



Research Article

Phytochemical Analysis of *Allium cepa* and *Allium fistulosum* by Gas Chromatography-Mass Spectrometry Analysis: A Comparative Study

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ABSTRACT

Allium cepa and *Allium fistulosum* are herbs used in many parts of the world as spice, food and medicine. To evaluate the phytochemical components, the aqueous extracts of the leaves of *Allium fistulosum* and fleshy leaves of *Allium cepa* were used. The phytochemical components of both plants were evaluated and compared using GC-MS analysis. There were ten (10) bioactive components in *Allium cepa* revealed by GC-MS analysis which are majorly 16-Deoxy-5,6-dihydrokryptogenin diformate (Retention Time (RT): 32.245 minutes, Molecular Weight (mol. wt.): 474, yield: 30.636%), acetic acid, dichloro- (RT: 3.280-3.349, mol. wt.: 128, yield: 12.710%), 9-eicosene, (E)- (RT: 33.033, mol. wt.: 280, yield: 7.139%) and others with less than 6% yield. Whereas, in *Allium fistulosum*, five (5) bioactive components were present and D-limonene (RT: 6.694, mol. wt.: 136, yield: 98.942%) as the main component. acetic acid, dichloro- was common in both plants but higher in *Allium cepa* (12.710%) than *Allium fistulosum* (0.484%). It is therefore concluded that *A. cepa* and *A. fistulosum* possess different and diverse phytochemical compounds that could be responsible for their biological activities.

Keywords: GC-MS, *Allium cepa*, *Allium fistulosum*, Bioactive components

INTRODUCTION

Onion (*Allium cepa*) and spring onion or Welsh onion (*Allium fistulosum*) are spices and culinary herbs used for food and medicine in many nations of the world. They belong to the plant family of Liliaceae and genus *Allium* (Singh and Ramakrishna, 2017). The *Allium* genus is very large and comprises of about 850 species with many of them having high economic values as vegetables, spices, ornaments and medicinal plants (Keller *et al.*, 2012). Apart from *Allium cepa* and *Allium fistulosum*, another species of the *Allium* genus with tremendous economic and medicinal value is *Allium sativum*, commonly known as garlic. Onions are mainly cultivated in the northern part of Nigeria and widely consumed raw or cooked in the entire country (Dawang *et al.*, 2016). The bulb of *A. cepa* and leaves of *A.*

fistulosum are used as spice in stew, rice, beans, cooked in soup or eaten raw (Salami *et al.*, 2012).

The *Allium* species are known to contain nutritionally favouring phytochemicals and a good percentage of sugar, protein, water, vitamins, fiber and fat (Upadhyay, 2016). *A. cepa* has been reported to have flavonoids that contains anthocyanins and flavonols (quercetin). Anthocyanin was noted to be responsible for the red or purple colour in some varieties of onions while quercetin was responsible for the yellow and brown compounds in other varieties (Ifesan, 2017). Chemical constituents reportedly isolated from *A. cepa* include sulfur-containing compounds such as allicin, allyl propyl-disulfide, organosulfur compounds like dimethyl-disulfide. It also contains important

polysaccharides such as saccharose and fructosans, peptides and essential oils (Upadhyay, 2016). However, the nutritional component analysis of *A. fistulosum* has been reported to contain low level of total fat and rich in iron and vitamins (niacin, folic acid, B2 and B6) (Sung et al., 2014; Sung et al., 2018).

