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GC - MS AND FTIR ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN DIFFERENT EXTRACTS OF *ERANTHEMUM PULCHELLUM* -ANDREWS

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ABSTRACT
Eranthemum pulchellum, a tropical evergreen shrub from the Acanthaceae family, is widely distributed in various parts of India, China and Nepal. Previously, various parts of this plant were reported for the treatment of various ailments but no GC-MS analysis of this plant has been reported. Thus, the aim of the present study was to identify the important functional groups and phytochemical contents in methanol, ethyl acetate, and n-hexane extracts of *E. pulchellum* using a Fourier transform infrared spectrometer (FTIR) and gas chromatography-mass spectrometry (GC-MS). Gas chromatography-mass spectrometry results revealed 48 phytocompounds in methanol extract, 42 phytocompounds in ethyl acetate and 47 phytocompounds in n-hexane extracts. The major bioactive components are i-Propyl alpha-linolenate (16.45%) and Squalene (12.34%) in methanol extract, Eicosyl trifluoroacetate (20.44%) and Squalene in ethyl acetate, Eicosyl trifluoroacetate (20.59%) and Squalene (12.86%) in n-hexane extract. The FTIR spectra revealed the presence of functional groups such as alcohols, carboxylic acids, aromatics, alkanes, alkenes, alkyl halides and amines which indicates the presence of various metabolites in the extracts. It is concluded that the significant bioactive compounds are present in plant extracts and these constituents are responsible for the therapeutic effects of the plant.

INTRODUCTION

Plants with medicinal properties have played a crucial role in various indigenous systems of medicine across the globe. Despite advancements in synthetic drugs and antibiotics, plants continue to be valued as significant sources of medicinal compounds in both modern and traditional medical practices (Gururani, *et al.*,2023).In past years, the crude extracts of various parts of medicinal plants, such as root, stem, flower, fruit, and twigs, were frequently utilized to cure some human ailments. The majority of research on medicinal plants is focused on their several phytochemicals which include flavonoids, alkaloids, tannins, and terpenoids and have a variety of biological properties (Gonelimali, *et al.*, 2018). The phytochemical compounds are not only encouraging the discovery of therapeutic prospects, but they also play a vital role in the development of innovative semi-synthetic and synthetic compounds (Starlin, *et al.*,2019). The screening of plant extracts is a novel approach for identifying medicinally active compounds in many plant species. Hence, gas chromatography-mass spectroscopy (GC-MS) and Fourier transform infrared (FTIR) spectroscopy are analytical techniques to find the phytochemical compounds and functional groups in the plant extract (Ghosh, *et al.*, 2015).

Uttarakhand, the northern state of India is well known for its rich resources of medicinal plants. There are about 7000 species of medicinal plants and 500 species of fauna in Uttarakhand state (Phartiyal and Jugran 2021). It also has a diverse range of medicinal plants, many of which have been extensively utilized by local communities to treat human diseases. In the present study, we have focused on one of the most common plants in India, *i.e.*, *Eranthemum pulchellum* Andrews, often known as blue sage or blue erathemum, which is a woody perennial shrub belonging to the Acanthaceae family. The genus *Eranthemum* has about 138 species found in tropical and subtropical regions of Asia. Plants from this genus have been traditionally used for various ethnomedicinal purposes (Prasanth, *et al.*, 2018). It is widely distributed in the northeast areas of India, China, Myanmar, Bhutan, Sikkim, and Nepal. The shrub attains a height of 4 to 6 feet. Leaves opposite simple, petiolate, lineolate, and usually entire flowers blue, purple, violet or purplish-white in terminal or axillary, simple or branched dense spikes. This shrub is cultivated in Indian gardens for its attractive foliage and the rich bright gentian violet flower, which occurs in terminal spikes (Duenas-Lopez 2019). The Indian traditional healers use all parts of this plant as folklore medicine to cure a variety of diseases. Local practitioners traditionally apply its leaf paste to treat blisters and skin cracks (Sharma *et al.*, 2013). Iridoid glucoside was isolated from *E. pulchellum*, which has demonstrated various biological activities (Fischer *et al.*,1987). Additionally, the plant's extract, which includes its leaves, stem, and root, is utilized as an antimicrobial and antiseptic agent. The antioxidant and antifungal activities of *E. pulchellum*

were evaluated by their green synthesis of AgNPs (Prakash *et al.*, 2021).

As per the literature review, only limited studies on the plant have been published, particularly in India, therefore phytochemical profiling and identification of its bioactive components for potential pharmacological and therapeutic purposes are significant. Analytical techniques such as GC-MS and FTIR were useful instruments for identifying and determining the bioactive compounds. The current study was carried out for the identification of bioactive compounds present in *Eranthemum pulchellum* in different extracts by GC-MS and FTIR methods, which may provide an insight into its use in traditional medicinal.

MATERIALS AND METHODS

Materials and Reagents

The fresh samples of *E. pulchellum* including leaves and stems, were taken in April 2022 from Dolmaar (Lat, 29.3200ºN; Long, 79.5126ºE) in Nainital district of Uttarakhand (India). The plant identification and authentication were done at the Botanical Survey of India (BSI), Northern Regional Centre, Dehradun, under accession number 1348.

Preparation of the Extract

The collected fresh leaves were shade dried at room temperature for a week. The dried material was ground into a fine powder. This powder was kept in an airtight container for further use (Aarti *et al.*, 2015). Extraction was conducted according to a method described by Ghosh *et al.*, 2023. About 50g of dried powdered leaves were placed in a Soxhlet apparatus and extracted separately with methanol, ethyl acetate and n-hexane for 24 hours in a 250 ml. The crude extracts were filtered and the filtrates were concentrated under reduced pressure at 40ºC using a rotary evaporator to obtain a viscous semi solid mass/extract. The crude extract was subjected to FTIR and GC-MS analyses.

GC-MS Analysis

The GC-MS analysis of bioactive compounds from the different extracts of the leaves of *Eranthemum pulchellum* was carried out in Shimadzu GCMS-QP2010 ultra equipment. The instrument was run under the following conditions: The injector temperature was maintained at 260ºC at a pressure 81.9kPa, linear velocity 40.5 cm/sec, purge flow 3.0 mL/min, total flow 16.3 mL/min, column flow 1.21 mL/min, split ratio of 10.0. The initial oven temperature program is maintained at 80 ºC and the final temperature at 280ºC with hold time of 20 min. Ion source temperature was maintained at 220 ºC and interface temperature at 270 ºC, solvent cut time 3.50 min. Other conditions are: detector gain mode-relative to the tuning result, threshold 1000, detector gain + 0.00 kV, event time 0.20 s, scan speed 3333, start time 4.00 min, end time 41.98 min, start m/z 40.00 and end m/z 650.00.

