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Ethno-botanical Verification and Phytochemical Profile of Ethanolic leaves Extract of Two Medicinal Plants (*Phragmenthera capitata* and *Lantana camara*) used in Nigeria using GC-MS Technique

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ABSTRACT

Background: *The present experiment was designed to determine the bioactive constituents from extracts of leaves of Phragmenthera capitata and Latana camara. The medicinal value of a plant species is dependent upon its various phytochemical constituents.*

Methodology: *The chemical compositions of the ethanolic extract of the leaves of P. capitata and L. camara were investigated using the Gas Chromatography-Mass spectrometry model GCMS-QP2010 PLUS SHIMADZU. The column used was a Perkin Elmer Elite - 5 capillary columns measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethylpolysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml / min.*

Results: *Fifteen (15) bioactive phytochemical compounds were identified for P. capitata and twenty (20) bioactive phytochemical compounds were identified for L. camara. The prevailing compounds in P. capitata were 3-H-pyrazol-3-1, Decanoic acid, n-Hexadecanoic acid, Pentanoic acid, Methyloctanoic acid, 4-octanoic acid, 15 -tetraosenoic acid, oleic acid, Octadecanoic acid, Pentafluoropropronic acid, (E)-13-docosenoic acid, Vitamin E, Lupeol and Chilosyphone. Also, in L. camara the following compounds were identified, 3-n-hexythiolane, Docosanoic acid, 1- nonanol, 1-hexacosanol, Divalonic acid, Decanoic acid, Octanoic acid, Eicosanoic acid, Hexadecanoic acid, Nonadecanoic acid, 9-12 octadecanoic acid, 11 -octadecadienoic acid, Pentadecanoic acid, Oleic acid, Methyl -Beta-D-arabinopyranoside, Acetic acid, Pentanoic acid, Heneicosanoic acid, methyl ester and 2H-pyran,2-(7-heptadecynloxytetrahydro.*

Conclusion: *Based on the results of findings, it could be concluded that extract from P. capitata and L. camara may have antioxidant, antimicrobial, anti-cancer, anti-diabetic, anti-fungal and anti-convulsant activities due to the presence of secondary metabolites in the ethanolic extract.*

Keywords: *Ethno-botanical verification, phytochemical profile, Phragmenthera capitata, Lantana camara, GC-MS Technique, Ethanolic leaves.*

1.0 INTRODUCTION

The knowledge of plants chemical constituents is key to the discovery of novel therapeutic values, hence the need for studies on plant phytochemical constituents cannot be over emphasized. All over Nigeria and the world beyond, medicinal plant use in folk medicine keeps increasing by the day even as Infectious diseases contribute significantly to high mortality rates judging from a global scale [4].

The therapeutic properties of plants have been used by mankind for centuries with zero or negligible signs of toxicity. These plants have been essential parts of indigenous traditional herbal medicine practice and their role in primary health care systems can never be underrated in developing countries including Nigeria becoming increasingly popular in the developed [35].

Research has revealed that the therapeutic values of plants more often than not are linked to the nature and number of hosts of active principles known as phytochemicals (which include alkaloids, tannins, flavonoids, steroids, terpenoids, saponins, and glycosides) which they contain.

Lantana Camara is one such invasive alien species and is considered by IUCN as one of the world's 100 most invasive species; and among the world's 10 worst weeds. Invasion by non-native exotic plant species poses a serious threat to native plant communities and ecosystem properties, such as population dynamics and community structure [29], and alters native vegetation and causes a threat to biodiversity [29].

Mistletoe plants are hemiparasitic plants that grow on trees such as kola nut (*Cola nitida*), Avocado (*Perseaamericana*), Grape (*Vitis vinifera*), Orange (*Citrus sinensis*) Pear (*Pyruscommunis*), etc. They are known scientifically for rich

nutritive contents such as carbohydrates, protein, fat, fiber, energy volume, and ash, hence their contribution to animals and human nutrition. Mistletoe leaves have been used in the treatment of some acute and chronic health diseases including hypertension, epilepsy, infertility, arthritis, and diabetes as well as diuretic agents [31].