The therapeutic uses and pharmaceutical effects of these onions have been age long. Athletes of ancient Greece were reported to traditionally consume large quantities of onion (*A. cepa*), drink onion juice and also rub onion on their bodies before competition in order to fortify themselves for the Olympic Games (Onion, 2020). India, in the sixth century, used onion as diuretic medicine good for digestion, eyes, hearts and joints, the Romans used onions to cure vision, induced sleep, and heal mouth sores, dog bites, dysentery and toothaches while the middle age European prescribed onions to treat snakebites, headaches and hair loss (Onion, 2020). Bioactive compounds isolated from *A. cepa* like the sulfur-containing compounds, quercetin, ferulic acid and many others were reported to possess chemopreventive properties such as anti-diabetic (Kadan et al., 2013; Mootoosamy and Mahomoodally, 2014), wound healing and anti-scar (Perez et al., 2010; Wananukul et al., 2013), anticancer (Oloyede et al., 2009; Lai et al., 2013), anti-genotoxic and anti-mutagenic (Fedel-Miyasato et al., 2014; Onwuamah et al., 2014), anti-parasitic (Mantawy et al., 2011), antimicrobial (Benmalek et al., 2013), anti-hyperlipidemic (Kumari and Augusti, 2007), anti-allergic and antihistaminic (Kaiser et al., 2009), anti-inflammatory (Shaik et al., 2006), analgesic (Sakakibara et al., 2008; Nasari and Anoush, 2012), hepatoprotective (Kumar et al., 2013), antioxidant (Kumar et al., 2013), cardioprotective (Kris-Etherton et al., 2002), insecticidal (Park and Shin, 2005) and antipyretic (Porchezian and Ansari, 2000). However, *A. fistulosum* has its traditional use as an herbal medicine for treating headache, abdominal pain, cold, dysentery, influenza, sores, arthritis, ulcers, parasitic infections and heart diseases (Chen et al., 2000). Fructans from *A. fistulosum* have been shown to have anti-influenza A virus activity (Lee et al., 2012) and bioactivities of D-Limonene are antioxidant and anticancer (Ajayi et al., 2019). Furthermore, *A. fistulosum* has been reportedly shown to possess anti-fungal (Sang et al., 2002), anti-platelet (Chen et al., 2000), anti-obesity (Sung et al., 2014) and antihypertensive (Yamamoto et al., 2005) properties.

This study was carried out to evaluate and compare the phytochemical compounds of *A. cepa* and *A. fistulosum* by gas chromatograph-mass spectrometry (GC-MS) analysis. Although, these herbs are extensively cultivated and used as spices and food in Nigeria, there has been paucity of information on the chemical composition of these plants

from Nigeria, hence we embark on the study of the two commonly and widely used *Allium* species.

MATERIALS AND METHODS

Plant Materials

The *A. cepa* bulbs and *A. fistulosum* leaves were bought fresh from Ile-Epo Market in Abule-Egba, Lagos State, Nigeria in February, 2018 (Figure 1A & B). The two plants were identified and authenticated by Mr. Adeleke, Department of Pharmacognosy, College of Medicine of the University of Lagos, Nigeria.

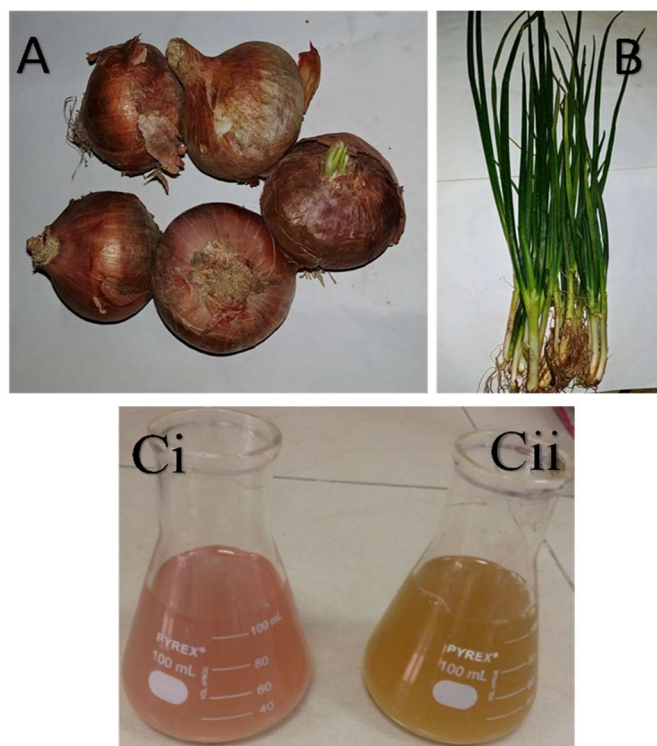


Figure 1: A-*Allium cepa* bulbs; B-*Allium fistulosum*; Ci-*Allium cepa* aqueous extract; Cii-*Allium fistulosum* aqueous extract.

