com/2017/03/12/bhandira-clerodendrum-infortunatum/, 27/07/2023, 16:51
2. Baid S: Evaluation of antihyperglycemic and hypolipidemic activities of *Clerodendrum infortunatum* Linn. leaf Extracts. Asian Journal of Complementary and Alternative Medicine 2013; 01(01): 01-08
3. Balaji K & Kilimozhi D: GC-MS analysis of various extracts of *Clerodendrum phlomidis* leaf. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(1): 226-232.
4. Banerjee R, Kumar HKS & Banerjee M: Medicinal significance of furan derivatives: a review. International Journal of Research in Phytochemistry and Pharmacology 2015; 5(3): 48-57.
5. Chauhan L & Gupta S: Creams: a review on classification, preparation methods, evaluation and its applications. Journal of Drug Delivery and Therapeutics 2020; 10(5): 281-289.
6. Das S, Bhattacharya S, Prasanna A, Suresh Kumar RB, Pramanik G & Haldar PK: Preclinical evaluation of antihyperglycemic activity of *Clerodendrom infortunatum* leaf against streptozotocin-induced diabetic rats. Diabetes Therapy 2011; 2: 92-100.
7. Dutta S, Chakraborty AK, Kar P, Dey P, Sen A & Chaudhuri TK: Stimulation of murine immune response by *Clerodendrum infortunatum*. Pharmacognosy Magazine 2018; 14(57): 417-429.
8. Ghosh G, Panda P, Rath M, Pal A, Sharma T & Das D: GC-MS analysis of bioactive compounds in the methanol extract of *Clerodendrum viscosum* leaves. Pharmacognosy Research 2015; 7(1): 110.
9. Gouthamchandra K, Riaz Mahmood, Basheer Mohamed, Khadeer Ahamed, Parameshwarappa, Suresh Babu and Venkatarangaiah Krishna: International Journal of Biomedical and Pharmaceutical Sciences 2009; 3(1): 21-25.
10. Jeong JB, Hong SC, Jeong HJ & Koo JS: Anti-inflammatory effect of 2-methoxy-4-vinylphenol via the suppression of NF-κB and MAPK activation, and acetylation of histone H3. Archives of Pharmacal Research 2011; 34: 2109-2116.
11. Khatun MS, Mia N, Al Bashera M, Murad MA, Zahan R, Parvin S & Akhtar MA: Evaluation of anti-inflammatory potential and GC-MS profiling of leaf extracts from *Clerodendrum infortunatum* L. Journal of Ethnopharmacology 2024; 320: 117366.
12. Kim AR, Zou Y, Kim HS, Choi JS, Chang GY, Kim YJ & Chung HY: Selective peroxynitrite scavenging activity of 3-methyl-1, 2-cyclopentanedione from coffee extract. Journal of Pharmacy and Pharmacology 2002; 54(10): 1385-1392.
13. Kumari A & Singh RK: Morpholine as ubiquitous pharmacophore in medicinal chemistry: Deep insight into the structure-activity relationship (SAR). Bioorganic Chemistry 2020; 96: 103578.
14. Lakshmi CHND, Raju BP, Madhavi T & Sushma NJ: Identification of bioactive compounds by FTIR analysis and *in-vitro* antioxidant activity of Clitoriaternatea leaf and flower extracts. Indo American Journal of Pharmacy and Research 2014; 4(9): 3894-3903.
15. Limsay RP, Somkuwar AP, Bonde SW, Kakde VK, Shirankar SS, Borekar VI & Jagdale RA: *In-vitro* anti-inflammatory activity of *Clerodendrum infortunatum* L. leaves 2021.
16. Manzoor-Khuda M & Sarela S: Constituents of Clerodendron infortunatum (bhat)—I: Isolation of clerodolone, clerodone, clerodol and clerosterol. Tetrahedron 1965; 21(4): 797-802.
17. Mashelkar RA: Part of Foreword from Drug Discovery and Development by Bhushan Patwardhan, New India Publishing Company, New Delhi, India 2007.
18. Molehin OR, Oloyede OI & Ajayi EI: GC–MS analysis of bioactive compounds in three extracts of *Clerodendrum volubile* P. Beauv leaves. J Med Plants Stud 2017; 5(5): 191-5.
19. Öhnstedt E, Lofton Tomenius H, Vågesjö E & Phillipson M: The discovery and development of topical medicines for wound healing. Expert Opinion on Drug Discovery, 2019; 14(5): 485-497.

20. Pandey SB, Nirmal SA & Pawar SP: Pharmacognostic study of *Clerodendrum splendens* flower and stem 2015.
21. Patle TK, Shrivas K, Kurrey R, Upadhyay S, Jangde R & Chauhan R: Phytochemical screening and determination of phenolics and flavonoids in *Dillenia pentagyna* using UV–vis and FTIR spectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2020; 242: 118717.
22. Pharmawati M & Wrasiati LP: Phytochemical screening and FTIR spectroscopy on crude extract from *Enhalus acoroides* leaves. Malaysian Journal of Analytical Sciences 2020; 24(1): 70-77.
23. Rajurkar BM: Phytopharmacological investigations of *Clerodendrum infortunatum* Gartn., International Research Journal of Pharmacy 2011; 2(11): 130-132.
24. Ram P, Limsay RP, Somkuwar AP, Adey KS & More GV: Studies on Phytochemistry and Wound Healing Activity of Leaf Extract Ointment of *Clerodendrum infortunatum* in Canines. The Indian Journal of Veterinary Sciences and Biotechnology 2023; 19(5): 38.
25. Sheel R, Nisha K & Kumar J: Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. IOSR Journal of Applied Chemistry 2014; 7(1): 10-13.
26. Shrivastava N and Tejas Patel: (2007) *Clerodendrum* and healthcare: An overview. Medicinal and Aromatic Plant Science and Biotechnology, 1: 142-150
27. Singh, A. (Ed.). Herbalism, phytochemistry and ethnopharmacology. CRC Press 2011.
28. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H: Phytochemical screening and extraction: a review. Internationale Pharmaceutica Sciencia 2011; 1(1): 98-106.
29. Vanitha V, Vijayakumar S, Nilavukkarasi M, Punitha VN, Vidhya E & Praseetha PK: Heneicosane—A novel microbicidal bioactive alkane identified from Plumbagozeylanica L. Industrial Crops and Products 2020; 154: 112748.
30. Verma S & Gupta R: Pharmacognostical and high performance thin layer chromatography studies on leaves of *Clerodendrum infortunatum* L. Ayu 2014; 35(4): 416.
31. Wang JH, Luan F, He XD, Wang Y & Li MX: Traditional uses and pharmacological properties of *Clerodendrum* phytochemicals. Journal of Traditional and Complementary Medicine 2018; 8(1): 24-38.

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