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D-Limonene: A major bioactive constituent in *Allium fistulosum* identified by GC-MS analysis

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ABSTRACT

This investigation was carried out to determine the bioactive components present in *Allium fistulosum* by gas chromatography-mass spectrometry (GC-MS) analysis. The GC-MS analysis of the aqueous extract of the plant identified D-Limonene, a cyclic monoterpene, as the most abundant bioactive compound in *A. fistulosum* with approximately 99% of the total yield. Minor bioactive constituents present in the plant as revealed by GC-MS analysis include dichloroacetic acid (0.48%), α-pinene (0.36%), 1-Buten-3-yne, 1-chloro-, (Z)- (0.14%) and thymol, TMS derivative (0.07%). D-Limonene has been known to be commonly present in citrus peels, however, it is the first time this compound will be identified by GC-MS analysis as the major bioactive compound in *A. fistulosum*.

Keywords: D-Limonene, *Allium fistulosum*, bioactive compounds, GC-MS.

INTRODUCTION

The use of plant in drug formulation and development has been as old as man existed. The early part of human civilization has seen human beings dependent on plants for their healthcare and food needs. This has made medicinal plants to be referred to as the nature's pharmacy [1]. According to the World Health Organization (WHO), 80% of the world's population depends on herbal medicine. In low economic country like Nigeria, herbal drugs are widely prescribed, even without knowing their biological components because of their effectiveness, fewer side effects and relatively low cost [2].

A. fistulosum is a non-bulbing, bunch forming and tender green onion species, belong to the plant family called Liliaceae. It is also known as spring onion, scallion, welsh onion, salad onion or Japanese bunch onion [3]. This plant is mostly cultivated in the Northern region of Nigeria and temperate to tropical regions of northern Hemisphere. The leaves are freshly consumed and its nutritional value is high, it is used to spice soups, rice, beans and other cookeries [4]. Almost the entire plant parts of the *A. fistulosum* that is, shoots, leaves and non-developed bulbs are eaten raw in salad, boiled as soup, cooked as vegetable or used as healing herbs [5]. It has medicinal properties such as antioxidant and antifungal due to sulphur-containing compounds, flavonoids and fatty acids [6]. It has also been reported that this plant can be used for the treatment of eyesight problems, common colds, headaches, heart problems, wounds and festering sores; reduces fat accumulation and serum lipid concentrations; and the root exudates in soil root-zone have anti-termite, anti-fungal and anti-microbial activities [5]. Onionins A_1, A_2 and A_3 are some of the bioactive compounds that have been successfully isolated and characterized from the leaves of *A. fistulosum* using various spectroscopic techniques [7].

D-Limonene is a naturally occurring monoterpene compound which has chemotherapeutic and chemopreventive activity against many rodent tumor types [8]. This compound is found in the rind of citrus fruits, such as oranges, lemons and limes. It is concentrated in orange peels, comprising approximately 97% of this rind's essential oils [9]. It belongs to a group of compounds called terpenes which offers several health benefits and its strong aromas protect plants from predators. D-Limonne has been reported to possess anti-oxidant [10], anti-inflammatory [11-12] and anti-carcinogenic [8] properties. The presence of this compound in *A. fistulosum* has not been reported before now. As a result of the health benefits reported from this plant, we ventured into investigating the bioactive components in the plant with the use of gas chromatography-mass spectrometry analysis.

MATERIAL AND METHOD

Collection and identification of Plant materials

Fresh *A. fistulosum* was bought from Ile-Epo local market, Abule-Egba, Lagos, Nigeria. The plant was identified and authenticated by Mr. Adeleke, Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria.