Identification of Bioactive Components

The components were identified after comparison with those available in the computer library (NIST and Willey) attached to the GCMS instrument and the results obtained. The compound name, molecular weight, retention time, percentage, nature of compound and structure of various compounds in the test materials were ascertained.

FTIR Spectroscopic Analysis

Fourier transform infrared spectrophotometer (FTIR) is presumably the most powerful tool for determining the functional groups present in the plant extract. For FTIR analysis, dried powders of different extracts of plant material were employed. In order to prepare a translucent sample disc,10 mg of dried extract powder was encapsulated in 100mg of KBr pellet. These pellets of the powdered mixture were packed in FTIR spectroscope (Pekin Elmer), having frequency range from 4000 to 400 cm^{-1}.

RESULTS AND DISCUSSION

Gas Chromatography and Mass spectrometry(GC-MS) Studies

GC-MS is one of the fast, more accurate and best techniques to identify the various bioactive constituents, like alcohols, alkaloids, long-chain, branched hydrocarbons, acids, esters, steroids, nitro compounds and amino acids present in plant species (Ramya, 2022). The phytochemical compounds of the presently investigated plants along with their retention time (RT), peak area % (concentration), molecular formula (MF), molecular weight (MW), structures and nature of compounds are presented in Tables 1, 2 and 3. In the methanolic extract, 48 compounds were identified (Figure 1 and Table1). The major constituents identified are i-propyl alpha -linolenate (16.45%) followed by squalene (12.34%), phytol (8.19%), n- hexadecanoic acid (7.02%), eicosyl trifluoroacetate (6.27%) andbetulin (5.13%). Other important compounds with less than 5% peak area are ergost-5-en-3-ol(3-beta.) (4.61%), methyl commate B (3.70%), 6-nitrocholesteryl acetate (3.34%), cis, cis, cis-7,10,13-hexadecatrienal (3.12), tridecanedial (2.83), neophytadiene(2.35%),1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid (2.79%), cycloartanol TMS (1.88%), olean-12-en-3-one (1.26%), pregnan-20-one,5,6-difluoro-3-hydroxy-, (3-beta) (1.14%), octacosanol (1.32%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (1.17%),

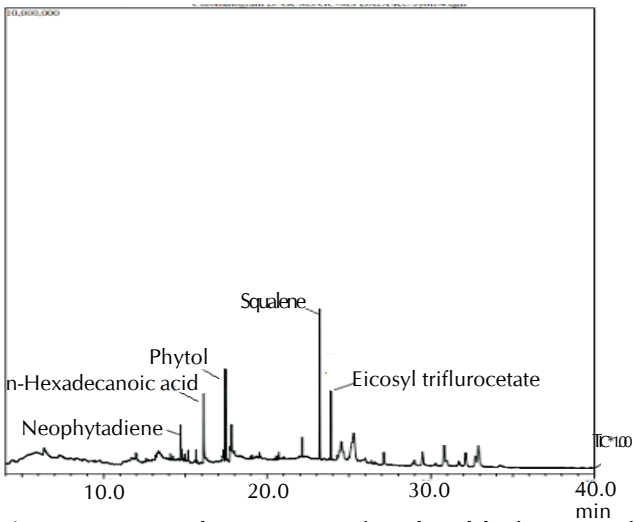
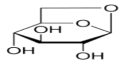
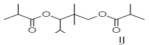
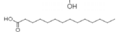
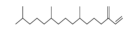
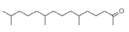
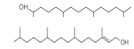
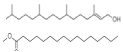
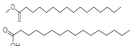
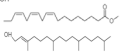
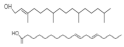
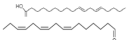
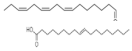
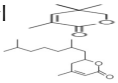
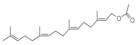
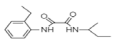
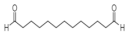
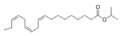
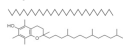
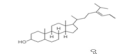
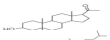
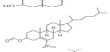


Figure 1: GC-MS Chromatogram of methanol leaf extract of *Eranthemum pulchellum*

Table 1: Bioactive compounds identified in the methanol leaf extract of *Eranthemum pulchellum* using gas chromatography-mass spectrometry