Within the study area, both plants are used by different communities in the treatment of malaria, hypertension, diabetes, epilepsy, infertility, arthritis, and skin infections. This is not properly verified and hence existing documentation is scanty. Attempts have been made by previous researchers on the phytochemical composition and histopathology of these plants [14, 15, 16, 17], but the results of such have not given a detailed characterization of the individual components of these species. Hence, the current study aims to determine the bioactive compounds present in the ethanolic extract of *Phragmenthera capitata* and *Lantana camara* using GC-MS techniques.

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Sample

Fresh leaves of mistletoe (*Phragmenthera Capitata*) growing on Avocado Leaf (Persea Americana) and *Lantana montevidensis* were collected for the study. The collection site of *Phragmenthera Capitata* was Ikot Udota in AfahaEket Local Government Area, Akwa Ibom State while *Lantana camara* was collected at Oron Road in Uyo Local Government Area, Akwa Ibom State. The date of Collection was 20th January; 2023. The plants material was taken for identification and authentication by plant systematics at the Department of Botany Herbarium, Akwa Ibom State University, Ikot Akpaden, Mkpat Enin Local Government Area.

Photos of Fresh Leaves of (A): Lantana camara and (B): Phragmenthera capitata

2.2 Preparation of Plant Material

After the identification, the leaves were washed and sun-dried. The leaves were shredded and spread on cellophane and allowed to dry for 72 hours at room temperature. The dried leaves were pulverized (ground) into fine powder using a wooden pestle and mortar.

2.3 Preparation of Ethanolic Extract (Maceration and Extraction)

Cold extraction method (Maceration) was used in this research according to [22], in the extraction procedure, 1000ml of 99% Concentrated Ethanol was used to Macerate 240g of the plant materials in an airtight container and kept in the laboratory under room temperature for 72 hours (3 days). On the due date of filtration, the mixture was filtered with Muslim cloth to acquire the filtrate. The filtrate was further extracted using funnels, Watman filter paper, a conical flask, and a vacuum pump. The extract was stored in 250ml conical flasks. The conical flask was well labelled, the mouth of the conical flask was covered with foil paper and masking tape was rapped around the mouth to ensure that it is tightly covered.

2.4 Extract Concentration

200ml of the extracted material was transferred into 250ml beakers and was then concentrated using a water bath at a temperature of 80 °C to disintegrate the filtrate to obtain the crude extract of the plants.

2.5 GC-MS Analysis of the Plant Extract

GC-MS analysis was carried out on GCMS-QP2010 PLUS SHIMADZU. The column used was Perkin Elmer Elite - 5 capillary columns measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethylpolysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml / min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250 °C. The oven temperature was programmed initially at 80 °C for 4 min, then an increased to 200 °C. And then programmed to increase to 280 °C at a rate of 20 °C ending with a 5 min. Total run time was 25 min. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS library [1; 20; 11].

3.0 RESULTS

3.1 GC-MS analysis of bioactive compounds identified from the fractions of ethanolic extract of *Phragmenthera capitata*.

The components present in the ethanolic extract of *P. capitata* were identified by GC-MS. The chromatogram is shown in (Fig. 1). The active principles with their retention time (RT) % peak area, % peak height, molecular formula, molecular weight (MW) and percentage composition in the ethanolic extract of *P. capitate* are presented in (Table 1). Fifteen (15) components were identified in the ethanolic extract of *P. capitata*. The compounds were Oleic acid (29.52 %) as the major component followed by Octadecanoic acid (19.31 %), n-hexadecanoic acid (8.29 %), methyloctanoic acid (5.75%), Tetradecanoic acid (4.80 %), Lupeol (2.38 %), (E)-13-Docosenoic acid (1.51 %), Pentafluoropropionic acid (1.40 %), 3H-Pyrazol-3-one, Decanoic acid (1.28 %), 15-Tetracosenoic acid (1.21 %), Vitamin E (1.17 %), Chiloscyphone (0.81 %), 4-Octenoic acid (0.73) and Pentanoic acid having (0.55 %) respectively. Major phyto-compounds obtained and their biological activities have been tabulated in (Table 2).