Preparation of *A. cepa* and *A. fistulosum* Extracts

The outer scaly leaf of *A. cepa* was removed, bulb thoroughly rinsed in a plastic bowl containing tap water and fleshy leaves cut into pieces. 500 g of cut fleshy leaves in 250 mL distilled water was blended with an electric blender and filtered with a clean white cloth to obtain the fresh aqueous extract of *A. cepa* (Figure 1Ci). The fresh aqueous filtrate extract was poured into a 100 mL Pyrex conical flask and stored in the refrigerator at -4°C until used. *A. fistulosum* leaves separated from the short stem were also thoroughly rinsed like the *A. cepa* and cut into pieces. Likewise, 500 g of cut leaves in 250 mL water was blended and filtered to obtain the fresh aqueous filtrate extract of *A. fistulosum*

(Figure 1Cii). This extract was poured into another 100 mL Pyrex conical flask and stored in the refrigerator at -4°C until used.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of *A. cepa* and *A. fistulosum* aqueous extracts was carried out as we have earlier reported (Ajayi et al., 2011). The Agilent Technologies Network Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector (FID) and Hewlett Packard 7683 series injector with MS transfer line of 250°C temperature was used. The carrier gas used was helium (99.999%) at a constant flow rate of 22 cm/s. Chromatographic separations were performed on a fused silica capillary column- HP-5MS with specification: length; 30 m, i.d; 0.25 mm, and thickness $1.0\ \mu\text{m}$. $1.0\ \mu\text{L}$ of extract was injected into the GC column at a split ratio of 1:30. Oven temperature was held at 50°C for 5 min holding time and gradually raised from 50 to 250°C at a rate of $2^{\circ}\text{C}/\text{min}$. Total elution time was 34 min. Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled to Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with NIST08 Library software database was used to carry out the MS analysis. Mass spectra were taken at $70\ \text{eV}/200^{\circ}\text{C}$ with scanning rate of 1 scan/s.

Identification of Compounds

Identification and interpretation of compounds were conducted using the database of National Institute Standard and Technology (NIST 08) Library. The mass spectrum of the unknown component was compared with the mass

spectrum of the known component in the repository of NIST library. The retention time, molecular weight, molecular formula, molecular structure and composition percentage of the sample material were recorded.

RESULTS

The results of the GC-MS analysis of aqueous extract of *A. cepa* are shown in Figure 2 and Table 1. The chromatogram (Figure 2) revealed 13 peaks with each peak representing a compound. However, some peaks depict same compound with different retention time and their percentage of yield were added as shown in Table 1. Table 1 shows the presence of 10 bioactive components in *A. cepa* as revealed by GC-MS analysis and their reported biological activities. The major compounds in *A. cepa* include 16-Deoxo-5,6-dihydrokryptogenin diformate (RT: 32.245, mol. wt.: 474, yield: 30.636%), Ethylphosphonic acid, Acetic acid, dichloro- (RT: 3.280-3.349, mol. wt.: 128, yield: 12.710%), 9-Eicosene, (E)- (RT: 33.033, mol. wt.: 280, yield: 7.139%) and other compounds with percentage yield of less than 6%. The structures of the 10 bioactive compounds is represented in Figure 3 while the GC-MS mass spectrum and molecular structures of acetic acid, and dichloro-, 16-Deoxo-5,6-dihydrokryptogenin diformate in *A. cepa* are shown in Figures 4, 5 and 6 respectively.

