International Journal of Food Science and Agriculture, 2022, 6(4), 403-408
https://www.hillpublisher.com/journals/jsfa/
ISSN Online: 2578-3475
ISSN Print: 2578-3467

Evaluation of Bioactive Compounds in *Afrostyrax Lepidophyllus* Seeds Using Gas Chromatography-Mass Spectrometry

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How to cite this paper: N. C. Oganezi, C. U. Okoronkwo, U. C. Ebere, P. O. Peter. (2022) Evaluation of Bioactive Compounds in *Afrostyrax Lepidophyllus* Seeds Using Gas Chromatography-Mass Spectrometry. *International Journal of Food Science and Agriculture*, 6(4), 403-408.
DOI: 10.26855/ijfsa.2022.12.007

Received: October 28, 2022
Accepted: November 26, 2022
Published: December 23, 2022

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Abstract

Afrostyrax lepidophylus seeds are utilized as spice in some African cuisines and for medicinal purposes in some cultures. The work was aimed at identifying compounds in methanol and dichloromethane/methanol extract of flour from whole seeds of *Afrostyrax lepidophylus*. Fourteen compounds were identified in the methanol extract while sixteen compounds were present in the dichloromethane/methanol extract. The most abundant compounds in the methanol extract were 9,12-Octadecadienoic acid, oleic acid, n-Hexadecanoic acid and 3H-Pyrazzole-3-one, 4-benzoyl-2,4-dihydro-5-methyl-2-phenyl which had relative abundance of 49.14, 10.26, 11.41 and 10.53% respectively. Results also indicated the presence of sulfur containing compounds in the methanol extract. *trans*-13-Octadecenoic acid and n-Hexadecanoic acid were the most abundant compounds in the dichloromethane/methanol extract and had relative abundance of 63.97 and 15.95% respectively. Dichloromethane/methanol extracted more of fatty acids, fatty acid derivatives and methyl esters of fatty acids while methanol extracted more of fatty acids, sulfur containing compounds and pyrazzole compound. The compounds identified in both extracts of *Afrostyrax lepidophylus* seeds have various beneficial bioactivities. As such, *Afrostyrax lepidophylus* seeds can serve as a good raw material from which bioactive compounds can be isolated and utilized in relevant food systems, as well as in the preparation of neutraceuticals and pharmaceuticals.

Keywords

Afrostyrax lepidophyllus seeds, bioactive compounds, relative abundance, gas chromatography/mass spectrometry

1. Introduction

Spices form an integral part of indigenous cultures but can be adopted by neighboring populace due to health benefits they can offer. Spices are plant parts used for culinary purposes and they serve to impart taste with varied flavor and aroma. They contain varied concentrations of macronutrients alongside, being laden with bioactive compounds associated with various physiological properties. Culinary herbs and spices are annual, biennial and perennial plants whose green parts, tender roots or ripe seed have an aromatic flavor and fragrance, due either to a volatile oil or to other chemically named substances peculiar to the individual spices [1]. A good number of these plants contain substances which have curative or protective properties against different diseases [2]. These compounds offer positive health benefits such as being antioxidants which have the ability of mopping up reactive oxygen species (ROS) or reactive nitrogen species (RNS) which induce oxidative stress causing various types of cancer in different body parts [3].

Spices are increasingly finding other important roles in healthcare aside their main use as organoleptic enhancers in

culinary [4]. Seed spices produce numerous secondary metabolites or phytochemicals which have overlapping actions including antioxidant, anti-cancer, anti-diabetic, antimicrobial activity, hypolipidemic effects etc all aimed at improving many human disorders [5]. Spices and herbs are reported to be excellent sources of antioxidants with high content of phenolic compounds and a frequent consumption of spicy foods are associated with lower risk of death from cancer, ischemic heart and respiratory diseases [6]. Currently, there are recommendations in support to the consumption of foods rich in bioactive components such as herbs and spices [7].

