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Identification of Bioactive Phytocompounds present in Methanolic Extract of *Neolamarckia cadamba* Leaves from Ranchi district of Jharkhand

Research Article

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Abstract

This study aimed to identify the bioactive phytocompounds present in the leaves of *Neolamarckia cadamba*, a plant traditionally used for ethnomedicinal purposes in Ranchi district, Jharkhand, India. In this study methanolic extract of *Neolamarckia cadamba* leaves was used for performing Chromatography-Mass Spectrometry (GC-MS) for the analysis of respective phytocompounds. The GC-MS data was interpreted using the NIST14.L (2020) database, where the spectrum of unidentified compounds was compared with the spectrum of existing identified compounds. The major phytocompounds identified were α- tocopherol (12.73%), n- hexadecanoic acid (11.23%), gamma.-Sitosterol (8.43%) and Squalene (7.32%). These identified phytocompounds possesses different kinds of biological activities such as anti- inflammatory, antioxidant, antimicrobial etc. These biological properties of identified phytocompounds recommends *Neolamarckia cadamba* as a plant of medicinal importance. The findings of this study validate the traditional use of *Neolamarckia cadamba* and highlight its potential as a source of natural remedies. The presence of these bioactive compounds suggests that *Neolamarckia cadamba* could be explored further for the development of novel therapeutic agents. This research provides a scientific basis for the ethnomedicinal use of *Neolamarckia cadamba* and underscores the importance of preserving and promoting traditional knowledge of medicinal plants. The study's results have implications for the discovery of new drugs and the development of sustainable healthcare solutions.

Keywords: *Neolamarckia cadamba,* Medicinal, Jharkhand, Solvent, GC- MS, NIST.

Introduction

Medicinal plants have been the basis of healthcare since ancient times and are still the first choice for medication purposes throughout the world. Various research on medicinal plants has also shown that single plants consist of different important phytocompounds and can be found even more with the combination of different plants. One of the major assets of using plants as medicine is the findings that have been gathered over thousands of years and traditional practices. Medicinal uses of plants have also been recognized by WHO (World Health Organization) (1). Different parts of plants possess different bioactive compounds. These bioactive compounds can be identified using various extraction techniques. Modern advanced instrumentation has made identification of bioactive compounds easier. GC- MS is one such instrumentation process which helps in identification of phytocompounds present in the plant. Solvents used for extraction also affect the extraction and quantification of bioactive compounds. Polar solvents are generally used for extraction of phenolic compounds, likewise non- polar are for fats and oils (2).

Neolamarckia cadamba is a commonly found tree in India and is known for its therapeutic properties and has been used traditionally for medicinal purposes since ages. Studies have shown the effectiveness of *Neolamarckia cadamba* leaves against various chronic diseases like cancer and diabetes (3-4). This present study aims at identification of bioactive phytocompounds present in leaves of *Neolamarckia cadamba* and methanol is used as solvent (polar) for extraction.

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Materials and Methods

Collection and Extraction

Leaves of *Neolamarckia cadamba* were collected from Ranchi ($23^0$22'N $85^0$20'E) district of Jharkhand, India. Collected leaves were washed properly and dried under the shade at room temperature. Dried plant material was transformed into powder using a mechanical grinder. 5g of powdered plant material was dissolved in 50 ml solvent (Methanol) making it at 1:10 (w/v) and kept in the shaker for 24 hrs. The mixture was filtered and dried to yield the extract.

GC- MS Analysis

GC- MS was used for the identification of different metabolites. In this process, a 2 µl prepared methanolic sample with helium as carrier gas was

injected at 1 ml/min in a GC- MS QP2010 model (Shimadzu®), SH- I- 5 Sil MS Capillary with split less injection mode. Ionization of the sample was done at 70 eV. For operating the GC- MS, the oven temperature was set at 45^0 C for 2 min and then increased to 140^0 C at 5^0 C per minute. Lastly, the temperature was increased to 280^0 C, which was isothermally held for 10 min.

Identification

Interpretation of GC- MS was conducted using the NIST (National Institute of Standards and Technology) library. The spectrum of unidentified compounds was compared with the spectrum of existing identified compounds. The table of compounds was formulated after their identification with the help of the NIST14. L (2020) database.