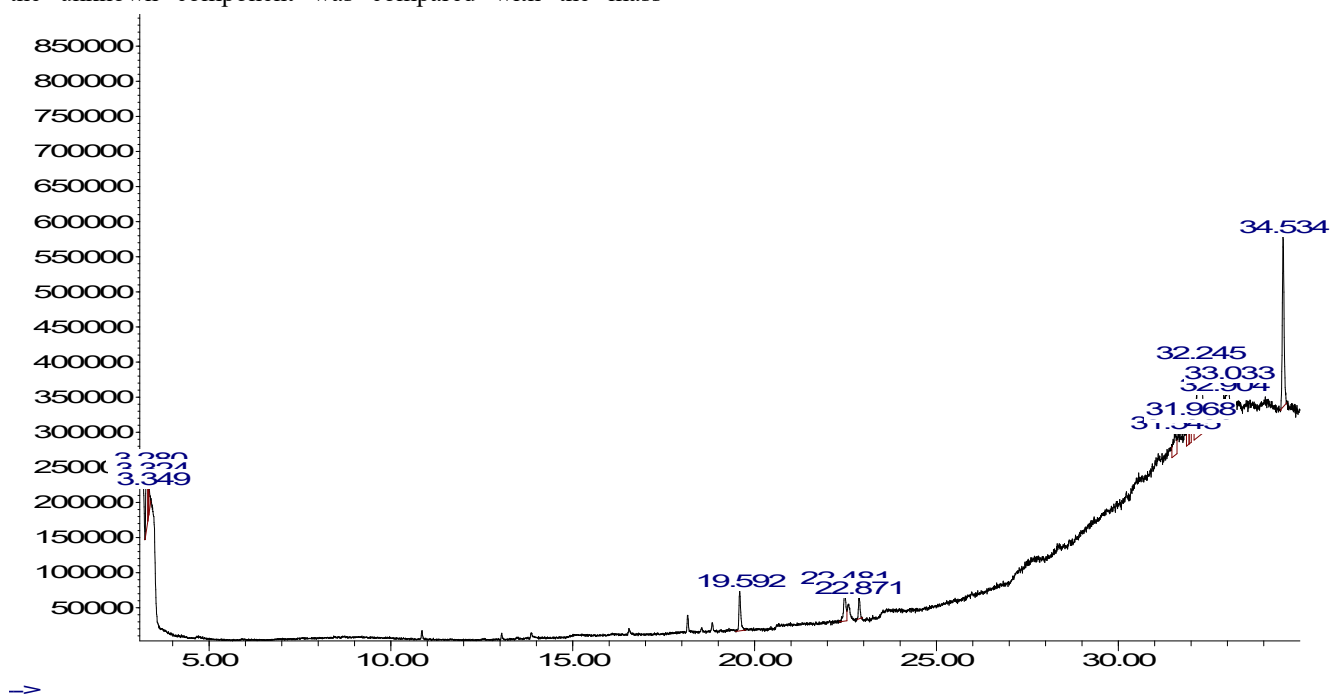
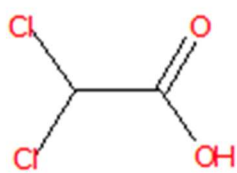


Figure 2. GC-MS Chromatogram of Aqueous Extract of *Allium cepa*.

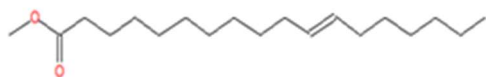
Table 1. Bioactive Components and Their Activities Identified in the Aqueous Extract of *Allium cepa* by GC-MS

Peaks	RT	Name of compound	Mol. wt.	% of yield	Biological activity
1-3	3.280-3.349	Acetic acid, dichloro-	128	12.710	Anticancer (Mamani and Alhaji, 2019)
4.	19.592	Hexadecanoic acid, methyl ester	270	5.684	Antioxidant, flavor, hypocholesterolemic Pesticide, 5-alpha reductase inhibitor (Danesh <i>et al.</i> , 2018), decrease blood cholesterol and anti-inflammatory (Belakhdar <i>et al.</i> , 2015)
5.	22.481	11-Octadecenoic acid, methyl ester	296	5.488	Not reported
6.	22.871	Methyl stearate	298	2.569	GABA aminotransferase inhibitor, anti-inflammatory, intestinal Lipid metabolism regulator, gastrin inhibitor, antihelminthic (nematodes) and antinociceptive (Kuppuswamy <i>et al.</i> , 2013)
7.	31.545	1-Tridecene	182	5.373	Not reported
8-9.	31.930, 32.904	17-Pentatriacontene	490	5.546	Antiinflammatory, anticancer, antibacterial and anti-arthritic (Kuppuswamy <i>et al.</i> , 2013)
10.	31.968	11,13-Dimethyl-12-tetradecen-1-ol acetate	282	2.691	Not reported
11.	32.245	16-Deoxo-5,6-dihydrokryptogenin diformate	474	30.636	Not reported
12.	33.033	9-Eicosene, (E)-	280	7.139	Anti-microbial and cytotoxic (Rivas da Silva <i>et al.</i> , 2012)

RT = Retention time; Mol. wt. = Molecular weight



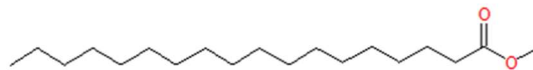
Acetic acid, dichloro-



11-Octadecenoic acid, methyl ester



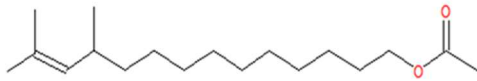
Hexadecanoic acid, methyl ester



Methyl estearate



1-Tridecene



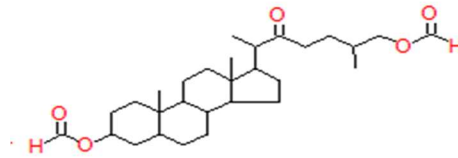
11,13-Dimethyl-12-tetradecen-1-ol acetate