Afrostyrax lepidophyllus also known as 'Bush onion' is a shrub like growing tree from the Huaceae family [8]. It has more restricted distribution occurring in the tropical forests of some Central and West African countries [9]. It is predominant in Cameroon where the seeds, bark and leaves are important as medicine [10]. The seeds are used as one of the spices in the preparation of a traditional Cameroonian food "Nkui" and is envisaged to have significant effects on female reproductive physiology [11]. The seeds are used as condiment in several African dishes [8] one of such is an African cuisine known as 'ofeakwu' or 'banga stew' which is popular in the Southern part of Nigeria.

Currently, many herbs and spices are investigated to ascertain bioactive phytochemicals which confer them with various therapeutic roles and nutraceutical activities. Identification of individual components of complex mixtures of phytochemicals requires the use of several techniques and one of the most popular method of studying phytochemical composition is the gas chromatography-mass spectrometry technique [12]. The identification and characterization of bioactive compounds in various food sources is useful in the production of neutraceuticals and allied products such as pharmaceutics useful for human existence. It is in this view that gas chromatography/mass spectrometry analysis was carried out to identify and characterize compounds present in whole seeds of *Afrostyrax lepidophyllus*. This will provide an in-depth knowledge on the usefulness of this spice.

2. Methods

2.1 Sourcing of Afrostyrax lepidophyllus seeds and extraction of bioactive compounds

Whole seeds of mature *Afrostyrax lepidophyllus* were sourced from Ahia Ohuru Market Aba in Obingwa Local Government Area, South-East Nigeria. They were dehulled, washed in clean portable water, drained of water and air dried. The air dried seeds were pulverized to fine flour and twenty (20) grams of the pulverized flour was dispensed into two different labeled conical flasks. Two hundred (200)mL absolute methanol and dichloromethane/methanol (1:1, v/v) were dispensed into the two different conical flasks respectively. The sample mixtures were shaked vigorously on a vortex mixer for 30mins and covered using aluminum foil. They were allowed to stand for 24h at room temperature and subsequently filtered through No1 Whatman filter paper respectively. Each sample extract was concentrated by heating over a boiling water bath to remove excess solvent. Hence, two extracts from whole seeds of *Afrostyrax lepidophyllus* were obtained. These extracts were subjected to gas chromatography/mass spectrometry (GCMS) analysis for the separation and identification of compounds.

2.2 Gas Chromatography Mass Spectrometry Analysis (GC–MS)

The GC–MS analysis of bioactive compounds from the two extracts were done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). Helium gas (99.995%) was used as carrier gas with flow rate of 1 mL/min. The initial column temperature was set at 50–150°C with increasing rate of 3°C/min and hold time of 10 min. Finally, the temperature was increased to 300°C at 10°C/min. One microliter (1μl) of each extract diluted with respective solvents was injected in a splitless mode into the gas chromatogram using hamilton syringe. The injector temperature was 250ºC while the mass spectrometer ion source temperature was 200ºC with an interface temperature of 280ºC and recorded over a scan range of 70-200m/z for the methanol extract and 40-650m/z for the dichloromethane/methanol extract but with electron impact ionization energy of 70 ev. Total run time of the gas chromatography-mass spectrometry analysis for the methanol extract was 25mins and 36mins for the dichloromethane methanol extract. The relative abundance of the chemical compounds present in each extract of was expressed as percentage based on peak area normalization produced in the chromatogram. Compounds were identified by mass spectroscopy. This was done by comparing retention indices and mass fragmentation patterns of the compounds with those stored in the computer library software of the National Institute of Standard Technology (NIST/EPA/NIH, Mass Spectral Library, Version 2.0). Quantitative determinations were made by relating respective peak areas to TIC areas of the GCMS.

3. Results

Gas chromatography/mass spectrometry (GCMS) analysis of whole seeds of *Afrostyrax lepidophyllus* revealed the presence of 14 compounds from the methanol extract and 16 compounds from the dichloromethane/methanol extract.

Table 1 shows results of compounds in the methanol extract of whole seeds of *Afrostyrax lepidophyllus* while Table 2 shows results of dichloromethane/methanol extract of *Afrostyrax lepidophylus* seeds.