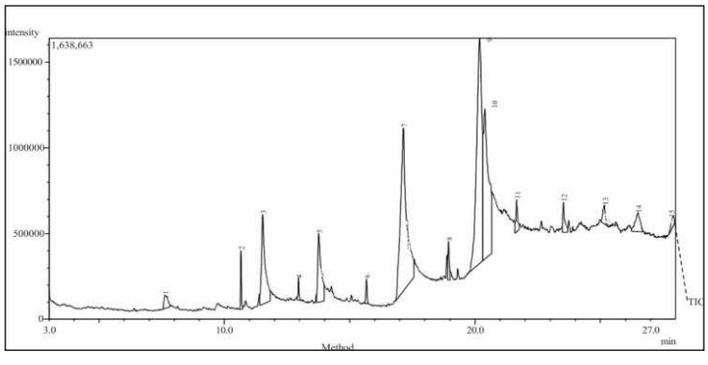

FIG. 1: GC-MS CHROMOTOGRAM OF ETHANOLIC EXTRACT OF P. capitata LEAF

Table.1: Bioactive compounds detected from ethanolic extract of leaves of Phragmenthera capitata.

Peak	Retention time	% Peak Area	% Peak Height	Compound Analyzed	Molecular Weight	Molecular formular
1.	7.628	1.28	1.41	3H-Pyrazol-3-one	126	$C_6H_{10}N_2O$
2.	10.658	1.28	6.08	Decanoic acid	186	$C_{11}H_{22}O_2$
3.	11.526	8.29	9.29	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
4.	12.956	0.55	2.36	Pentanoic acid	130	$C_7H_{14}O_2$
5.	13.761	5.75	7.14	Methyloctanoic acid	158	$C_9H_{18}O_2$
6.	15.667	0.73	2.54	4-Octenoic acid	156	$C_9H_{16}O_2$
7.	17.139	4.80	16.86	Tetradecanoic acid	228	$C_{14}H_{28}O_2$
8.	18.941	1.21	4.02	15-Tetracosenoic acid	380	$C_{25}H_{48}O_2$
9.	20.178	29.52	23.28	Oleic acid	282	$C_{18}H_{34}O_2$
10.	20.389	19.31	15.51	Octadecanoic acid	284	$C_{18}H_{36}O_2$
11.	21.658	1.40	3.36	Pentafluoropropionic acid	332	$C_{15}H_{25}F_5O_2$
12.	23.524	1.51	3.11	(E)-13-Docosenoic acid	338	$C_{22}H_{42}O_2$
13.	25.156	1.17	1,96	Vitamin E	430	$C_{29}H_{50}O_2$
14.	26.499	2.38	2.05	Lupeol	426	$C_{30}H_{50}O$
15.	27.913	0.81	1.04	Chiloscyphone	218	$C_{15}H_{22}O$
Total		**100.0**	**100.0**			

Table 2: Biological Activities of Phytocomponents Identified in the Ethanolic extract of Phragmenthera capitate