Result and Discussion

This present GC- MS analysis of *Neolamarckia cadamba* leaves showed 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6, Benzoic acid, Catechol, 4-Vinylphenol, 5-Hydroxymethylfurfural, 2-Methoxy-4-vinylphenol, 4-Vinylbenzene-1,2-diol, Phenol, 4-ethenyl-2,6-dimethoxy-, 1-acetate, 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl, Tetradecanoic acid, Neophytadiene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 1,4-Dibutyl benzene-1,4-dicarboxylate, Phytol, Linolenic acid, cis-7-Dodecen-1-yl acetate, Octadecanoic acid, Undec-10-ynoic acid, tetradecyl ester, gamma.-Sitosterol, n-Propyl 9,12-octadecadienoate, 9, 12, 15- Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-, Octadecanoic acid, 2,3-dihydroxypropyl ester, Squalene, Hexacontane, α-Tocopherol and 5-Docosyldihydrofuran-2(3H)-one as bioactive phytocompounds. The peak report of chromatogram which includes retention time, area %, height % and molecular formula of compounds are depicted in the table (table 1).

Table 1: Identified phytocompounds from methanolic extracts of *Neolamarckia cadamba* leaves

Sl. No.	Name of compounds	R. Time	Area %	Height %	Molecular Formula
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 methyl	14.597	1.05	0.92	$C_6H_8O_4$
2	Benzoic acid	15.625	2.29	0.61	$C_7H_6O_2$
3	Catechol	16.103	1.91	1.07	$C_6H_6O_2$
4	4-Vinylphenol	16.751	0.93	1.25	C_8H_8O
5	5-Hydroxymethylfurfural	16.999	0.88	0.75	$C_6H_6O_3$
6	2-Methoxy-4-vinylphenol	19.378	0.88	1.38	$C_9H_{10}O_2$
7	4-Vinylbenzene-1,2-diol	23.046	2.47	1.78	$C_8H_8O_2$
8	Phenol, 4-ethenyl-2,6-dimethoxy-, 1-acetate	27.665	0.53	0.67	$C_{12}H_{14}O_4$
9	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl	30.313	0.08	0.17	$C_{13}H_{22}O_3$
10	Tetradecanoic acid	32.076	5.77	5.10	$C_{14}H_{28}O_2$
11	Neophytadiene	33.088	1.06	2.40	$C_{20}H_{38}$
12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	33.650	0.35	0.83	$C_{20}H_{40}O$
13	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	33.964	0.48	0.97	$C_{17}H_{24}O_3$
14	Hexadecanoic acid, methyl ester	34.206	0.62	1.50	$C_{17}H_{34}O_2$
15	n- Hexadecanoic acid	34.780	11.23	8.00	$C_{17}H_{34}O_2$
16	1,4-Dibutyl benzene-1,4-dicarboxylate	34.586	0.78	1.78	$C_{16}H_{22}O_4$
17	Phytol	36.953	2.69	3.76	$C_{20}H_{40}O$
18	Linolenic acid	37.533	5.53	4.24	$C_{18}H_{30}O_2$
19	cis-7-Dodecen-1-yl acetate	37.412	2.69	1.82	$C_{14}H_{26}O_2$
20	Octadecanoic acid	37.994	3.64	3.19	$C_{18}H_{36}O_2$
21	Undec-10-ynoic acid, tetradecyl ester	39.909	0.99	1.19	$C_{25}H_{46}O_2$
22	gamma.-Sitosterol	43.093	8.43	4.33	$C_{29}H_{50}O$
23	n-Propyl 9,12-octadecadienoate	44.597	0.61	1.16	$C_{21}H_{38}O_2$
24	9, 12, 15- Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	44.681	0.44	0.99	$C_{21}H_{36}O_4$
25	Octadecanoic acid, 2,3- dihydroxypropyl ester	44.908	1.54	2.84	$C_{21}H_{42}O_4$
26	Squalene	46.026	7.32	10.34	$C_{30}H_{50}$
27	Hexacontane	47.137	0.95	1.27	$C_{60}H_{122}$
28	α- Tocopherol	51.175	12.73	6.26	$C_{28}H_{48}O_2$
29	5-Docosyldihydrofuran-2(3H)-one	51.907	0.89	0.82	$C_{26}H_{50}O_2$