4. Discussion

Results from the methanol extract (Table 1) indicated the presence of sulfur containing compounds, pyrazzole derivatives, fatty acids, methyl ester of fatty acids. The most abundant compound was 9,12-Octadecadienoic acid (i.e linoleic acid) and had a relative abundance of 49.14%. Other compounds found in appreciable quantities were oleic acid, n-Hexadecanoic acid and 3H-Pyrazzole-3-one, 4-benzoyl-2,4-dihydro-5-methyl-2-phenyl had relative abundance of 10.26, 11.41 and 10.53% respectively. All these compounds have been reported to have varied health benefits. Linoleic acid is reported to have anti-inflammatory, hypocholesterolemic , cancer preventive, hepatoprotective, nematicide, insectifuge, anti-histaminic, anti-eczemic, antiacne, 5-alpha reductase inhibitor, anti-androgenic, anti-arthritic and anti-coronary activities [13]. Oleic acid has cancer preventive, anemiagenic, insectifuge, anti-androgenic and dermatitigenic properties [14]. n-Hexadecanoic acid has antioxidant property [15]. 3H-Pyrazzole-3-one, 4-benzoyl-2, 4-dihydro-5-methyl-2-phenyl (also known as 4-(benzoyl)-5-methyl-2-phenyl-1,2-dihydro-3H-pyrrazole-3-one) is a pyrrazole derivative. Pyrazzoles are described to bestow a wide range of biological activities such as antimicrobial, antifungal, antitubercular, anti-inflammatory, anti-convulsant, antiviral, angiotensin converting enzyme (ACE) inhibitor activity, neuroprotective, cholecystokinin 1 receptor antagonist and estrogen receptor antagonist and estrogen receptor ligand activity [16]. The presence of pyrrazole nucleus in different structures leads to various applications in areas such as technology, medicine and agriculture [17]. This entails that the presence of 3H-Pyrazzole-3-one, 4-benzoyl-2,4-dihydro-5-methyl-2-phenyl and the other aforementioned compounds in *Afrostyrax lepidophyllus* seeds will proffer a number of health benefits that encompass not only its usefulness for food but also in the development of pharmaceutics, hence the application in medicine.

The relative abundance of sulfur containing compounds was 8.82%. [18] reported the presence of sulfur containing compounds which induced the typical garlic and onion-like odour in *Afrostyrax lepidophyllus* oil. They reported the major sulfur constituents in the oil to be 2,4,5,7-tetrathiadecane, 2,4,5,7,9-pentathiadecane, 2,3,5-trithiadecane and 6-methyl-2,4,5,7,9-pentathiadecane. However, the sulfur compounds we found in the whole seeds of *Afrostyrax lepidophyllus* were 2-Chloroethyl methyl sulfoxide, dimethyl sulfoxide, 2-Chloroethyl chlorobromoethyl sulfide, S-methyl methane thiosulfinate. There is not much pharmacological activities reported for most of these compounds except dimethyl sulfoxide. Dimethyl sulfoxide is reported to be used to treat certain types of melanoma, metastatic anaplastic thyroid cancer, may have analgesic and anti-inflammatory properties [19].

Table 1. Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds in the Methanol Extract of *Afrostyrax lepidophylus* seeds