S/N	Name of the compound	Nature of Compound	Biological activity	References
1.	3H-Pyrazol-3-one	Heterocyclic organic compound	Anti-microbial, anti-fungal, anti-inflammatory, anti-cancer, anti-viral, anti-convulsant	[13]
2.	Decanoic acid	Straight chain saturated fatty acid	Antibacterial, anti-inflammatory, human metabolites	[13; 5]
3.	n-Hexadecanoic acid	Saturated fatty acid	Anti-oxidants, nematicide and pesticides	[12;33]
4.	Pentanoic acid	Organic acid	anticonvulsant	[13, 6]
5.	Methyloctanoic acid	Carboxylic acid	Anti-bacterial	[13, 5]
6.	4-Octenoic acid	Unsaturated fatty acid	Anti-bacterial, human metabolite	[13, 5]
7.	Tetradecanoic acid	Straight chain saturated fatty acid	Anti-fungal, anti-bacterial	[13, 5]
8.	15-Tetracosenoic acid	Monounsaturated Omega-9-fatty acid	Anti-bacterial	[13, 5]
9.	Oleic acid	Unsaturated fatty acid	Antibacterial	[38]
10.	Octadecanoic acid	Saturated fatty acid	Anti-inflammatory	[6]
11.	Pentafluoropropionic acid	Carboxylic acid	Efficient catalyst	[6]
12.	(E)-13-Docosenoic acid	Monounsaturated Omega-9-fatty acid	Anti-bacterial	[13]
13.	Vitamin E	Fat-soluble vitamin	Anti-oxidant	[12, 32]
14.	Lupeol	Organic compound	anti-inflammatory, anti-carcinogenic, Antibacterial, anti-oxidant, anti-diabetic	[12; 13; 23]
15.	Chiloscyphone	Organic compound	Antibacterial	[13]

3.2 GC-MS analysis of bioactive compounds identified from the fractions of ethanolic extract of *P. capitata*

The results pertaining to GC-MS analysis lead to the identification of a number of chemical constituents from the GC fractions of ethanolic extract of *L. montevidensis*. Twenty (20) bioactive compounds were identified and their, retention time, % peak area, % peak height, molecular formula, molecular weight, and percentage composition in the ethanolic extract of *L. montevidensis* is presented in (Fig. 2, Table 3). The compounds were Oleic acid (26.04 %) as the major component followed by Nonadecanoic acid (21.07 %), Methyl-.beta.-D-arabinopyranoside (12.82 %), 11-octadecanoic acid (8.04 %), 9,12-Octadecadienoic acid (5.17 %), n-Hexadecanoic acid (5.00 %), Hexadecanoic acid (4.93 %), Eicosanoic acid (4.85), Pentadecanoic acid (2.04 %), 3-n-Hexylthiolane, S,S-dioxide (1.96 %), 1-Hexacosanol acid (1.21 %), 2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro- (1.19 %), Octanoic acid (1.10 %), Decanoic acid (1.04 %), Decanoic acid (0.95 %), Acetic acid (0.72 %), Heneicosanoic acid, methyl ester (0.57 %), 1-Nonanol (0.55 %), Divalonic acid (0.45 %) and Pentanoic acid having (0.28 %) respectively. Major phyto-compounds obtained and their biological activities have been tabulated in (Table 4).