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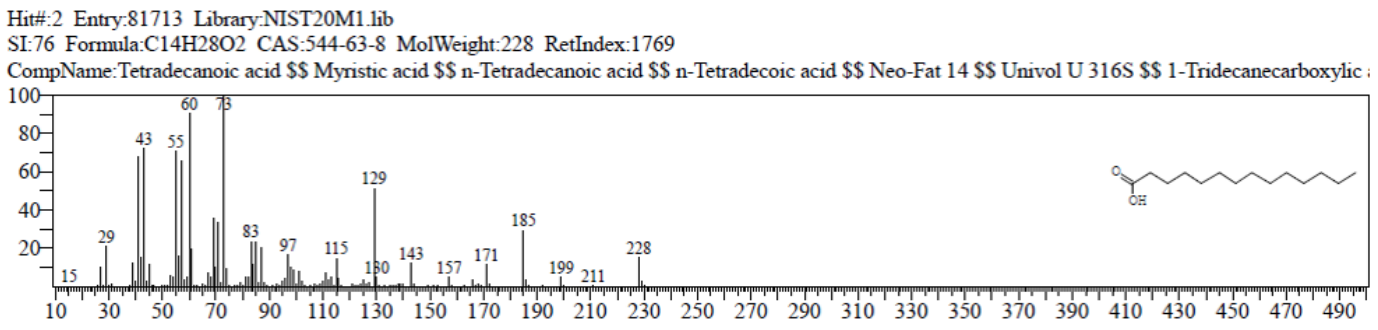
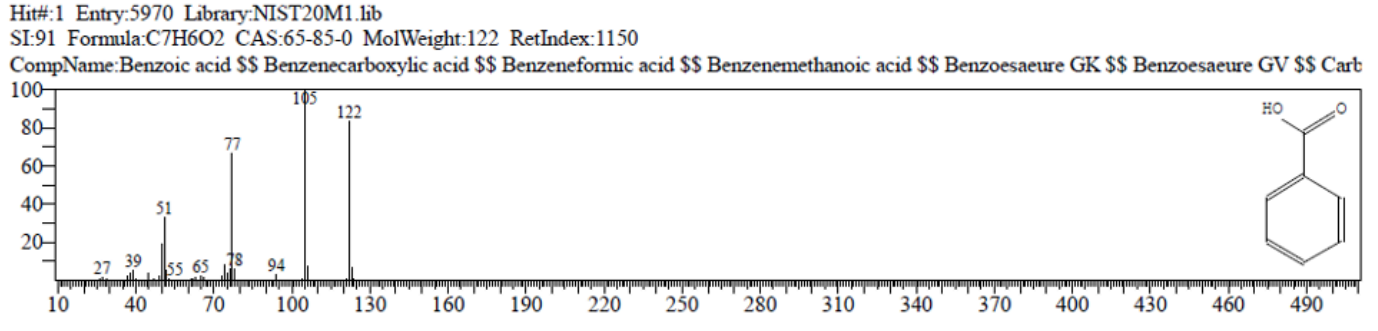
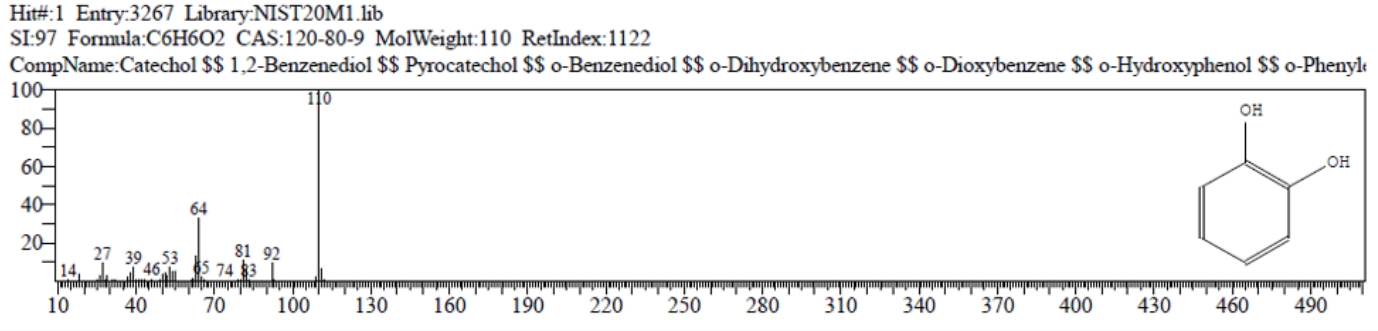
Graph 1: GC- MS chromatogram data of Tetradecanoic acid