Peak Number	Retention Time (Mins)	Name of Compound	Relative abundance (%)	Molecular Formula	Molecular weight
1.	5.777	2-Chloroethyl methyl sulfoxide	3.44	C_3H_7ClOS	126.61
2.	7.089	Dimethyl sulfoxide	1.32	C_2H_6OS	78.14
3.	9.221	Phthalic anhydride	3.69	$C_8H_4O_3$	148.1
4.	9.849	2-Chloroethyl chlorobromoethylsulfoxide	1.92	-	-
5.	12.896	Dimethyl sulfoxide	0.60	C_2H_6OS	78.14
6.	15.404	Carbonic acid, butyl dodecyl ester	1.49	$C_{17}H_{34}O_3$	286.4
7.	16.954	Benzene sulfonyl chloride-,4-dinitro	0.87	$C_6H_3ClN_2O_6S$	266.62
8.	17.403	n-Hexadecanoic acid	2.40	$C_{16}H_{32}O_2$	256.42
9.	17.533	n-Hexadecanoic acid	9.01	$C_{16}H_{32}O_2$	256.42
10.	18.412	S-Methyl methanethiosulfinate	0.67	$C_2H_6OS_2$	256.42
11.	18.592	(S)-(-)-2-Chloropropionic acid	1.48	$C_3H_5ClO_2$	110.20
12.	18.745	9-Oxabicyclo [6.1.0] nonane, cis	3.06	$C_8H_{14}O$	126.19
13.	18.983	Hepadecanoic acid, 10-methyl,methyl-ester	0.22	$C_{19}H_{38}O_2$	298.29
14.	19.308	9,12-Octadecadienoic acid (Z,Z)	28.37	$C_{18}H_{32}O_2$	280.40
15.	19.496	Oleic	10.26	$C_{18}H_{34}O_2$	282.47
16.	19.737	9,12-Octadecadienoic (Z,Z)	9.81	$C_{18}H_{32}O_2$	280.40
17.	19.979	9,12-Octadecadienoic (Z,Z)	3.41	$C_{18}H_{32}O_2$	280.40
18.	20.128	9,12-Octadecadienoic (Z,Z)	6.53	$C_{18}H_{32}O_2$	280.40
19.	23.193	3H-Pyrazole-3-one,4-benzoyl-2,4-dihydro-5-methyl-2-phenyl	6.34	$C_{17}H_{14}N_2O_2$	278.31
20.	23.449	3H-Pyrazzole-3-one,4-benzoyl-2,4-dihydro-5-methyl-2-phynyl	4.19	$C_{17}H_{14}N_2O_2$	278.31
21.	23.779	9,12-Octadecadienoic acid (Z,Z)	1.02	$C_{18}H_{32}O_2$	280.40

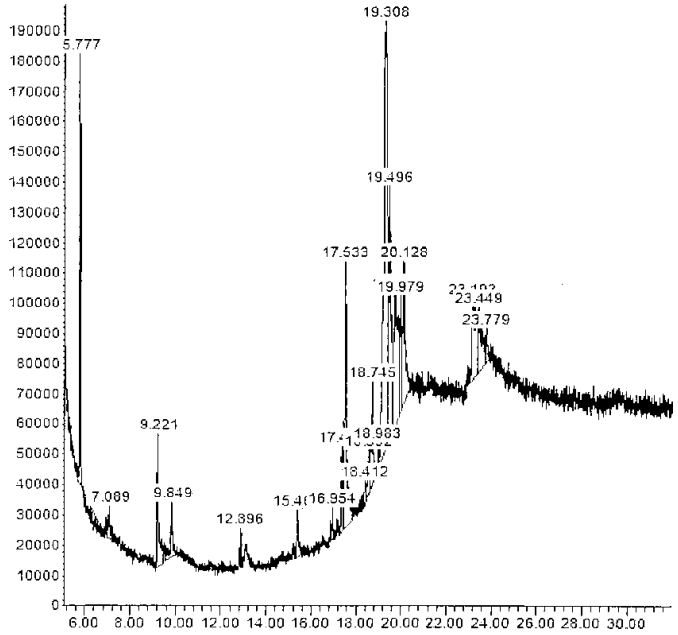

Figure 1. Gas Chromatograph of Methanol extract of *Afrostyrax lepidophylus* seeds.

Results from dichloromethane/methanol extract shown in Table 2 indicated the presence of alkanes, ketones, fatty acid derivatives, methyl esters of fatty acids, fatty acids, unsaturated aldehyde and fatty alchohols. The most abundant compound was *trans*-13-octadecenoic acid and n-Hexadecanoic acid which had relative abundance of 63.97 and 15.95% respectively. Erucic acid was observed to be present and it had a relative abundance of 2.54%. Erucic acid is a monounsaturated omega-9 fatty acid. Excessive intake of erucic acid is attributed to lead to adverse health effects such as reducing the contractile force of the heart muscles [20]. Trans-13-octadecenoic acid has pharmacological activity as being anti-inflammatory [21]. 9,17-Octadecadienal, (Z) is reported to have antimicrobial property [22]. Compounds that were common to both methanol and dichloromethane/methanol extracts were oleic acid, n-Hexadecanoic acid and 9,12-Octadecenoic acid (Z,Z) but they varied in relative abundance.