Preparation of plant extracts

The plant was thoroughly rinsed with clean tap water in a plastic bowl to remove dirt and sand. The long leaves were separated from the fibrous root, then the leaves were cut into pieces. 500 g of the plant was macerated in 250 ml of distilled water. This was filtered with clean white cloth and the fresh filtrate extract obtained was put inside a pyrex glass tube, covered and kept in the refrigerator until use.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was carried out on aqueous extract of *A. fistulosum* according to the method described earlier by Ajayi *et al*. [13]. A Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector and Hewlett Packard 7683 series injector with MS transfer line temperature of 250°C was used. The GC was equipped with a fused silica capillary column- HP-5MS (30 x 0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50°C for 5 min holding time and raised from 50 to 250°C at a rate of 2°C /min, employing helium gas (99.999%) as a carrier gas at a constant flow rate of 22 cm/s. 1.0 micron of extract (1 mg dissolved in 1 ml absolute alcohol), at a split ratio of 1:30 was injected. MS analysis was carried out on Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled to Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200°C, scanning rate of 1 scan/s. Identification of compounds was conducted using the database of NIST08 Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Library.

RESULT

A total of 5 compounds were identified in the aqueous extract of *A. fistulosum* by the GC-MS analytical method (Table 1). These compounds were Dichloroacetic acid (0.48%), 1-Buten-3-yne, 1-chloro-, (Z)- (0.14%), α-Pinene (0.36%), D-Limonene (98.94%) and Thymol, TMS derivative (0.07%). The gas chromatogram shown in Figure 1 shows one major peak which was identified through the NIST08 L. database as D-Limonene with retention time of 6.694 and % of total as approximately 99% (Table 1). Figure 2 shows the mass spectrum of D-Limonene which gives the molecular structure of the compound and molecular weight as 136. The properties of the compound is shown in Table 2 as a monoterpene with antioxidant and anticancer properties while the % of yield of bioactive compounds that is, the phytoconstituents in *A. fistulosum* is represented in Figure 3 showing D-Limonene as a major and almost the only bioactive compound in the plant.

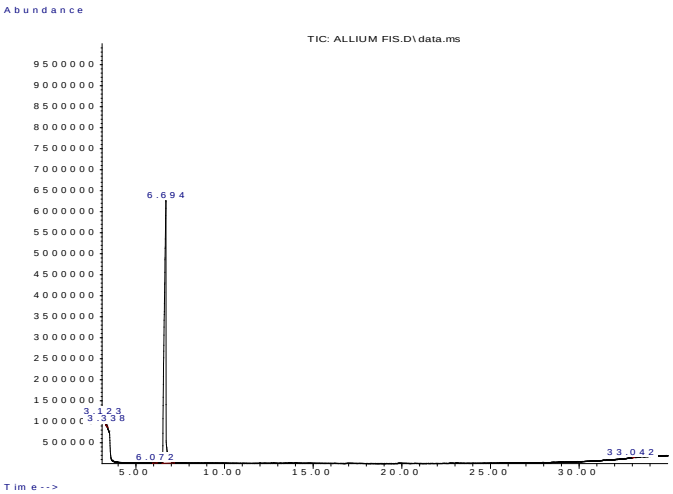

Figure 1: Chromatogram of *A. fistulosum* by GC-MS analysis

Table 1: Chemical Constituents in *A. fistulosum* by GC-MS analysis

Peak	Retention Time	Name of compounds	Molecular formula	Molecular weight	% of yield
1	3.123	Dichloroacetic acid	$CHCl_2COOH$	128	0.484
2	3.338	1-Buten-3-yne, 1-chloro-, (Z)-	C_4H_3Cl	86	0.140
3	6.072	A-Pinene	$C_{10}H_{16}$	136	0.362
4	6.694	D-Limonene	$C_{10}H_{16}$	136	98.942
5	33.042	Thymol, TMS derivative		222	0.072