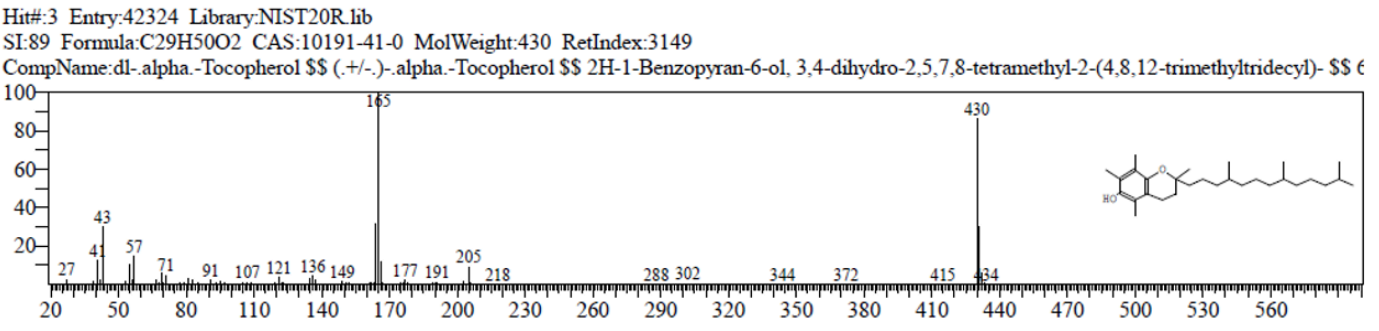
Graph 2: GC- MS chromatogram data of Benzoic acid



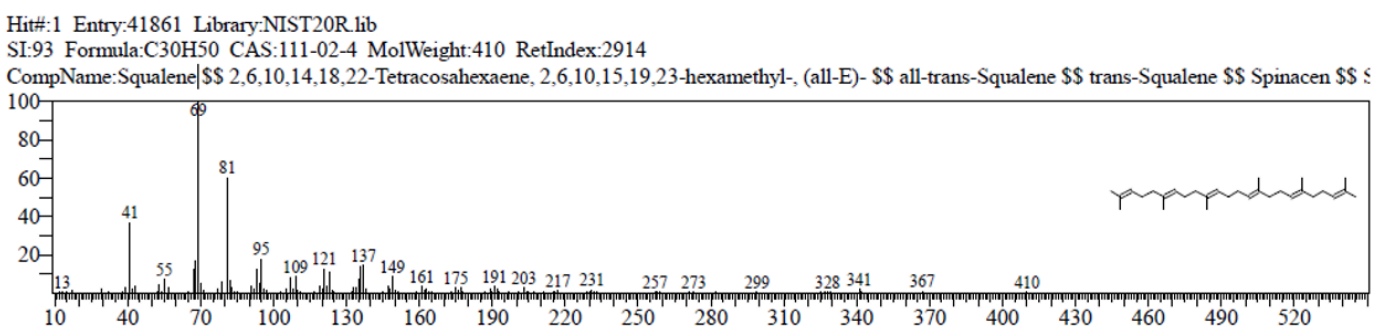
Graph 3: GC- MS chromatogram data of Catechol