Table 2. Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds in the Dichloromethane/Methanol Extract of *Afrostyrax lepidophylus* seeds

Peak Number	Retention Time (Mins)	Name of Compound	Relative Abundance (%)	Molecular Formula	Molecular weight
1.	8.120	Hexadecane	0.33	$C_{16}H_{34}$	226.44
2.	8.926	Methyl tetradecanoate	0.24	$C_{15}H_{30}O_2$	242.40
3.	10.821	Tetradecanal	0.63	$C_{14}H_{28}O$	212.37
4.	12.280	2-Heptadecanone	1.36	$C_{17}H_{34}O$	254.50
5.	12.676	Hexadecanoic-acid, methyl ester	0.71	$C_{17}H_{34}O_2$	270.45
6.	13.818	n-Hexadecanoic acid	15.95	$C_{16}H_{32}O_2$	256.42
7.	15.142	9,12-Octadecadienoic acid (Z,Z)	1.72	$C_{18}H_{32}O_2$	280.44
8.	15.425	9-Octadecenoic acid, methyl ester (E)	2.74	$C_{19}H_{36}O_2$	296.50
9.	15.755	Methyl stearate	0.42	$C_{19}H_{38}O_2$	298.50
10.	16.614	Trans-13-Octadecenoic acid	63.97	$C_{18}H_{34}O_2$	282.50
11.	17.682	9,17-Octadecadienal (Z)	3.12	$C_{18}H_{32}O$	264.44
12.	19.692	Oleic acid	0.89	$C_{18}H_{34}O_2$	282.47
13.	20.378	9-Octadecenal, (Z)	0.46	$C_{18}H_{34}O$	266.46
14.	21.127	2-methyl-Z,Z-3,13,-Octadecadienol	1.27	$C_{19}H_{36}O$	280.50
15.	24.150	Oleic acid	0.48	$C_{18}H_{34}O_2$	282.47
16.	27.057	Erucic acid	2.54	$C_{22}H_{42}O_2$	338.58
17.	30.006	Z-2-Octadecen-1-ol	0.96	$C_{18}H_{36}O$	268.50
18.	30.256	Oleic acid	1.06	$C_{18}H_{34}O_2$	282.47
19.	33.585	Oleic acid	0.85	$C_{18}H_{34}O_2$	282.47
20.	34.956	Oleic acid	0.29	$C_{18}H_{34}O_2$	282.47