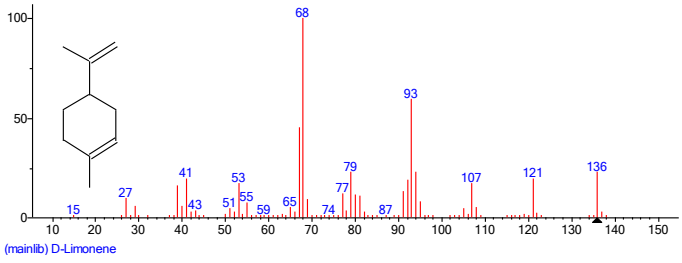

Figure 2: Mass spectrum and molecular structure of D-Limonene by GC-MS

Table 2: D-Limonene properties

Properties	Known as
Nature of compound	Terpene (Monoterpene)
Molecular weight	136
Bioactivity	Antioxidant, anticancer
IUPAC ID	1-Methyl-4-(1-methylethyl)-cyclohexene

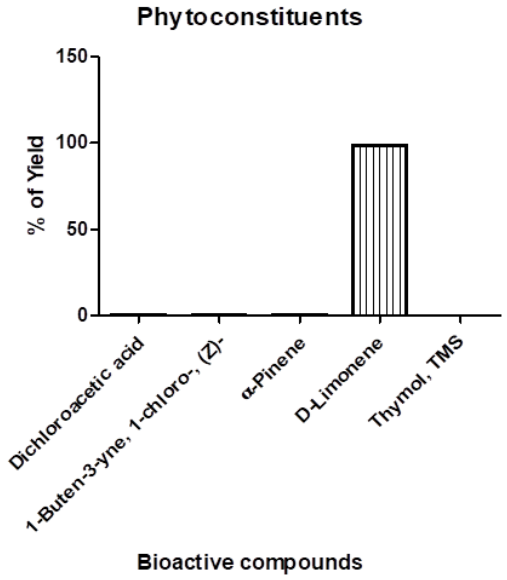

Figure 3: Percentage of Bioactive compounds in *A. fistulosum* by GC-MS

DISCUSSION

Having the knowledge of phytochemical constituents of plants nowadays is important, not only to discover the therapeutic agents, but also because the new information may be of economic value in obtaining new sources of materials as precursors for the synthesis of complex chemical substances [14-15]. *A. fistulosum* has been reported as one of the spices and culinary herbs that are economically, nutritionally and medicinally important in Nigeria [16]. The use of GC-MS in identification of bioactive compounds in plants is a valuable technique in that it suggests the possible curative properties of the plants [17]. In this study, the GC-MS analysis revealed the presence of 5 compounds of which D-Limonene is approximately 99% of total compound.

D-Limonene has an enormous industrial use. According to WHO, 1998, Limonene is useful as a substitute for chlorinated hydrocarbons, chlorofluorocarbons, and other solvents. It is also used in removing grease from metals (30% limonene) before industrial painting, in the electronic industry for cleaning (50–100% limonene), in the printing industry for cleaning (30–100% limonene), and in paint as a solvent. Limonene is useful as a solvent in histological laboratories and as a flavour and fragrance additive in food, household cleaning products, and perfumes [18].

Recently, D-Limonene has been reported to be a well-tolerated, lipophilic constituent of citrus peel with *in vitro* and *in vivo* anti-cancer potency. It belongs to a group of compounds called terpenoids which also includes carvone, carveol, oleanic acid, ursolic acid, a- and b-carotene, lutein, lycopene, zeaxanthine, and cryptoxanthineis. This group of compounds has shown great promises in promoting overall good health such as anti-cancer, cardio-protective, and anti-oxidant effects [19]. According to a recent updated report, among the benefits of D-Limonene include helping to treat or prevent cancer, promotion of weight loss, relieving symptoms of bronchitis and heartburn, reducing inflammation and oxidative stress, and probably aids in treating heartburn and gastroesophageal reflux by neutralizing gastric acid and promoting normal peristalsis [20].

CONCLUSION

The GC-MS analysis revealed that the leaf aqueous extract of *Allium fistulosum* contained very high level of D-Limonene (98.9%). The presence of this phytocompound in the plant is higher than the already known natural sources, the citrus peels which contained about 78% of D-Limonene. The presence of this bioactive compound in *A. fistulosum* root extract is a significant finding in this present study.

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