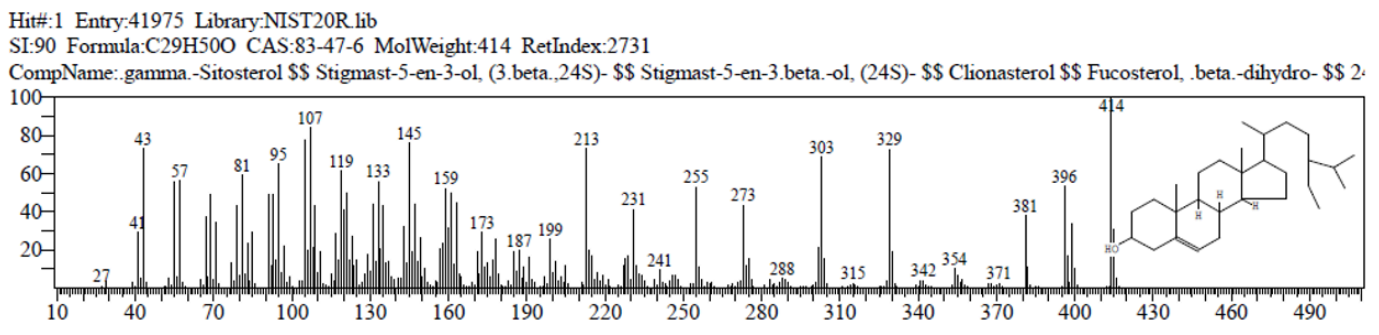
Graph 4: GC- MS chromatogram data of α- Tocopherol



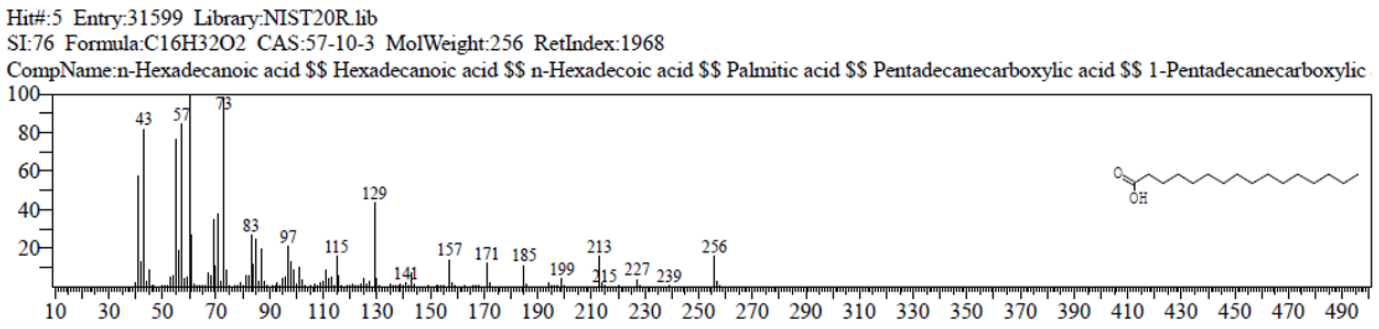
Graph 5: GC- MS chromatogram data of Squalene