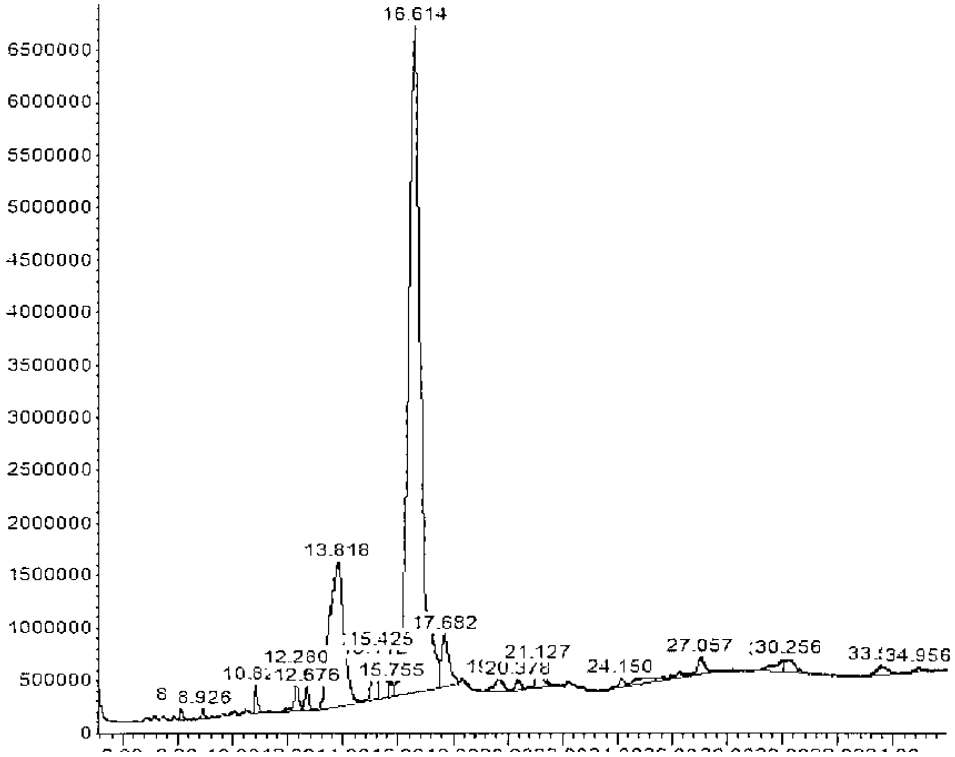

Figure 2. Gas Chromatograph of Dichloromethane/Methanol extract of *Afrostyrax lepidophylus* seeds.

5. Conclusion

In the present study, fourteen compounds from methanol extract and sixteen compounds from dichloromethane/ methanol were identified from *Afrostyrax lepidophylus* seeds. The polarity of solvents used for extraction resulted to variations in the class and relative abundance of compounds extracted. Results revealed that methanol extract of *Afrostyrax lepidophylus* seeds have good quantities of 9,12-Octadecadienoic acid, oleic acid, n-Hexadecanoic acid and 3H-Pyrazzole-3-one, 4-benzoyl-2,4-dihydro-5-methyl-2-phenyl with 9,12-Octadecadienoic acid being the most abundant. Results also revealed the presence of sulfur containing compounds in the methanol extract and these compounds could be such that confer *Afrostyrax lepidophylus* seeds the characteristic onion/garlic-like odor. *trans*-13-Octadecenoic acid is a linoleic acid derivative and was the most abundant compound in the dichloromethane/methanol extract of *Afrostyrax lepidophylus* seeds. The presence of all these compounds at varied concentrations/relative abundance with their associated beneficial biological activities depicts the usefulness of *Afrostyrax lepidophylus* seeds used as spice in human nutrition and can serve as raw material in the preparation of neutraceuticals and pharmaceuticals.

References

[1] Samphina Academy (SA). (2022). The importance of local spices and herbs to the human health (Accessed 12-7-22).

[2] Ong, M C., Yusuf, S.N.A.M and Lim, V. (2016). Pharmacognostic and Antioxidant Properties of *Dracaena sanderiana* Leaves. *Antioxidants*, 5(28), 9pages.

[3] Amaechi, N.C and Chijioke, C.L. (2018). Evaluation of bioactive compounds in *Pseudarenthemum tunicatum* leavesusing gas chromatography-mass spectrometry. *Scientific paper series management, Economic Engineering in Agriculture and Rural Development*, 18(1), 53-59.

[4] Ogbunugafor, H.A., Ugochukwu, C.G and Kyrian- Ogbonna, A.E. (2017). The Role of Spices in Nutrition and Health: A Review of Three Popular Spices Used in Southern Nigeria. *Food Quality and Safety*, 1(3), 171-185.

[5] Saxena, S and Singh, B. (2013). Potential Health Benefits of Major Seed Spices. *International Journal of Seed Spices*, 3, 1-12.

[6] Jiang, T.A. (2019). Health Benefits of Culinary Herbs and Spices. *Journal of Association of Official and Analytical Chemists International*, 102(2): 395-411. doi:10.5740/jaoacint.

[7] Tapsell, L.C., Hemphil, I., Cobiac, L., Patch, C.S., Sullivan, D.R., Fenech, M., Roodenrys, S., Keogh, J.B., Clifton, P. M., Williams, P.G., Fazio, V., and Inge, K.E. (2006). Health Benefits of Herbs and Spices: The past, the present, the future. *Medical Journal of Australia*, 185(34), S1-S24

[8] Moukette, B.M., Pieme, C.A., Biapa, P.C.N., Moor, V.J.A., Berinyuy, E., and Ngogang J.Y. (2015). *Afrostyrax lepidophyllus* Extracts Exhibit In Vitro Free Radical Scavenging, Antioxidant Potential and Protective Properties Against Liver Enzymes Ion Mediated Oxidative Damage. *BMC Research Notes*, 8, 344.doi./0.7186/S13104015-1304-8.