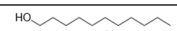
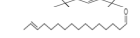
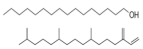
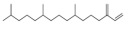
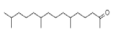
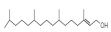
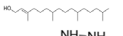
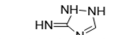
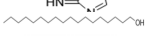
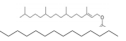
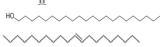
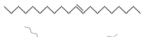
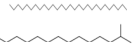
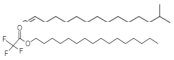
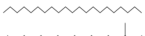
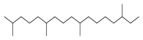
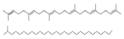
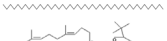
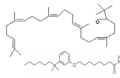
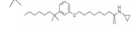
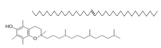
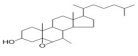
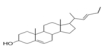
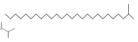
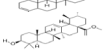
RT	Area %	Compound name	Structure	Compound nature	MF	MW
6.367	1.17	3,5-Dihydroxy-6-Methyl-2,3-Dihydro-4H-Pyran-4-One		Hetrocyclic compound	$C_6H_8O_4$	144
9.475	0.19	1-Tetradecene		Alkene	$C_{14}H_{28}$	196
11.723	0.51	1,6-Anhydro-.Beta.-D -Glucopyranose (Levoglucosan)		Anhydrohexose	$C_6H_{10}O_5$	162
11.964	0.76	2,2,4-Trimethyl-1,3-Pentanediol diisobutyrate		Diester	$C_6H_{30}O_4$	286
12.611	0.38	Megastigmatrienone		Terpene	$C_{13}H_{18}O$	190
13.365	2.79	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid		Quinic acid	$C_7H_{12}O_6$	192
14.082	0.78	Tetradecanoic Acid		Fatty acid	$C_{14}H_{28}O_2$	228
14.257	0.3	1-Octadecene		Alkene	$C_{18}H_{36}$	252
14.713	2.35	Neophytadiene		Diterpene	$C_{20}H_{38}$	278
14.794	0.58	Hexahydrofarnesyl acetone		Terpene	$C_{18}H_{36}O$	268
14.869	0.19	6,10,14-Trimethy1-Pentadecan-2-ol		Fatty alcohol	$C_{18}H_{38}O$	270
15.162	1.1	3,7,11,15-Tetramethy1-2-Hexadecen-1-ol		Terpene	$C_{20}H_{40}O$	296
15.638	0.86	Hexadecanoic acid, methyl ester		Fatty acid, methyl ester	$C_{17}H_{34}O_2$	270
16.11	7.02	n- Hexadecanoic acid		Fatty acid	$C_{16}H_{32}O_2$	256
17.329	0.68	Linolenic acid, methyl ester		Fatty acid, methyl ester	$C_{19}H_{32}O2$	292
17.437	8.19	Phytol		Diterpene	$C_{20}H_{40}O$	296
17.726	0.67	9,12-Octadecadienoic Acid(Z,Z)-		Fatty acid	$C_{18}H_{32}O_2$	280
17.799	3.12	Cis,cis,cis-7,10,13-Hexadecatrienal		Fatty aldehyde	$C_{16}H_{26}O$	234
17.979	0.23	9-Octadecenoic acid (Z)-		Fatty acid	$C_{18}H_{34}O_2$	282
18.972	0.19	Octanoic acid ,2-dimethylaminoethyl ester		Fatty acid, ethyl ester	$C_{12}H_{25}NO_2$	215
19.081	0.29	2-Methyloctacosane		Alkane	$C_{29}H_{60}$	408
19.257	0.13	2H-Pyran-2-one, 5,6-dihydro-3,5,5-trimethyl		Heterocyclic compound	$C_8H_{12}O_2$	140
19.52	0.52	6-(2,6-Dimethylheptyl)-4-methyl-5,6-dihydro-2H-pyran-2-one		Heterocyclic compound	$C_{15}H_{26}O_2$	238
20.454	0.21	Hexanoic acid, 2-dimethylaminoethyl ester		Fatty acid, ethyl ester	$C_{10}H_{21}NO_2$	187
20.579	0.29	9-Tricosene, (Z)-		Alkene	$C_{23}H_{46}$	322
20.717	0.73	2-Octyldodecanol		Fatty alcohol	$C_{20}H_{42}O$	298
21.02	0.21	Tetradecanal		Fatty aldehyde	$C_{14}H_{28}O$	212
22.124	1.32	Octacosanol		Fatty alcohol	$C_{28}H_{58}O$	410
23.216	12.34	Squalene		Triterpene	$C_{30}H_{50}$	410
23.802	0.16	1-Heneicosanol		Fatty alcohol	$C_{21}H_{44}O$	312
23.884	6.27	Eicosyl trifluoroacetate		Fatty acid ester	$C_{22}H_{41}F_3O_2$	394
24.28	0.35	3,7,11,15-Tetramethyl-2,6,10,14 Hexadecatetraenyl Acetate		Monoterpenoid	$C_{22}H_{36}O_2$	332
24.397	0.35	N-1-(Sec-Butyl)-N-2-(2-Ethylphenyl) Ethanediamide		Acetamide	$C_{14}H_{20}N_2O_2$	248
24.519	2.83	Tridecanedial		Fatty aldehyde	$C_{13}H_{24}O_2$	212
25.289	16.45	i-Propyl alpha.-linolenate		Fatty acid	$C_{21}H_{36}O_2$	320
26.561	0.3	Octacosane		Alkane	$C_{28}H_{58}$	394
27.134	2.06	(+)-.Alpha.-Tocopherol		Vitamin E	$C_{29}H_{50}O_2$	430
28.862	0.27	Cholest-5-En-3-ol,24-Propylidene-,(3.Beta.)-Hydroxy-,(3.Beta.)		Steroids	$C_{30}H_{50}O$	426
28.996	1.14	Pregnan-20-One,5,6-Difluoro-3-		Steroids	$C_{21}H_{32}F_2O_2$	354
29.495	3.34	6-Nitrocholesteryl acetate		Steroids	$C_{29}H_{47}NO_4$	473
30.271	0.51	Stigmasterol		Steroids	$C_{29}H_{48}O$	412
30.815	4.61	Ergost-5-En-3-ol		Steroids	$C_{28}H_{48}O$	400
30.977	0.43	Methyl Commate D		Triterpene	$C_{31}H_{50}O_4$	486
31.721	1.26	Olean-12-En-3-one		Pentacyclic triterpene	$C_{30}H_{48}O$	424
32.127	3.7	Methyl Commate B		Triterpene	$C_{31}H_{50}O_3$	470
32.709	1.88	Cycloartanol TMS		Triterpene	$C_{33}H_{58}0Si$	442
32.909	5.13	Betulin		Pentacyclic triterpene	$C_{30}H_{50}O_2$	442
34.269	0.86	7-(2-Hydroxy-1-Methylethyl)-1,4a-Dimethyl-2,3,4,4a,5,6,7,8-octahydro-2-naphthalenol		Organic compound	$C_{15}H_{26}O_2$	238

RT- Retention Time, MF- Molecular formula, MW-Molecular weight

3,7,11,15-tetramethy1-2- hexadecen-1-ol (1.10%) and remaining are the compounds less than 1%.

The GC-MS analysis has shown to identify the 42 compounds (Figure 2 and Table 2) in the ethyl acetate extract. The major chemical compound which have higher than 10% peak area are eicosyl trifluoroacetate (20.44%) followed by squalene (14.75%) while remaining chemical constituents which are in between 1% and 7% peak area phytol (6.54%),

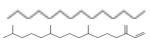
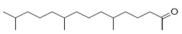
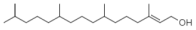
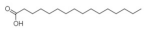
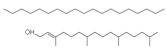
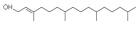
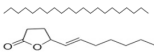
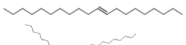
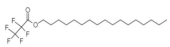
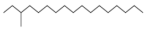
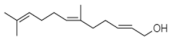
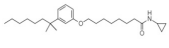
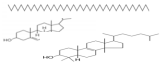
Table 2: Bioactive compounds identified in the ethyl acetate leaf extract of *Eranthemum pulchellum* using gas chromatography-mass spectrometry