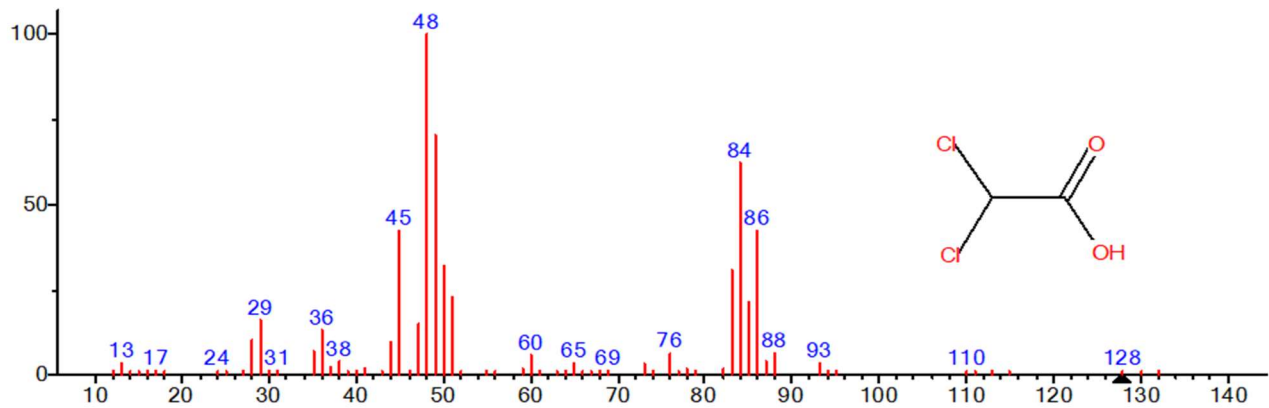
17-Pentatriacontene



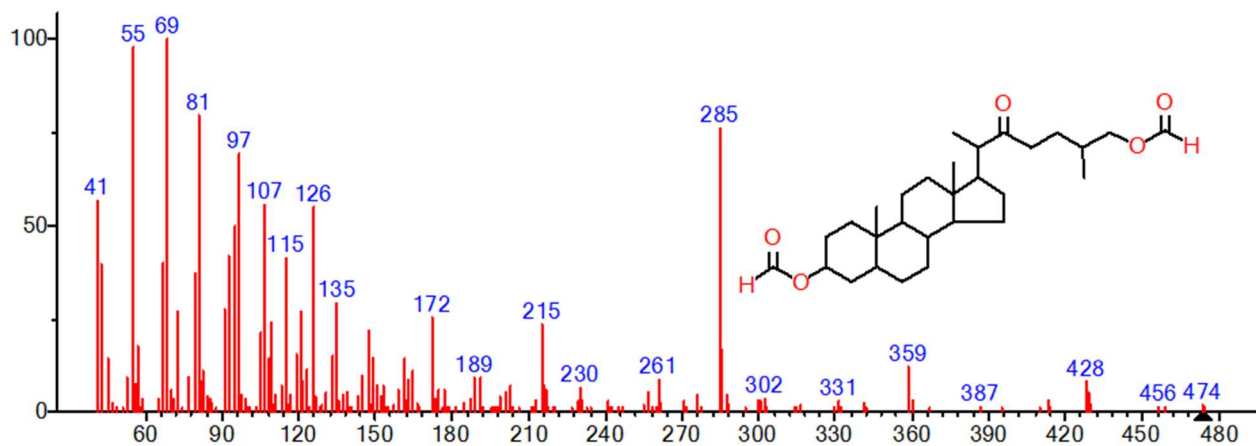
9-Eicosene, (E)-



16-Deoxy-5,6-dihydrokryptogenin diformate

Figure 2. Structures of Bioactive Compounds in *Allium cepa* as Revealed by GC-MS

(mainlib) Acetic acid, dichloro-

Figure 4: GC-MS mass spectrum and molecular structure of Acetic acid, dichloro- in *A. cepa*

(mainlib) 16-Deoxy-5,6-dihydrokryptogenin diformate

Figure 5. GC-MS Mass Spectrum and Molecular Structure of 16-Deoxy-5,6-dihydrokryptogenin Diformate in *A. cepa*