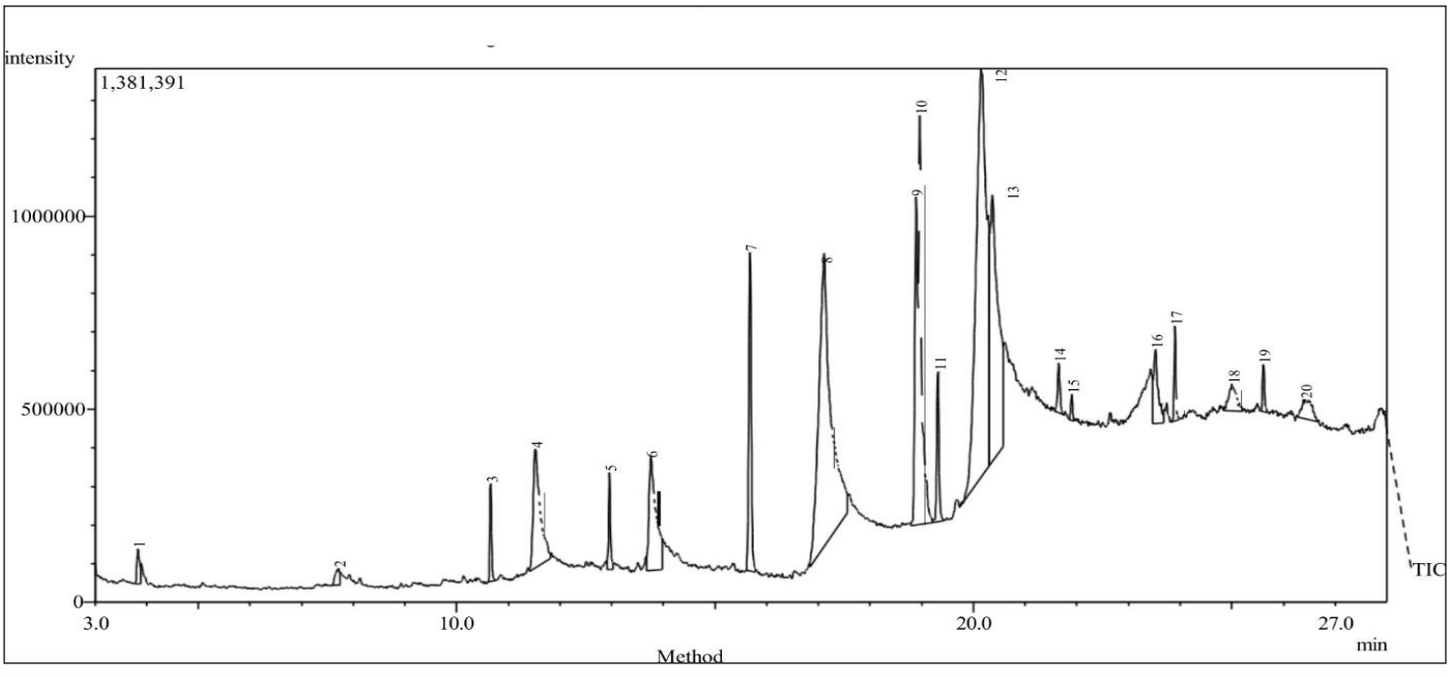

FIG. 2: GC-MS CHROMOTOGRAM OF ETHANOLIC EXTRACT OF L. montevidensis LEAF

Table 3: Bioactive compounds detected from ethanolic extract of leaves of Lantana montevidensis

Peak	Retention time	% Peak Area	% Peak Height	Compound Analyzed	Molecular Weight	Molecular formular
1.	3.835	0.55	1.17	1-Nonanol	144	$C_9H_{20}O$
2.	7.713	0.45	0.56	Divalonic acid	130	$C_6H_{10}O_3$
3.	10.656	1.04	3.29	Decanoic acid, methyl ester	186	$C_{11}H_{22}O_2$
4.	11.524	5.00	3.97	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
5.	12.957	1.10	3.25	Octanoic acid	158	$C_9H_{18}O_2$
6.	13.760	4.85	3.82	Eicosanoic acid	312	$C_{20}H_{40}O_2$
7.	15.677	4.93	10.65	Hexadecanoic acid	270	$C_{17}H_{34}O_2$
8.	17.111	21.07	9.81	Nonadecanoic acid	298	$C_{19}H_{38}O_2$
9.	18.893	5.17	10.95	9,12-Octadecadienoic acid	294	$C_{19}H_{34}O_2$
10.	18.932	8.04	13.65	11-Octadecenoic acid	296	$C_{19}H_{36}O_2$
11.	19.314	2.04	5.03	Pentadecanoic acid	270	$C_{17}H_{34}O_2$
12.	20.147	26.04	13.64	Oleic Acid	282	$C_{18}H_{34}O_2$
13.	20.365	12.82	8.94	Methyl-.beta.-D-arabinopyranoside	164	$C_6H_{12}O_5$
14.	21.650	0.72	1.65	Acetic acid	200	$C_{12}H_{24}O_2$
15.	21.902	0.28	0.87	Pentanoic acid, 4-methyl-, methyl	130	$C_7H_{14}O_2$
16.	23.521	1.96	2.48	3-n-Hexylthiolane, S,S-dioxide	204	$C_{10}H_{20}O_2S$
17.	23.900	0.95	3.18	Docosanoic acid, methyl ester	354	$C_{23}H_{46}O_2$
18.	25.001	1.21	0.90	1-Hexacosanol	382	$C_{26}H_{54}O$
19.	25.611	0.57	1.59	Heneicosanoic acid, methyl ester	340	$C_{22}H_{44}O_2$
20.	26.384	1.19	0.63	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	336	$C_{22}H_{40}O_2$
Total		**100.0**	**100.0**			