RT	Area %	Compound name	Structure	Compound nature	MF	MW
9.509	0.33	1-Undecanol		Fatty alcohol	$C_{11}H_{24}O$	172
11.155	0.18	Phenol, 3,5-di-tert-butyl-		Phenols	$C_{14}H_{22}O$	206
12.051	0.55	E-14-Hexadecenal		Fatty aldehyde	$C_{16}H_{30}O$	238
14.276	0.62	1-Hexadecanol		Fatty alcohol	$C_{16}H_{34}$	242
14.725	2.93	Neophytadiene		Diterpene	$C_{20}H_{38}$	278
14.814	0.39	2-Pentadecanone, 6,10,14-trimethyl		Sesquiterpene	$C_{18}H_{36}O$	268
15.174	0.85	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Terpene alcohol	$C_{20}H_{40}O$	296
16.127	1.98	n-Hexadecanoic acid		Fatty acid	$C_{18}H_{32}O_2$	256
16.308	0.6	10-Heneicosene(c,t)		Alkene	$C_{21}H_{42}$	294
17.308	0.31	Pentadecane		Alkane	$C_{15}H_{32}$	212
17.439	6.54	Phytol		Diterpene	$C_{20}H_{40}O$	296
17.74	0.14	1H-1,2,4-Triazol-3-Amine		Hetrocyclic compound	$C_2H_4N_4$	84
18.166	0.39	1-Octadecanol		Fatty alcohol	$C_{18}H_{38}O$	270
18.34	0.26	Phytol, acetate		Diterpene	$C_{22}H_{42}O_2$	338
19.083	0.49	Heptadecane		Alkane	$C_{17}H_{36}$	240
19.534	0.26	3-Butylcycloheptanone		Cyclic ketone	$C_{11}H_{20}O$	168
19.88	0.17	Behenic alcohol		Fatty alcohol	$C_{22}H_{46}O$	326
20.526	0.14	9-Eicosene,(E)		Alkene	$C_{20}H_{40}$	280
20.583	0.4	9-Tricosene,(Z)-		Alkene	$C_{23}H_{46}$	322
20.72	1.51	Octacosane		Alkane	$C_{28}H_{58}$	394
21.021	0.15	13-Methyltetradecanal		Fatty aldehyde	$C_{15}H_{30}O$	226
22.071	0.14	Heptadecyl trfluoroacetate		Fatty acid	$C_{19}H_{35}F_3O_2$	352
22.13	2.5	n-Tetracosanol		Fatty alcohol	$C_{24}H_{50}O$	354
22.249	2.07	Heneicosane		Alkane	$C_{21}H_{44}$	296
22.753	0.16	Heptadecane,2,6,10, 15-tetramethyl-		Alkane	$C_{21}H_{44}$	296
23.226	14.75	Squalene		Triterpene	$C_{30}H_{50}$	410
23.659	3.23	2-methyloctacosane		Alkane	$C_{29}H_{60}$	408
23.902	20.44	Eicosyl trifluoroacetate		Fatty acid ester	$C_{22}H_{41}F_3O_2$	394
24.04	5.54	Tetratetracontane		Alkane	$C_{44}H_{90}$	618
24.414	0.24	Oxirane,2,2-dimethy1-3-(3,7, 12,16,20-pentamethyl-3,7,11		Epoxide alkane	$C_{30}H_{50}O$	426
26.001	1.07	CB-86		Resorcinol-anandamide	$C_{26}H_{43}NO_3$	417
26.362	1.85	17-Pentatriacontene		Alkene	$C_{35}H_{70}$	490
27.14	1.84	(+)-.Alpha.-Tocopherol		Vitamin E	$C_{29}H_{50}O_2$	430
27.787	0.51	Hentriacontane		Alkane	$C_{31}H_6$	436
28.991	0.67	5.Beta.,6.beta.-Epoxy-7.alpha. -bromocholestan-3.beta.-ol		Steroid	$C_{27}H_{45}BrO_2$	480
29.502	3.4	26,27-Dinorcholesta-5,2 2-dien-3-ol,(3.beta.,22		Steroid	$C_{25}H_{40}O$	356
30.35	1.01	2-Methylhexacosane		Alkane	$C_{27}H_{56}$	380
30.82	3.6	Ergost-5-en-3-ol		Steroid	$C_{28}H_{48}O$	400
31.72	0.94	24-Noroleana-3,12-diene		Nortriterpene	$C_{29}H_{46}$	394
32.149	3.54	Methyl Commate B		Triterpene	$C_{31}H_{50}O_3$	470
32.728	1.92	Cycloartenol		Triterpene	$C_{30}H_{50}O$	426
32.931	4.1	Betulin		Triterpene	$C_{30}H_{50}O$	442
34.28	1	(-)-Globulol		Sesquiterpene alcohol	$C_{15}H_{26}O$	222

tetratetracontane (5.54%), betulin (4.10%), ergost-5-en-3-ol (3.60%), methyl commate B (3.54%), 26,27-dinorcholesta-5,22-dien-3-ol,(3.beta.,22)(3.40%),2-methylo ctacosane(3.23%), neophytadiene (2.93%), n-tetracosanol (2.50%), heneicosane (2.07%), tetratetracontane (2.05%), n-hexadecanoic acid(1.98%), cycloartenol (1.92%), 17-pentatriacontene(1.85%), alpha.-tocopherol(1.84) , CB-86 (1.07), 2-methylhexacosane (1.01%) and (-)-globulol(1.00%).

47 compounds were identified (Figure 3 and Table 3) in the n-hexane extract of *E. pulchellum*. The three major compounds are eicosyl trifluoroacetate (20.59%), squalene (12.86%), and tetratetracontane (5.40%). Phytol (4.98%), betulin (3.82%), methyl commate B (3.33%), lanost-8-en-3-ol (2.93%), ergost-5-en-3-ol (2.75%), tetratetracontane (2.71%) heneicosane (2.20%), 1-tetracosanol (2.30%) octacosanol (1.78%) cycloartanol TMS (1.72%), CB-86 (1.39%), octacosane (1.35%), alpha.-tocopherol(1.28%) and tetratetracontane (1.13%) are the fourteen minor compounds in the n-hexane extract of *E. pulchellum*. The remaining bioactive compounds were present at low concentrations and their names, retention

Table 3: Bioactive compounds identified in the n-hexane leaf extract of *Eranthemum pulchellum* using gas chromatography-mass spectrometry