The GC-MS analysis results of *A. fistulosum* are shown in Figure 7 and Table 2. The chromatogram (Figure 7) revealed 5 peaks depicting different compounds while the list of the 5 compounds and their reported biological activities are represented in Table 2. The major bioactive compound in *A. fistulosum* is D-Limonene (RT: 6.694, mol. wt.: 136, yield: 98.942%). Other bioactive compounds present are Acetic acid, dichloro- in both plants with percentage of yield much higher in *A. cepa* (12.710%) than *A. fistulosum* (0.484%).

acid (RT: 3.123, mol. wt.: 128, yield: 0.484%), dichloro-, 1-Buten-3-yne, 1-chloro-, (Z)- (RT: 3.338, mol. wt.: 86, yield: 0.140%) and alpha.-pinene (RT: 6.072, mol. wt.: 136, yield: 0.362%). The structures of these compounds are shown in Figure 8 while the GC-MS mass spectrum and molecular structure of D-Limonene in *A. fistulosum* is shown in Figure 9. GC-MS analysis also revealed the

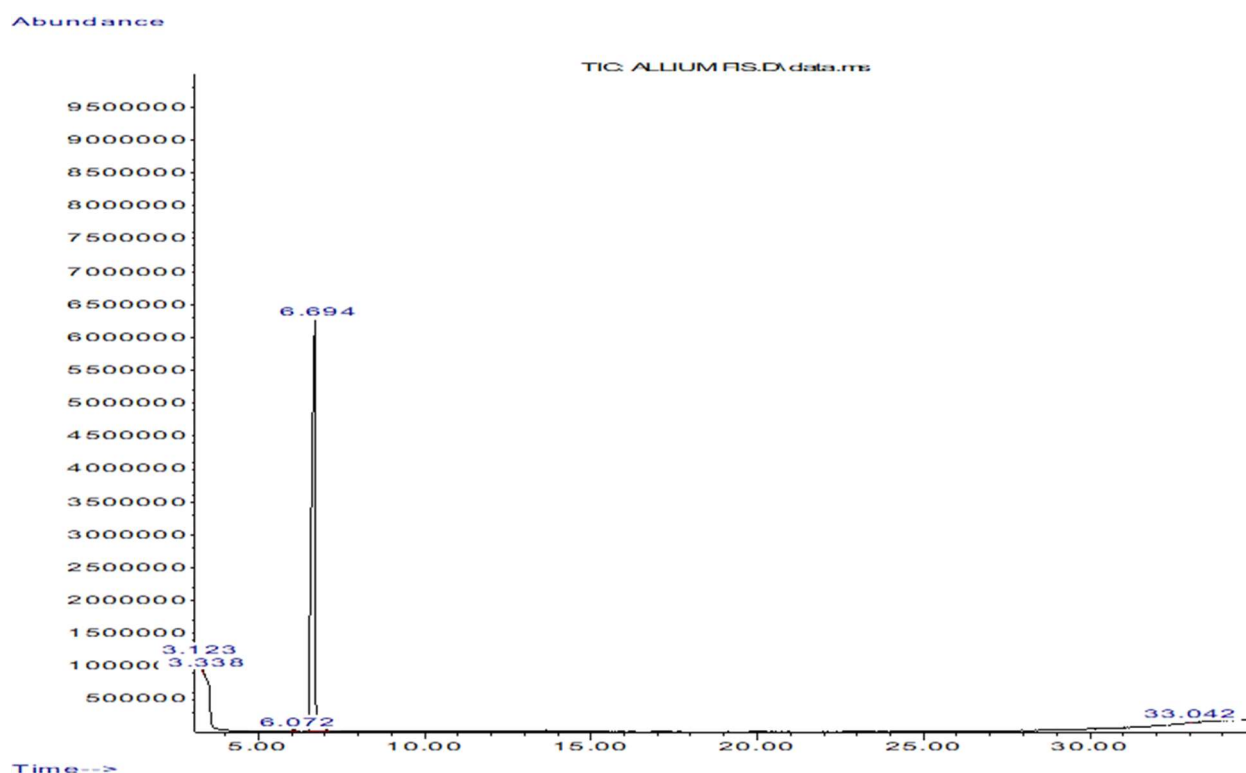


Figure 7: GC-MS chromatogram of aqueous extract of *Allium fistulosum*.

Table 2. Bioactive Components and Their Activities Identified in the Aqueous Extract of *A. fistulosum* by GC-MS.

