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Phytochemical and Gc-Ms Analysis of Bioactive Components in Ethanolic Bulb Extract of *Allium Porrum*

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ABSTRACT

The present study investigated the phytochemical and biochemical composition of the crude ethanolic extracts of *A. porrum* plant. High polarities solvents (ethanol) were used for extraction of *A. porrum*. Qualitative phytochemical analysis of the plant extracts was followed by gas chromatography mass spectrophotometry (GC-MS) to determine the bioactive constituents. The phytochemical investigation of crude ethanolic extract of *A. porrum* plant showed the presence of flavonoids, alkaloids, saponins, cardiac glycoside and terpenoids. GC-MS indicated the presence of (ten) 10 different types of high and low molecular weight biocompounds including fatty acids, esters and heterocyclic compounds. Hexadecanoic acid ethyl ester (38.63%), octadecanoic acid (18.11%) and docosanoic acid ethyl ester (16.88%), constituted the major biocomponents of the ethanolic fraction of *A. porrum.* This study established the biochemical composition of the bulb of *A. porrum* which have a commendable bioactivity and can be advised as a plant of phytopharmaceutical importance as used in traditional medicine.

Keywords: Phytochemical, Bioactive compound, Ethanolic extract, GC-MS analysis, *A. porrum*

INTRODUTION

Plants with various bioactive compounds have provided the basis for traditional treatment for different types of diseases in man and animals and still offer enormous potential sources of new chemotherapeutic agents [1]. Medicinal plants are rich sources of secondary metabolites including saponins, flavanoids, glycocides, terpenes, alkaloids and coumarines which are known to possess exciting biological activities [2,3].

In the recent past, there has been growing interest in the use of a medicinal plant with desirable biological activities and 85-90% of the world population including Nigeria consumes traditional herbal medicines [4,5]. This is due to their natural origin, cost effectiveness and lesser side effects [6]. Moreover, medicinal plants have been used all over the world particularly in developing countries with high rate of infectious diseases and inadequate modern health facilities [7].

Screening active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including infectious diseases, cancer and alzheimer's disease [8,9]. In spite of the rapidly expanding literature on phytochemistry, only a small percentage of the total plant species have been examined chemically for their bioactivity [10]. Gas Chromatography Mass Spectroscopy (GC-MS) is the most widely used techniques for identification and quantification the bioactive constituents including long chain hydrocarbons, alcohols, acids esters, alkaloids, steroids, amino acid and nitro compounds [11,12]. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [13].

The Allium genus includes more than 700 species widely distributed all over the world and they are the most important genus of the Alliaceae family [14]. *Allium porrum* (Leek) also known as "Ogede odo" in Southwestern Nigeria have been known for their medicinal properties [15,16]. They have been utilized in folk medicine for the treatment of varied disorder such as burns, wound, headaches, chestcold, rheumatism antimicrobial and antioxidants [17,18]. Unfortunately, the leek, *Allium porrum*, has received less research attention than other Allium vegetables (especially garlic and onion) and for this reason there is less documentation of their likely health benefits [17]. Despite the wide usage of this plant in traditional medicine especially in Nigeria, information on the analysis of its bioactive constituents has not been documented in literature. Therefore, the aim of this work was to isolate, investigate and characterize the bioactive chemical constituents in the ethanolic crude extract of *A porrum* using Gas Chromatography Mass Spectroscopy (GC-MS)

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh bulbs of *A. porrum* were purchased from the market at Ibadan, Oyo State. The bulb were taken to the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, where it was identified and a voucherspecimen reference number (2196) was assigned. The bulbs were then chopped, allowed to dry in the shade until a constant weight was obtained after which it was pounded to powder using pestle and mortar

Preparation of extract

Plant extracts were prepared using Soxhlet extractor as described by [19]. Powdered *A. porrum* weighing 500 g was transferred into a cotton bag, and inserted into the thimble of soxhlet apparatus. Thereafter, 1.5 L of ethanol was poured into the thimble and heated to reflux until the solvent vapor travelled up a distillation arm and flooded into the chamber housing the thimble of solid. The non-soluble portion was discarded while the extracted material was concentrated by evaporation at room temperature on warm water bath (HH-S 21.6, double row six holes, HINOTEK China) to separate the solvent from the extract (oily liquid) at a temperature of 93.3^{O}C.