Table 4: Biological Activities of Phytocomponents Identified in the Ethanolic extract of Lantana camara

S/N	Name of the compound	Nature of Compound	Biological activity	References
1.	1-Nonanol	Straight chain fatty acid	Anti-bacterial, anti-fungal	[26; 27]
2.	Divalonic acid	Cyclic organic compound	Anti-bacterial	[26; 27]
3.	Decanoic acid	Straight chain saturated fatty acid	Antibacterial, anti-inflammatory, metabolites	[26; 27]
4.	n-Hexadecanoic acid	Saturated fatty acid	Anti-oxidants, nematicide and pesticides	[26]
5.	Octanoic acid	Saturated fatty acid	Anti-bacterial and human metabolite	[6; 33]

6.	Eicosanoic acid	Straight chain saturated fatty acid	Anti-bacterial	[6]
7.	Hexadecanoic acid	Saturated fatty acid	Anti-oxidant, nematicide and pesticides	[6; 29]
8.	Nonadecanoic acid	Straight chain saturated fatty acid	Anti-fungal	[36]
9.	9,12-Octadecadienoic acid	Poly-unsaturated omega-6- fatty acid	Anti-bacterial	[6]
10.	11-Octadecadienoic acid	Unsaturated monocarboxylic acid	Anti-bacterial	[6]
11.	Pentadecanoic acid	Straight chain saturated fatty acid	Anti-bacterial, antifungal	[27]
12.	Oleic Acid	Unsaturated fatty acid	Antibacterial	[6]
13.	Methyl-.beta.-D-arabinopyranoside	Organic compound	Antibacterial	[6]
14.	Acetic acid	Organic compound	Anti-bacterial	[6]
15.	Pentanoic acid	Organic acid	anticonvulsant	[6]
16.	3-n-Hexylthiolane	Organic compound	Antibacterial	[6]
17.	Docosanoic acid	Long-chain saturated fatty acid	moisturizers	[33]
18.	1-Hexacosanol	Saturated fatty acid	Anti-microbial, anti-bacteria,	**[27; 18]**
19.	Heneicosanoic acid, methyl ester	Fatty acid	Plant metabolite	[33]
20.	2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro-	Organic compound	Antibacterial	[6]

4.0 DISCUSSION

The study of organic chemicals found in plants, as well as their actions, has grown in popularity. The combination of GC (best separation technique) with MS (best identification technique) made GC-MS one of the ideal techniques for quantitative analysis of volatile and semi-volatile compounds, [21].

The identified compounds in both extracts of *P. capitata* and *L. camara* showed a wide range of potent bioactivity. These phytochemicals are responsible for various pharmacological actions like antioxidants and antimicrobial activities, [37]. Among the fifteen compounds identified in *P. capitata*, 10 showed Anti-microbial activity, 4 showed Anti-inflammatory, 2 showed anti-fungal, 1 showed anti-cancer, 1 exhibited anti-viral, 2 showed anti-convulsant, 3 showed anti-oxidant and also showed activities such as anti-carcinogenic, anti-diabetic, nematicides, pesticides, metabolites and as a catalyst. In *L. camara* 20 bioactive compounds were identified, with similar biological activity as observed in *P. capitata.*

The results of the GC-MS analysis of ethanolic extract of *P. capitata* and *L. camara* are in deviant from reports of previous authors in related studies who reported lower bioactive compounds as compared to the results of the present study. [30] reported on GC-MS profiling and bioactivity prediction of compounds from *Momordica charantia* (L.) extract and identified only five (5) bioactive compounds, [20] in his studies on GC-MS analysis of *Majideazanquebarica* extract using gas chromatography and mass spectrophotometry technique identified eight (8) bioactive compounds, [10] when working on qualitative phytochemical and GC-MS analysis of some commonly consumed vegetables, identified nine (9) bioactive compounds each in *T. occidentalis* and *O. gratissimum* respectively, and reported eight (8) bioactive compounds for *T. triangulare,* [2] during their studies on the GC-MS analysis of amethanolic extract of *vernonia cinerea* identified nine (9) bioactive compounds and [34] identified nine (9) bioactive compounds when working on an extract of *Guiera senegalensis* using GC-MS analysis. Similarly, [28] reported eight (8) bioactive compounds during their studies on GC-MS analysis of methanolic extract of *H. Africana*.