Peaks	RT	Name of compound	Mol. Wt.	% of Total	Biological activity
1.	3.123	Acetic acid, dichloro-	128	0.484	Anticancer (Mamani and Alhaji, 2019)
2.	3.338	1-Buten-3-yne, 1-chloro-, (Z)-	86	0.140	Not reported
3.	6.072	alpha.-Pinene	136	0.362	Antimicrobial (Russo, 2011), anti-inflammatory and acetylcholinesterase inhibitor (Erasto an Viljoen, 2008)
4.	6.694	D-Limonene	136	98.942	Antimicrobial, anti-inflammatory, antinociceptive, anticancer, insecticidal (Marchese et al., 2016) and antioxidant (Marchese et al., 2016; Ajayi et al., 2019b)

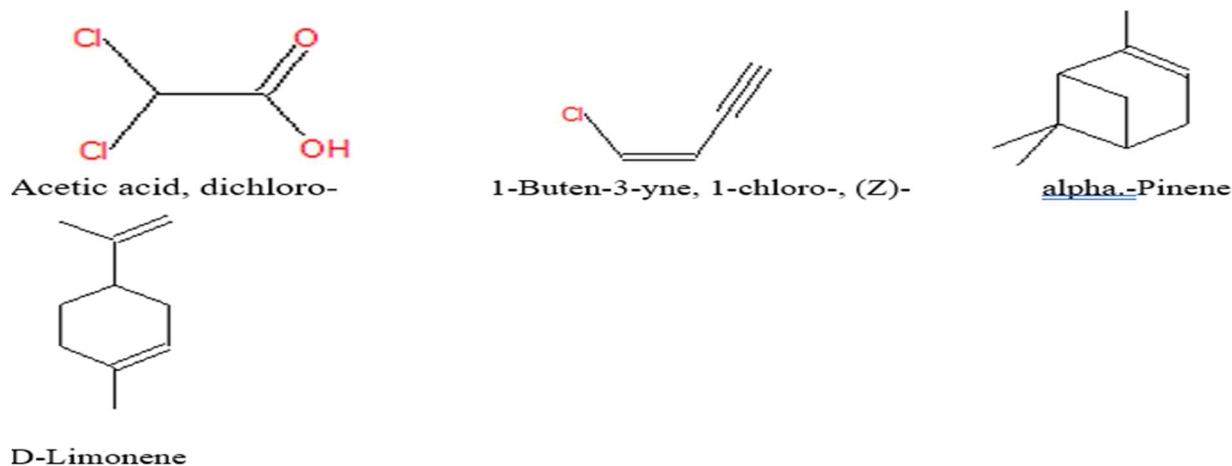


Figure 8. Structures of Bioactive Compounds in *Allium fistulosum* as Revealed by GC-MS

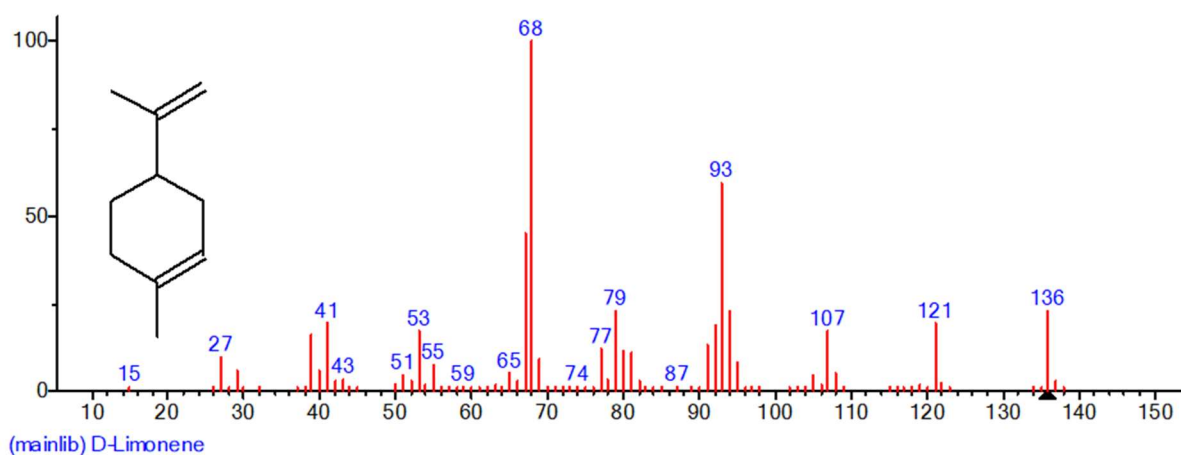


Figure 9. GC-MS Mass Spectrum and Molecular Structure of D-Limonene in *A. fistulosum*

DISCUSSION

The GC-MS analysis has been used in more recent times to determine the biologically active constituents of medicinal plants. Information obtained through GC-MS on these bioactive compounds has also been known to be precise and very reliable. Most of these substances are secondary metabolites of which many have been isolated and estimated. These substances are known to serve as defense mechanism in plants against insects and herbivores. They also exhibit various biological activities such as antifungal, anti-inflammatory, antiulcer, antioxidant and anti-hepatotoxic (De-Fatima *et al.*, 2006).