Phytochemical screening of the experimental plant

Phytochemical analysis of the extracts were conducted to determine the presence of secondary metabolites such as saponins, alkaloids, glycosides, flavonoids, anthroquinones, tannins, volatile oils and triterpenoids, in the extract using standard procedures as described by [20].

Gas chromatography-mass spectometry (GC-MS) analysis of crude extracts

Gas Chromatography-Mass Spectometry (GC-MS) analyses were carried out on a GCMS-QP 2010 PLUS (Shimadzu, Japan) to determine the bioactive compounds of the extracts. The GC-MS was equipped with a split injector and an ion – trap mass spectrometer detector together with a Column Elite-1 fused – silica capillary column having a thickness of 1.00 µm, dimensions of 20 m x 0.22 mm and temperature limits of 60^{o}C to 325^{o}C For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 46.3 cm/sec. An injection volume of 1ml was employed (Split Ratio of 10:1), injector temperature -250^{o}C and an ion-source temperature of 230^{o}C. The oven temperature was programmed from 80^{o}C (Isothermal for 1 min) with an increase of 10^{o}C /min to 200^{o}C

then 10°C/min to 280°C/min ending with a 5min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 600 Da. Total GC running time was 28 min. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatogram was a turbomass (version 2.4). The detection employed the NIST05 library.

Identification of biocomponents

Interpretation of mass spectrum of GC-MS was done using the computer-aided matching of unknown spectra with spectra of known compounds from the Library of spectra from the National Institute of Standards, Washington, USA having more than 62,000 patterns. In addition, the hit quality (which indicates how closely matched the compound are with the Library data) was used to further verify the identity of the compounds in the sample. The name, molecular weight and the structure of the components of the test materials were ascertained. The relative percentage composition of each component was calculated by comparing its average peak area to the total area. The biological activities of the components of the extracts were accessed from Phytochemical and Ethnobotanical Database [21].

RESULTS AND DISCUSSION

The Qualitative analysis of phytochemical constituents of crude ethanolic extract *A. porrum* (Table 1) showed that there is presence of alkaloids, flavanoids, saponins, terpenoids, glycosides and steroids in while tannins and anthracens were not detected.

Table 1: Qualitative analysis of phytochemical constituents of the ethanolic bulb extract of *Allium porrum*

Metabolites	**Availability**
Alkaloids	+
Flavanoids	+
Saponins	+
Terpenoids	+
Steroids	+
Tannins	_
Anthracens	_
Glycosides	+

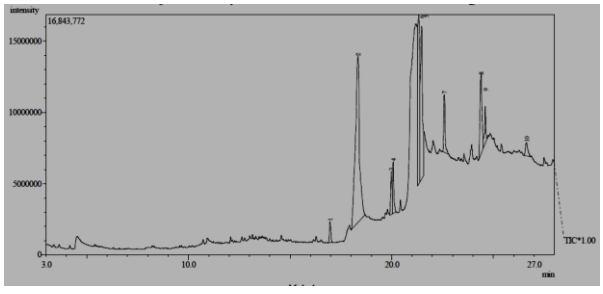


Figure 1: Total Ion Chromatogram (TIC) of ethanol extract of *A. porrum*

The total ion chromatogram (TIC) of ethanol extract of *A. porrum*, showing the GC-MS profile of compounds identified is given in Figures 1.

The peaks in the chromatogram when integrated and compared with database of spectrum of known components stored in the GC-MS library showed the presence of 10 bioactive constituents in ethanolic extracts of *A. porrum*. The active principles with their retention time (RT), molecular formula, molecular weight (MW), percentage composition and the chemical structure of the active principle in the ethanolic extract is given in Table 2 and Table 3.