RT	Area%	Compound name	Structure	Compound nature	MF	MW
12.124	0.16	Hexadecane		Alkane	$C_{16}H_{34}$	226
14.339	0.15	Tetradecane		Alkane	$C_{14}H_{30}$	198
14.722	0.67	Neophytadiene		Diterpene	$C_{20}H_{38}$	278
14.809	0.36	Hexahydrofarnesyl acetone		Terpene	$C_{18}H_{36}O$	268
15.871	0.21	Phytol		Diterpene	$C_{20}H_{40}O$	296
16.127	0.52	n-Hexadecanoic acid		Fatty acid	$C_{16}H_{32}O_2$	256
17.306	0.32	Nonadecane		Alkane	$C_{19}H_40$	268
17.44	4.98	Phytol		Diterpene	$C_{20}H_{40}O$	296
18.212	0.13	Tricosane		Alkane	$C_{23}H_{48}$	324
19.081	0.47	Heneicosane		Alkane	$C_{21}H_{44}$	296
19.531	0.24	(E)-Docec-5-en-4-olide lactones		Unsaturated	$C_{12}H_{20}O_2$	196
20.527	0.1	9-Eicosene,(E) -		Alkene	$C_{20}H_{40}$	280
20.584	0.34	9-Tricosene,(Z)-		Alkene	$C_{23}H_{46}$	322
20.722	1.35	Octacosane		Alkane	$C_{28}H_{58}$	394
21.495	0.28	Tetratetracontane		Alkane	$C_{44}H_{90}$	618
22.068	0.13	Pentafluoropropionic acid ,heptadecyl ester		Fatty acid methyl ester	$C_{20}H_{35}F_5O_2$	402
22.131	2.3	1-Tetracosanol		Fatty alcohol	$C_{24}H_{50}O$	354
22.253	2.2	Heneicosane		Alkane	$C_{21}H_{44}$	296
22.753	0.15	Octacosane		Alkane	$C_{28}H_{58}$	394
2.843	0.39	3-Methylheptadecane		Alkane	$C_{18}H_{38}O$	254
23.083	0.96	Heneicosane		Alkane	$C_{21}H_{44}$	296
23.233	12.86	Squalene		Triterpene	$C_{30}H_{50}$	410
23.664	3.51	2-methyloctacosane		Alkane	$C_{29}H_{60}$	408
23.81	0.36	17-Pentatriacontene		Alkene	$C_{35}H_{70}$	490
23.919	20.59	Eicosyl trifluoroacetate		Fatty acid ester	$C_{22}H_{41}F_3O_2$	394
24.055	9.67	Tetratetracontane		Alkane	$C_{44}H_{90}$	618
24.292	0.29	1,6,10,14,18,22-Tetracosahexaen -3-ol,2,6,10,15,19,23 -hexamethyl-, (all-E)-		Fatty acid	$C_{30}H_{50}O$	426
24.416	0.18	Oxirane,2,2-dimethyl-3-(3,7,12, 16,20-pentamethyl-3-7,11		Epoxide alkane	$C_{30}H_{50}O$	426
24.563	0.17	6,11-Dimethy-2,6,1 0-dodecatrien-1-ol		Sesquiterpene alcohol	$C_{14}H_{24}O$	426
25.178	1.13	Tetratetracontane		Alkane	$C_{44}H_{90}$	618
26.007	1.39	CB-86		Resorcinol-anandamide	$C_{26}H_{43}NO_3$	417
26.238	0.12	17-Pentatriacontene		Alkene	$C_{35}H_{70}$	490
26.361	1.78	Octacosanol		Fatty alcohol	$C_{28}H_{58}O$	410
26.575	5.4	Tetratetracontane		Alkane	$C_{44}H_{90}$	618
27.144	1.28	(+)-.Alpha.-Tocopherol		Vitamin E	$C_{29}H_{50}O_2$	430
27.779	0.66	Triacontane		Alkane	$C_{30}H_{62}$	422
28.999	0.7	Campesterol		Steroids	$C_{28}H_{48}O$	400
29.512	2.93	Lanost-8-en-3-ol		Triterpene	$C_{30}H_{52}O$	428
30.385	2.71	Tetratetracontane		Alkane	$C_{44}H_{90}$	618
30.844	2.75	Ergost-5-en-3-ol		Steroids	$C_{28}H_{48}O$	400
31.003	0.36	Olean-12-en-3- one		Pentacyclic Triterpenes	$C_{30}H_{48}O$	424
31.738	0.84	24-Noroleana-3,12-Diene		Nortriterpene	$C_{29}H_{46}$	394
32.156	3.33	Methyl Commate B		Triterpene	$C_{31}H_{50}O_3$	470
32.761	1.72	Cycloartanol TMS		Triterpene	$C_{33}H_{58}OSi$	498
32.952	3.82	Betulin		Pentacyclic Triterpene	$C_{30}H_{50}O_2$	442
34.306	0.62	11.alpha.-Hydroxy-17. alpha.-methyltestosterone		Steroids	$C_{20}H_{30}O_3$	318
38.004	0.17	Phytol acetate		Diterpene	$C_{22}H_{42}O_2$	338

RT- Retention Time, MF- Molecular formula, MW-Molecular weight

times and percentages are listed in Table 3.

The percentage abundance of the classes of bioactive chemicals found in the methanol, ethyl acetate and n-hexane extracts is shown, respectively in Figures 4, 5 and 6. In methanol extracts, fatty acids had a total abundance of 18.13%, diterpene 10.54%, pentacyclic triterpene 6.39%, triterpene 18.35%, fatty alcohol 2.40%, fatty aldehyde 6.16 %, vitamin E 2.06%, fatty acid ester 6.27%, fatty acid methyl ester1.94%,

Table 5: Bioactive compounds common to methanol, ethyl acetate and n-hexane extracts of *Eranthemum pulchellum*

Bioactive compound	Percentage abundance in extraction solvents%		
	Methanol	Ethyl acetate	n-Hexane
Phytol	8.19	6.54	4.98
Betulin	5.13	4.1	3.82
Squalene	12.34	14.75	12.86
Eicosyl trifluoroacetate	6.27	20.44	20.59
(+)-.Alpha.-Tocopherol	2.06	1.84	1.28
Neophytadiene	2.35	2.93	0.67
2-Methyloctacosane	0.29	3.23	3.51
9-Tricosene, (Z)-	0.29	0.4	0.34
Methyl Commate B	3.7	3.54	3.33
Octacosane	0.3	1.51	1.35

Figure 2: GC-MS Chromatogram of ethyl acetate leaf extract of *Eranthemum pulchellum*

