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

Gabriel O Ajayi, Mushaufa A Akinsanya, Adedoyin T Aghabia, Kayode S Oyebanjo, Temitope D Hungbo, Joseph A Olagunju

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, 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 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 eye sight problems, common colds, headaches, heart problems, wounds and festering sores; reduces fat accumulation and serum lipid concentrations; and the root exudates in soil root and antimicrobial activities [3]. Onionins A1, A2 and A3 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-Limonene 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.

| Peak | Retention Time | Name of compounds | Molecular formula | Molecular weight | % of yield |
|------|----------------|-------------------|-------------------|-----------------|------------|
| 1    | 3.123          | Dichloroacetic acid | CHCl₂COOH         | 128             | 0.484      |
| 2    | 3.338          | 1-Buten-3-yne, 1-chloro-, (Z)- | C₇H₆Cl        | 86              | 0.140      |
| 3    | 6.072          | A-Pinene           | C₁₀H₁₆          | 136             | 0.362      |
| 4    | 6.694          | D-Limonene         | C₁₀H₁₆          | 136             | 98.942     |
| 5    | 33.042         | Thymol, TMS derivative | 222         | 0.072          |

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.

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

![Figure 1: Chromatogram of *A. fistulosum* by GC-MS analysis](image)

![Figure 2: Mass spectrum and molecular structure of D-Limonene by GC-MS](image)

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 |
REFERENCES

1. Yamuna P, Abirami P, Vijayashalini P, Sharma M. GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of Gomphrena decumbens Jacq. Journal of Medicinal Plants Studies. 2017; 5(3):31-37.

2. Kumar R, Kumar S, Patra A, Jayalakshmi S. Hepatoprotective activity of aerial parts of Plumbago zeylanica Linn against carbon tetrachloride-induced hepatotoxicity in rats. Int. J. Pharm. Pharm. Sci. 2009; 1(1):171-175.

3. Pleasant B, Spring Onions, Green Onions, Welsh Onions or Scallions?, 2013 https://www.growveg.com/guides/spring-onions-green-onions-welsh-onions-or-scallions/ downloaded on 2/8/2019.

4. Dawang SN, Affiahu DU, Lanka NJ, Fannap LM. Preliminary Checklist of Spices and Culinary Herbs Sold in Jos, Plateau State, Nigeria. IOSR Journal of Pharmacy and Biological Sciences. 2016; 11(4):24-29.

5. Singh BK, Ramakrishna Y, Welsh Onion (Allium fistulosum L): A Promising Spicing-Culinary Herb of Mizoram. Indian Journal of Hill Farming. 2017; 30(2):201-208.

6. Vlase L, Parvu M, Parvu EA, Toui A. Phytochemical analysis of allium fistulosum L. and A. ursinum L. Digest Journal of Nanomaterials and Biostuctures. 2013; 8(1):457-467.

7. Nohara T, Fujiwara Y, Kudo R, Yamaguchi K, Ikeda T, Murakami K, Oto M, Kajimoto T, Takeyab M. Isolation and Characterization of New Onions A2 and A3 from Allium cepa, and of Onionsins A3, A6, and A7 from Allium fistulosum. Chem. Pharm. Bull. 2014; 62(11):1141-1145.

8. Crowell PL, Gould MN. Chemoprevention and therapy of cancer by d-limonene. Crit Rev Oncog. 1994; 5(1):1-22.

9. Sobel A. What Is Limonene? Everything You Need to Know. https://www.healthline.com/nutrition/d-limonene. 2019; downloaded 2/8/2019

10. Yu L, Yan J, Sun Z. D-limonene exhibits anti-inflammatory and antioxidant properties in an ulcerative colitis rat model via regulation of iNOS, COX-2, PGE2 and ERK signaling pathways. Molecular Medicine Reports. 2017; 15:2339-2346.

11. Souza MC, Siani AC, Ramos MF, Menezes-de-Lima OJ, Henriques MG. 2003. Evaluation of anti-inflammatory activity of essential oils from two Asteraceae species. Pharmazee. 2003; 58(3):582-586.

12. Yilmaz BS, Özbek H. Investigation of the Anti-inflammatory, Hypoglycemic Activity and Medinan Lethal Dose (LD50) Level of Limonene in Mice and Rats. Acta Pharm. Sci. 2018; 56(1):35-94.

13. Ajayi GO, Olagunju JA, Ademuyiwa O, Martins OC. Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of Plumbago zeylanica. Linn. Journal of Medicinal Plants Research. 2011; 5(9):1756-1761

14. Akrouit A, El Jani H, Zammmouri T, Mighri N, Neffati M. Phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analysis of phytochemical constituents and anti-inflammatory activity of Aerva lanata (L.) leaves. African Journal of Pharmacy and Pharmacology. 2014; 8(5):126-135.

15. Dawang SN, Affiahu DU, Lanka NJ, Fannap LM. Preliminary Checklist of Spices and Culinary Herbs Sold in Jos, Plateau State, Nigeria. IOSR Journal of Pharmacy and Biological Sciences. 2016; 11(4):24-29. www.iosrjournals.org

16. Uraku AJ. Determination of chemical compositions of Cymbopogon citratus leaves by gas chromatography-mass spectrometry (GC-MS) method. Research Journal of Phytochemistry. 2015; 9(4):175-187.

17. World Health Organization. 1998. Limonene. Concise International Chemical Assessment (CICA) Document. 1998; 5:1-36.

18. Miller JA, Thompson PA, Hakim IA, Chow HHS, Thomson CA. D-Limonene: a bioactive food component from citrus and evidence for a potential role in breast cancer prevention and treatment. Oncol. Rev. 2011; 5:31-42. DOI 10.1007/s12156-010-0066-8

19. Wong C. The Benefits of D-Limonene Extract. 2019. https://www.wellhealth.com/the-benefits-of-d-limonene-89444-downloaded-2/8/2019.

HOW TO CITE THIS ARTICLE

Ajayi GO, Akinsanya MA, Agbabiaka AT, Oyebanjo KS, Hugbod TD, Olagunju JA. D-Limonene: A major bioactive constituent in Allium fistulosum identified by GC-MS analysis. J Phytopharmacol 2019; 8(5):257-259.