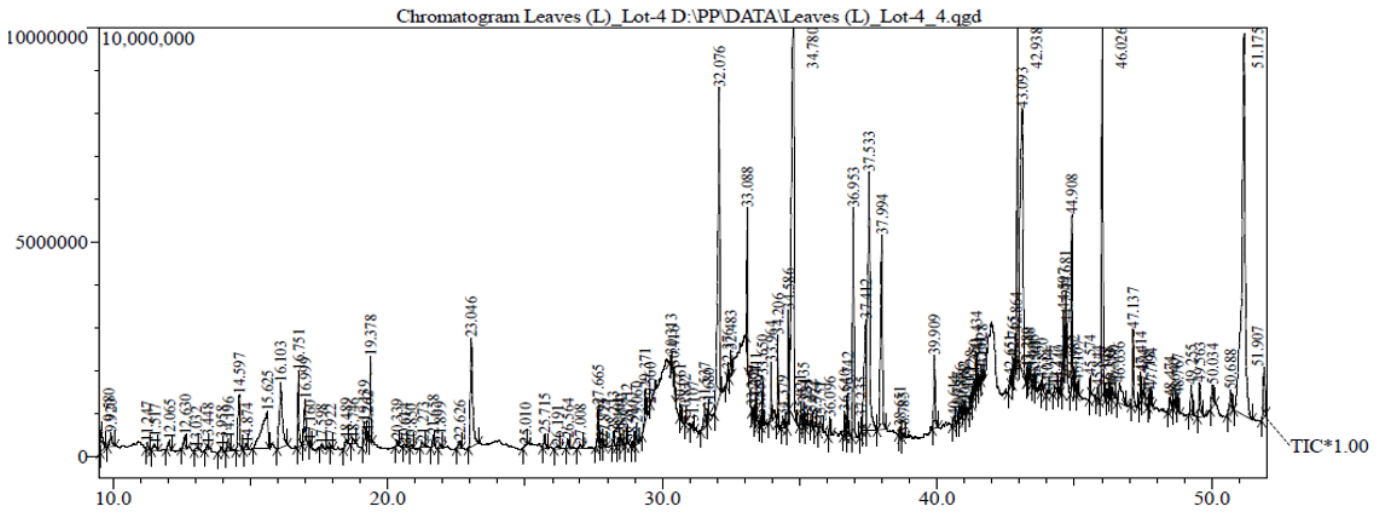
Graph 6: GC- MS chromatogram data of gamma.-Sitosterol



Graph 7: GC- MS chromatogram data of n- hexadecanoic acid



Graph 8: GC- MS chromatogram of *Neolamarckia cadamba* leaves



Phytocompound differs in every plant depending on their genetic, chemical and physiological environmental conditions. Different metabolite pathways specify the elucidation of different phytocompounds which directly or indirectly affect the nature of response of the plants to the changing of their surrounding environment which could be physical, chemical or genetic. Since Gas chromatography mass spectrometry (GC-MS) is a very sensitive instrument hence, considered one of the best developed techniques for phytocompound analysis. GC-MS based methodologies were the first in profiling phytocompounds and in analysing target metabolites. GC-MS technique also helps in simplifying data processing and managing the specificity of metabolites because of its sensitive nature (6).

The above table (table 1.) revealed that methanolic extracts of *Neolamarckia cadamba* are rich in phytocompounds. α- tocopherol (12.73%) and n-hexadecanoic acid (11.23%) was found as the major compound in leaves. Zayed *et al.* (2014) also studied phytocompounds of *Neolamarckia cadamba* from Malaysia in different solvents (Hexane, Petroleum ether, Chloroform, Ethyl acetate and methanol). He identified 26 important phytochemicals from all the studied solvents (7).

α- Tocopherol from the above finding is a compound of vitamin E which is generally present in the chloroplast of plants and plays an important role in plant stress tolerance (6). According to a study, α-Tocopherol has antioxidant, anti- inflammatory and anticancerous properties (7-9). In Ayurveda, oils rich in n- hexadecanoic acid were used against rheumatic symptoms (10). Ravi & Krishna (2017) worked on n-hexadecanoic acid which was extracted from *Kigelia pinnata* and showed cytotoxic activity of the same (11).

Squaline is known to be found in human skin. It protects from UV and other harmful radiation which cause lipid peroxidation. Hence, it is used in cosmetics. It is also used as supplements and in pharmaceutics due to its nutraceutical properties (12-13). Butyl 9,12,15-octadecatrienoate found in *Carica papaya* leaves whereas octadecanoic acid was found in leaves of *Epihyllum oxypetalum* and is known for antimicrobial properties (14-15). Phytols are one of the abundant biological compounds found in nature produced by photosynthetic organisms mainly used for fragrance purposes. Intense study on phytol has revealed its various biological properties, which include antibacterial, antioxidant, anticonvulsant, antinociceptive, anxiolytic and anti- inflammatory properties (16).

Conclusion

In conclusion, GC- MS profiling of compounds from methanolic extracts of *Neolamarckia cadamba* identified several phytocompounds with various biological properties. A major phytocompound with 12.73% peak area was found at a retention time of 51.175. Most of the compounds found were free radical scavengers, which indicates the plant as high in antioxidants, which supports the work of previous workers on *Neolamarckia cadamba* as a potent antioxidant agent.

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