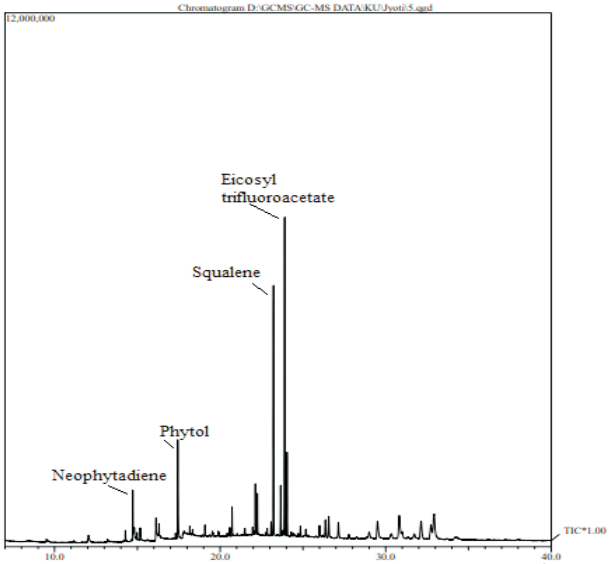
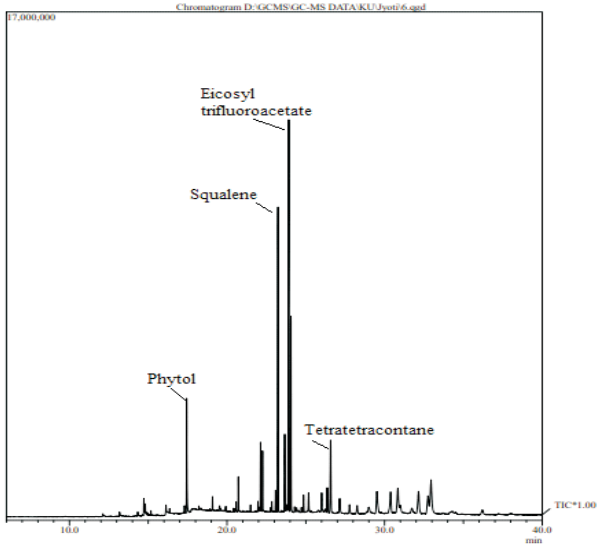


Figure 3: GC-MS Chromatogram of n-hexane leaf extract of *Eranthemum pulchellum*



quinic acid2.79%, steroids 9.87%, heterocyclic compound 1.82% and terpene1.68%. The least abundant class of

Figure 4: Percentage abundance of classes of phytocompounds in *Eranthemum pulchellum* methanol leaves extract

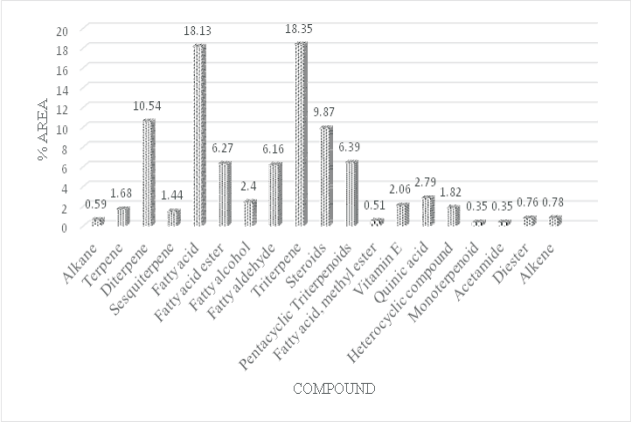


Figure 5: Percentage abundance of classes of phytocompounds in *Eranthemum pulchellum* ethyl acetate leave extract

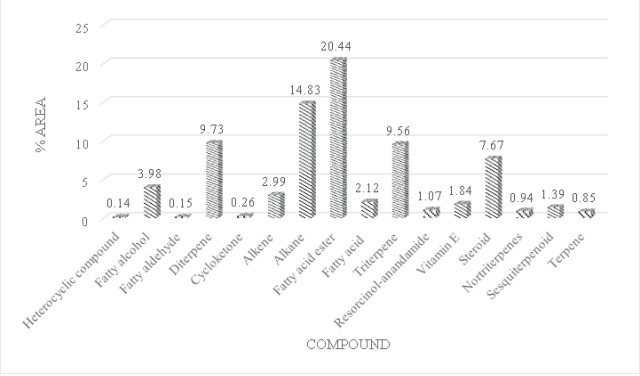
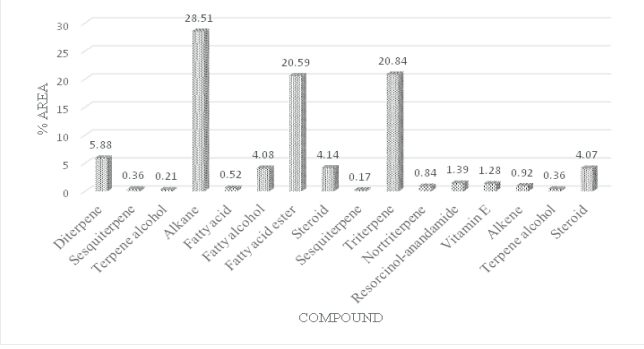


Figure 6: Percentage abundance of classes of phytocompounds in *Eranthemum pulchellum* n-hexane leaves extract



bioactive compounds was monoterpenoid 0.35%, acetamide 0.35%, diester 0.76%, anhydrohexose 0.51%, alkane 0.59, and alkene 0.78%. In ethyl acetate extracts, alkane had a total abundance of 14.83%, triterpene 9.56%, fatty acid ester 20.44%, diterpene 9.73%, steroid 7.67%, fatty alcohol 3.98%, alkene 2.99%, fatty acid 2.12%, vitamin E 1.84%, sesquiterpene 1.39%, resorcinol anandamide 1.07%. The least abundance class of bioactive compounds was nortriterpenes 0.94%, fatty aldehyde 0.15%, heterocyclic compound 0.14%, cycloketone 0.26% and terpenes 0.85%.