Ten compounds were identified in *Allium cepa* by GC-MS analysis. Quite a number of these compounds have been reported to possess various biological activities while the activities of others have not been reported as shown in Table 1. Reports have shown that acetic acid, dichloro- and 17-Pentatriacontene possessed anticancer activity (Dinesh *et al.*, 2018; Tataranni and Piccoli, 2019), Hexadecanoic acid, methyl ester; Methyl stearate and 17-Pentatriacontene possessed anti-inflammatory activity (Belakhdar *et al.*, 2015;

Dinesh *et al.*, 2018), 17-Pentatriacontene and 9-Eicosene, (E)- have antibacterial and antimicrobial respectively (Kuppuswamy *et al.*, 2013; Dinesh *et al.*, 2018). The antioxidant effect of hexadecanoic acid, methyl ester that were present in this sample have also been reported by Mamani and Alhaji, 2019. Whereas the biological activities of 11-Octadecenoic acid, methyl ester; 1-Tridecene; 11,13-Dimethyl-12-tetradecen-1-ol acetate and 16-Deoxo-5,6-dihydrokryptogenin diformate have not been reported.

In *Allium fistulosum*, five compounds were revealed by GC-MS analysis. Four of these compounds reportedly have biological activities while the activity of one compound has not been reported (Table 2). Acetic acid, dichloro- and D-Limonene are reported as anticancer (Erasto and Viljoen, 2008; Tataranni and Piccoli, 2019), alpha-Pinene and D-Limonene reportedly have antimicrobial and anti-inflammatory activity (Erasto and Viljoen, 2008; Russo, 2011; Rivas da Silva *et al.*, 2012). D-Limonene was also reported to have additional activity like antioceptive, insecticidal and antioxidant properties (Erasto and Viljoen,

2008; Ajayi et al., 2019). The high performance-liquid chromatography (HPLC) analysis of aqueous and ethanolic extracts of *A. fistulosum* in literature, showed that both extracts contain ferulic acid and quercetin (Sung et al., 2018). Ajayi et al., (2019) using GC-MS analysis, have recently reported D-limonene as the major bioactive component found in *A. fistulosum*.

D-limonene which is almost 99% of total yield in *A. fistulosum* has reportedly possessed significant chemopreventive and pharmacological properties. This has raised a lot of research interest on its anticancer, antimicrobial, antiparasitic and other properties (Erasto and Viljoen, 2008). However, the activity of 1-Buten-3-yne, 1-chloro-, (Z)- is yet to be reported. Moreso, 11-Octadecenoic acid, methyl ester; 1-Tridecene; 11,13-Dimethyl-12-tetradecen-1-ol acetate; and 16-Deoxo-5,6-dihydrokryptogenin diformate found in *A. cepa* were absent in *A. fistulosum*. In addition, D-limonene a major constituent of *A. fistulosum* is absent in *A. cepa*. This study revealed the presence of more biochemical compounds of biological activities in *A. cepa* than in *A. fistulosum* which could not be collaborated in literature.

CONCLUSION

In this study, GC-MS analysis was used to profile the presence of the bioactive compounds in *Allium cepa* and *Allium fistulosum* and do a comparative analysis to justify the medicinal usage and nutritional benefits of these plants. Majority of the identified compounds in the two plants though differ in nature and character except acetic acid, dichloro- that is found in both plants, were reportedly possessed anticancer, anti-inflammatory, antioxidant, antibacterial, antimicrobial, hypocholesterolemic, antifungal and other properties that support previous investigations and therefore could be found useful in potential drug development.

AUTHORS' CONTRIBUTIONS

GOA: Conceptualization, Methodology, Writing of Original Draft and Editing; MAA: Methodology, Writing of Review and Editing. Both Authors have read and approved the manuscript for publication.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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