The results of the present study are in agreement with the reports of [25] who identified twenty-two (22) bioactive compounds when investigating the extract of leaves and fruits of *Trichosanthesischoica* using GC-MS analysis, [7] identified 19 bioactive compounds when reporting on the GC-MS analysis of a methanolic extract of tubers of *P. tuberose*, [8] reported 15 phyto-compounds when working on *Latana camara* and [9] identified 11 bioactive compounds when working on *Withaniasomnifera* (L.) extract using GC-MS analysis.

Phytochemicals are biologically active chemical compounds in plants that wield both medicinal and nutritional potentials [19]. This is evident by the fact that n-hexadecanoic acid, one of the major compounds in the extract of both plant species has been described as a potent antioxidant as well as a dependable anticancer agent [3]. Also, the presence of lupeol in the extract of *P. capitata* has earlier been described as having both anti-cancer and anti-inflammatory properties [23]. Oleic acid is found to have anti-bacterial activity; Vitamin E has been confirmed to have anti-oxidant properties [32].

The therapeutic potentials of bioactive compounds isolated in the extract of *P. capitata* and *L. camara*is are presented in Table 2 and Table 4 with relevant citations to confirm its therapeutic activities. The results of the present study showed that extract from *P. capitata* and *L. camara* has pharmacological potentials based on the bioactive compounds isolated from the two extracts. The identified compounds possess some important biological potentials for future nematicide, pesticides, antibacterial, anti-inflammatory, anti-fungal, anti-convulsant antioxidants and metabolites. The results of the bioactive compounds obtained in *L. camara* has been confirmed by [26] when reporting on Comparative LC–LTQ–MS–MS analysis of the leaf extracts of*Lantana camara* and *Lantana montevidensis* growing in Egyptwith insights into their antioxidant, anti-Inflammatory,andcytotoxic activities.

4.1 CONCLUSION

In conclusion, the major components of the ethanolic extract of *L. camara* and *Phragmenthera capitata,* as indicated in this studycontains bioactive compounds of numerous biological or therapeutic importance. The fifteen and twenty phytochemical constituents identified by the GC-MS analysis of both plant

extract are therefore, medicinally valuable and possess various pharmaceutical applications. The biological activities of each of the identified phyto-components range from antimicrobial, anti-inflammatory, antioxidant, anti-cancer, anti-convulsant, and anti-fungal activities. The research findings have shown that the extract from these plants species is extensively rich in secondary metabolites. The plant's extract has a high potential for a vast number of bioactive compounds which justifies its use for various ailments by traditional practitioners. These findings have provided a scientific basis to the ethno-medical usage of the plant. However, isolation of the individual phytochemical constituents, and subjecting it to biological activity and toxicity profile will give fruitful results. Therefore, further studies isolating individual phyto-compounds to confirm it potential is recommended.

List of Abbreviations
P. capitata- *Phragmenthera capitata*
L. camara – Latana camara
GC – Gas Chromatography
MS – Mass Spectrophotometry
EE – Ethanolic Extract

Declarations

Ethics approval and consent to participate: NIL

Consent for publication: We, the authors have given consent for publication.

Availability of data and materials: Yes, at the request of the authors

Competing interests: No competing interest

Funding: No funding

Authors' contributions: Author 1: Conception, design, and development of the topic, data collection, analysis of data, initial drafting and reviewing the manuscript and final approval of the prepared manuscript. Author 2: Conception, design, and development of research protocol. Author 3: supervision of the experiments, data analysis, and review of the manuscript. Author 4: Student who actually played a supporting role in the experiments and reviewing the manuscript.

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