Table 6. FTIR interpretation of compounds of methanol extract of *Eranthemum pulchellum*

S. No.	Peak ranges (cm^{-1})	Peak Value (cm^{-1})	Appearance	Vibration/bond	Specific functional group
1	3550-3200	3209	Strong, Broad	O-H stretching	Alcohol
2	3000-2840	2945.05	Medium	C-H stretching	Alkane
3	3000-2840	2847.49	Medium	C-H stretching	Alkane
4	1670-1600	1621.56	Strong, broad	C=C stretching	α ,β - unsaturated ketone
5	1465	1492.44	Medium	C-H bending	Alkane
6	1420-1330	1397.61	Medium	O-H bending	Phenol
7	1420-1330	1333.04	Medium	O-H bending	Phenol
8	1310-1250	1259.18	Medium	C-O stretching	Aromatic ester
9	1250-1020	1143.54	Medium	C-N stretching	Amine
10	1250-1020	1116.98	Medium	C-N stretching	Amine
11	1085-1030	1033.61	Strong, Broad	C-O stretching	Primary Alcohol
12	895-885	898.28	Medium	C=C bending	Alkene
13	840-600	830.13	Medium	C=C bending	Alkene
14	500-790	508.95	Medium	C-I stretching	Halo compounds

Table 7. FTIR interpretation of compounds of ethyl acetate extract of *Eranthemum pulchellum*

S. No.	Peak ranges (cm^{-1})	Peak Value (cm^{-1})	Appearance	Vibration/bond	Specific functional group
1	3550-3200	3365.68	Broad	O-H stretching	Alcohol
2	3000-2800	2917.43	Strong, Sharp	N-H stretching	Amine salt
3	3000-2840	2849.36	Medium	C-H stretching	Alkane
4	1720-1706	1710.1	Strong, Sharp	C=O stretching	Carboxylic acid
5	1550-1500	1516.32	Medium	N-O stretching	Nitro Compound
6	1600-1300	1446.14	Medium	C-H bending	Alkane
7	1400-1000	1373.4	Strong, Sharp	C-F stretching	Fluoro compound
8	1275-1200	1238.64	Strong Sharp	C-O stretching	Alkyl aryl ether
9	1250-1020	1158.88	Medium	C-N stretching	Amine
10	1070-1030	1043.79	Strong, sharp	C-O stretching	Primary alcohol
11	840-790	815.25	Medium	C=C bending	Alkene
12	690-515	606.72	Medium	C-Br stretching	Halo compound

Table 8. FTIR interpretation of compounds of n-hexane extract of *Eranthemum pulchellum*

S. No.	Peak ranges (cm^{-1})	Peak Value (cm^{-1})	Appearance	Vibration/bond	Specific functional group
1	3000-2840	2956.6	Medium	C-H stretching	Alkane
2	3000-2800	2916.27	Strong, Sharp	N-H stretching	Amine salt
3	3000-2800	2848.56	Strong, Sharp	N-H stretching	Amine salt
4	1720-1706	1710.78	Medium	C=O stretching	carboxylic acid
5	1650-1566	1576.85	Medium	C=C stretching	Cyclic alkene
6	1600-1300	1461.94	Medium	C-H bending	Alkane
7	1390-1310	1376.29	Medium	O-H bending	Phenol
8	1275-1200	1260.25	Medium	C-O stretching	Alkyl aryl ether
9	1124-1087	1092.27	Medium, Broad	C-O stretching	Secondary alcohol
10	1250-1020	1021.49	Medium, Broad	C-N stretching	Amine
11	840-790	803.06	Medium	C=C bending	Alkene
12	730-665	719.69	Medium	C=C bending	Alkene

For the n-hexane extract, alkane had a total abundance of 28.51% followed by fatty acid ester 20.59%, triterpene 20.84%, diterpene 6.03%, pentacyclic triterpene 4.18%, steroid 4.14%, fatty alcohol 4.08%, steroids 4.07% resorcinol-anandamide 1.39% and vitamin E 1.28%. The least abundant class of bioactive compounds were nortriterpenes 0.84%, fatty acid 0.29%, alkene 0.92%, sesquiterpenes 0.17% and terpene alcohols 0.36%.

Table 5 shows the bioactive compounds common in methanol, ethyl acetate and n- hexane extracts of *E. pulchellum*. Squalene was prevalent in ethyl acetate extract 14.75% compared to methanol 12.34% and n-hexane extract 12.86%. In n-hexane extracts, eicosyl trifluoroacetate is predominated 20.59% compared to ethyl acetate 20.44% and methanol extracts 6.27%. Alpha -tocopherol was more commonly found in the extract of methanol 2.06% compared to ethyl acetate 1.84% and n-hexane extracts 1.28%. The diterpene was more abundant in methanol extract 10.54% compared to ethyl acetate 9.47% and n-hexane extract 5.65 %. The triterpene was found in higher concentrations in methanol extract 8.83% compared to ethyl acetate 7.64% and n-hexane 7.15%. The fatty acids were more prevalent in the ethyl acetate extracts 6.54% than in the n- hexane 6.12% and methanol extracts 0.88%. The different concentrations of these bioactive compounds in the three extracts underscored the importance of selecting the right extraction solvents while doing research, keeping the target bioactive compounds in mind.

A total of 137 phytochemical compounds have been identified in methanol ethyl acetate, and n- hexane extracts of *E. pulchellum* with 39 hydrocarbons, 11 fatty alcohols, 12 triterpenes, 11 steroids four pentacyclic triterpenes, nine fatty