[9] Yirankinyuki, F.F and Danbature, W.L. (2017). Characterization and Determination of Antifungal Activities of Essential Oil Extracted From the Bark of *Afrostyrax lepidophyllus* 'Country Onion' or Shium. *World Journal of Biological Chemistry*, 2, 29-33.

[10] Thomas, R.C. (2009). 'Country Onion' (*Afrostyrax lepidophyllus*).Spice and Medicine of the Cameroon rainforest. *Proceedings of the 194th ESA Annual Meeting,* Albuquerque Convention, Central Albuquerque, New Mexico.

[11] Tchoupang E., Ateba, S., Zingwe, S., Zehi, M., Krenn, L., and Njamen, D. (2016). Estrogenic Properties of Spices of the Traditional Cameroonian Dish 'Nkui' in Ovarietomized Wistar Rats. *Journal of Complementary and Integrative Medicine*, 13(2), 151-162.

[12] Anand-Gideon, V. (2015). GC-MS Analysis of Phytochemical Components of *Pseudoglochidion anamalaynum* Gamble: An Endangered Medicinal Tree. *Asian Journal of Plant Science and Research*, 5(12), 36-41.

[13] Jegadeeswari, P., Nishanthini, A., Muthukumarasamy, S and Mohan, V.R. (2012). GC-MS Analysis of bioactive compounds of *Aristolochia krysagathra* (Aristolochiaceae). *Journal of Current Chemical and Pharmaceutical Sciences*, 2(4):226-232.

[14] Vijisaral, E.D and Arumugam, S. (2014). GCMS Analysis of Bioactive Constituents of *Indigofera suffruticosa* Leaves. *Journal of Chemical and Pharmaceutical Research*, 6(8), 294-300.

[15] Karthika, K and Paulsamy, S. (2014). Phytochemical Profiling of Leaf, Stem and Tuber Parts of *Solena amplexicaulis* (Lam) Ghandi Using GC-MS. *International Scholarly Research Notes*. 13 pages. http://dx.doi.org/10.1155/2014/567409.

[16] Naim, M.J., Alam, O., Nawaz, F., Alam, M.J., and Alam, P. (2016). Current Status of Pyrazzole and Its Biological Activities. *Journal of Pharmacy and Bioallied Sciences*, 8(1), 2-17.

[17] Karrouchi, K., Radi, S., Ramil, Y., Taoufik, J.A., Maokhot, Y.N., Ai-aizari, F.A and Ansar, M. (2018). Synthesis and pharmacological activities of pyrazzole derivatives: A review. *Molecules*, 23(1): 134.doi.10.3390/molecules.23010134.

[18] Fogang, H.P.D., Maggi, F.,Tapondjou, L.A., Womeni, H.M., Papa, F., Quassinti, L., Bramucci, M., Vitali, L.A., Petrelli, D., Lupidi, G., Vittori, S., and Barboni, L. (2014). In vitro biological activities of seed essential oils from the Cameroonian Spices *Afrostyrax lepidophyllus* Mildbr and *Scorodophloeus zenkeri* Harms Rich in Sulfur Containing Compounds. *Chemistry and Biodiversity*, 11, 161-169.

[19] Guo, Z and Prakash, I. (1990). Synthesis and Purification of 3,3-dimethylbutyraldehyde via Oxidation of 1-Chloro-3,3-dimethyl butane with Dimethyl sulfoxide. US Patent US590-05175. (Accessed 4/8/2022)

[20] Suen, L. (2020). Erucic Acid in Edible Fats and Oils. Food Safety Focus, Issue 162, Food Safety Platform, Center for Food Safety.

[21] Hussein, H.J., Ubaid, J.M., and Hameed, I.H. (2016). Gas Chromatography-Mass Spectrum Analysis of Methanolic Leaves Extract of *Cordia myxa*. *International Journal of Current Pharmaceutical Review and Research*, 7(6), 400-406.

[22] Rajeswari, G., Munigan, M and Mohan, V.R. (2013). GCMS Analysis of Bioactive Components of *Hugonia mystax* L (Linaceae). *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 29(299), 818-824.