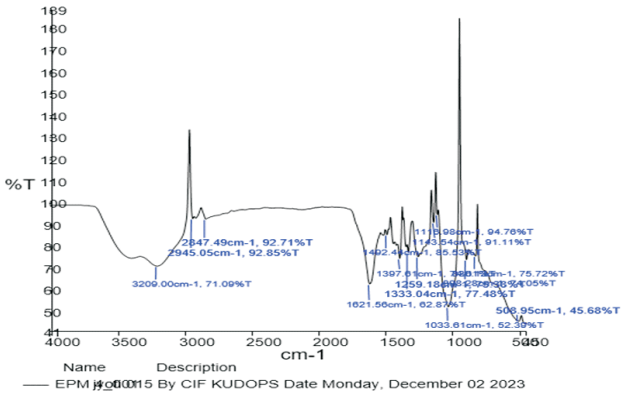


Figure 7: FTIR Spectra of methanol extract of *Eranthemum pulchellum*

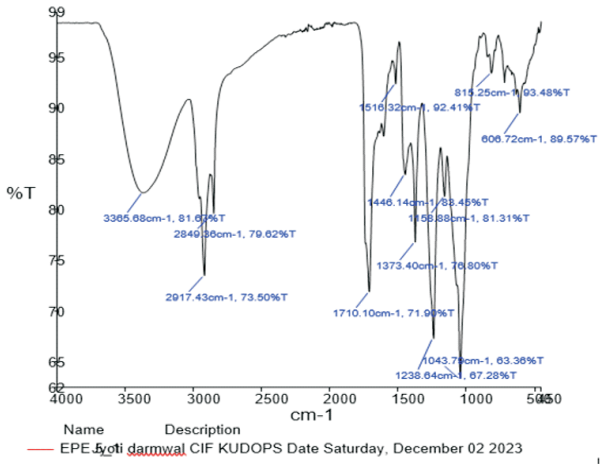


Figure 8: FTIR Spectra of ethyl acetate extract of *Eranthemum pulchellum*

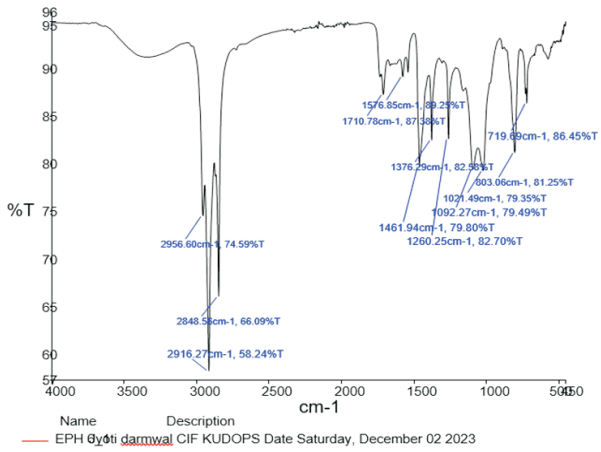


Figure 9: FTIR Spectra of n-hexane extract of *Eranthemum pulchellum*

acids, three fatty acid esters, five fatty aldehydes, nine diterpenes, three sesquiterpenoids, three vitamin E and two nortriterpenes. A comparison of the compounds examined in this study with existing literature indicates that most of them are responsible for various medicinal properties. Squalene is reported for its antioxidant and antitumour properties and is used as emollient in the skincare products (Huang *et al*., 2009). Eicosyl trifluoroacetate is known for antimicrobial and antifungal properties (Jeevitha *et al*., 2018) whereas phytol and its acetate have been shown to possess antioxidant, anti-inflammatory, anticancer and antidiuretic properties(Swamy *et al*., 2017). i-Propyl alpha-linolenate is known for antibacterial properties (Kusumah *et al*., 2020) while n-hexadecanoic acid have reported to exhibit the antioxidant, anti-inflammatory, hypocholesterolemic and cancer prevention activities. Neophytadiene has been reported to reveal anti-inflammatory, analgesic, antipyretic, antimicrobial, and antioxidant properties (Swamy *et al*., 2017).Tetratetracontane exhibit antibacterial activity (Rhetso *et al*., 2020). Betulin is known to inhibition of human immunodeficiency virus (HIV) and also possess antibacterial, antimalarial, anti-inflammatory, anthelmintic and antioxidant properties (Yogeeswari *et al*., 2005).

Fourier-Transform Infrared Spectroscopy (FTIR) Studies

Fourier transformed infrared (FTIR) spectroscopy is a reliable and sensitive method for finding the functional groups in plant extracts. The functional groups are analyzed using IR region in the range of 400–4000 cm^{-1}. The functional groups were determined by matching the frequency range with the Sigma-Aldrich reference table.

The results of FTIR spectra confirmed the presence of functional groups in the methanolic leaf extract of *E. pulchellum*. The broadest peak observed at 3209 cm^{-1}, indicates strong O-H stretching, suggesting the presence of alcohol or phenol compounds. This strongly suggests the presence of phenolic compounds, known for their antioxidant activities (Mabasa *et al*., 2021). Peak at 2945.05cm^{-1} and 2847.49cm^{-1} correspond to alkane (C-H stretch). The strong peak at 1621.56cm^{-1} corresponds to C=C stretching representing α ,β -unsaturated ketone. The peak at 1492.44cm^{-1} suggests the presence of an alkane compound (C=H bend). The peak at 1397.61cm^{-1} and 1333.04cm^{-1} indicate phenols assigned to O-H bending. The peak at 1259.18cm^{-1} indicated C-O stretching of an aromatic ester group. The peak at 1143.54$cm^{-1}$and 1116.98 cm^{-1} suggest the presence of C-N stretching in the amine group while a strong, broad peak at 1033.61cm^{-1} represented C-O stretching of primary alcohol. Peak at 898cm-1 and 830.13cm^{-1} represent C=C bending of alkene compounds. A peak at 508.95cm^{-1} indicates C-I stretching of a halo compound (Figure 7 and Table 6).

The ethyl acetate leaf extract of *E. pulchellum* exhibits broad peak at 3365.68cm^{-1} indicating O-H stretching of alcohols in the extract. A strong peak at 2917.43cm^{-1} indicates that the presence of alkaloids due to N-H stretch representing primary and secondary amines (Mabasa *et al*., 2021). A peak at 2849.36cm^{-1} signifies C-H stretching in alkane groups. The strong peak at 1710.1cm^{-1} corresponds to C=O stretch representing carboxylic acids. A peak at 1516.32cm^{-1} denotes N-O stretching of a nitro compound and a peak at 1446.14cm^{-1} indicate the presence of alkanes (C-H bend). The strong band at 1373.4cm^{-1} indicates C-F stretching of a fluoro compound. A strong peak at 1238.64cm^{-1} represents C-O stretching of an alkyl aryl ether. A peak at 1158.88cm^{-1} corresponds to C-N stretching of an amine. A very strong peak

at 1043.79cm^{-1} signifies C-O stretching of primary alcohol. Peak at 815.25cm^{-1} and 606.72cm^{-1} denotes C=C bending and C-Br stretching of alkene and halo compounds, respectively (Figure 8 and Table7).

The peaks at 2956.6 cm^{-1} indicate the presence of alkane (C-H stretch), while a very strong peak at 2916.27 cm^{-1} and 2848.56 cm^{-1} show strong N-H stretching, indicating the presence of an amine salt. A peak at 1710.78cm^{-1} corresponding to medium C=O stretching indicates the presence of a carboxylic acid. A peak at 1576.85cm^{-1} corresponds to a cyclic alkene with C=C stretching. Furthermore, a medium peak at 1461.94cm^{-1} represents C-H bending of an alkane group and 1376.29cm^{-1} denotes O-H bending of a phenol compound. Another peak includes 1260.25cm^{-1} indicating C-O stretching of an alkyl aryl ether. The broad peak observed at 1092.27cm^{-1} signifies C-O stretching of a secondary alcohol, and 1021.49cm^{-1}corresponds to C-N stretching of an amine. Lastly, the peak at 803.06cm^{-1} denotes C=C bending of an alkene compound and 719.69cm^{-1} represents C=C bending of an alkene in the n-hexane leaf extract of *E. pulchellum* (Figure 9 and Table 8).

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