The bioactive compounds identified in the extracts included

different fatty acids, esters and heterocyclic compounds. However, hexadecanoic acid ethyl ester (38.63%), octadecanoic acid (18.11%) and docosanoic acid ethyl ester (16.88%) constituted the major components of the ethanolic fraction of the *A. porrum.*

Table 2: GC-MS analysis of bioactive constituents of ethanol extract of *A. porrum*

Peak	RT	Compound name (Formula)	MW	% Comp.	Reported Biological activities
1	16.968	Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$)	270	1.59	Antiinflammatory, Antioxidant, Antiandrogenic, Hypocholesterolenic, Pesticide, Nematicide, flavor, haemolytic,
2	18.342	Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$)	284	38.63	Antioxidant, Antiandrogenic, Hypocholesterolenic, Pesticide, Nematicide, flavor, haemolytic
3	19.978	9,12-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$)	294	3	Antimicrobial, trypanocidal
4	20.066	9-Octadecanoic acid, methyl ester, (E)- ($C_{19}H_{36}O_2$)	296	3.45	Antioxidant, Anticancer
5	21.325	Docosanoic acid, ethyl ester ($C_{24}H_{48}O_2$)	368	16.88	No Activity
6	21.473	Octadecanoic acid ($C_{18}H_{36}O_2$)	284	18.11	Flavor, Hypocholesterolemic, Suppository, Cosmetic
7	22.582	Decane, 1-fluoro- ($C_{10}H_{21}F$)	160	4.76	No activity
8	24.406	Nonanoyl chloride ($C_9H_{17}ClO$)	176	9.25	Anticancer
9	24.596	Dicotyl ether ($C_{16}H_{34}O$)	242	2.64	Antiinflammatory, antimicrobial.
10	26.62	Oleic acid, hexyl ester ($C_{24}H_{46}O_2$)	366	1.7	No activity

RT = retention time, MW = molecular weight, % Comp. = percentage composition

The GC-MS analysis of *A. Porrum* shows various essential oils especially fatty acids which are most abundant. The GC-MS profile of *A. Porrum* in this study differs from profile presented by [22,23] for similar plant. This difference probably could be due to variations in the solvents used by the authors for extraction which may have different polarity [24]. Extraction in this study was done with ethanol while

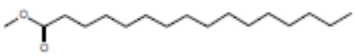
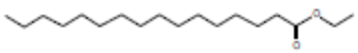
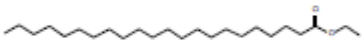
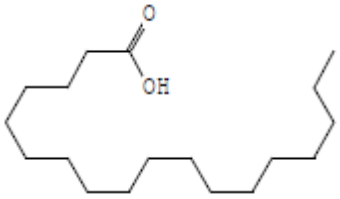
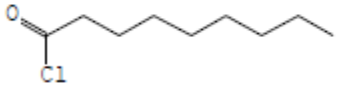
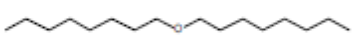
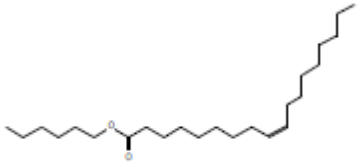
previous report used crude aqueous extract [18]. The major constituents of ethanolic fraction of *A. porrum* recorded in this study are reported to have trypanocidal, antioxidant, antiandrogenic, antiinflammatory, antibacterial, cancer preventive, hypocholesterolenic, pesticide, nematicide, flavor and haemolytic activities; and are well documented in Phytochemical and Ethnobotanical Database [21]. It is also

noteworthy that several active principles of the *A. porrum* detected in this study have toxic biological activities some of which includes neurotoxicity, hepatotoxicity and nephrotoxicity [25]. The Lilicaea have been reported to have abundant palmitic acids which have been reported to be more toxic to the mammalian cells than trypanosome parasites [26]. The cytotoxicity of the essential oils of *A. porrum* have been suggested to be due to the fact that fatty acids, fatty acid ester and aliphatic chains (long chain alkanes and alkenes) normally accumulates in the lipid layer of the cell membrane and mitochondria, consequently disturbing the integrity of the cell structure which becomes permeable and ultimately lead to cell death [27].

CONCLUSION

This study established the biochemical composition of the bulb of *A. porrum* which have a commendable bioactivity and can be advised as a plant of phytopharmaceutical importance as used in traditional medicine.

Table3: Chemical structure of compounds present in ethanolic extract of *A.porrum*

 Hexadecanoic acid, methyl ester	 Hexadecanoic acid, ethyl ester
 9,12-Octadecadienoic acid, methyl ester	 9-Octadecanoic acid, methyl ester, (E)-
 Docosanoic acid, ethyl ester	 Octadecanoic acid
 Decane, 1-fluoro-	 Nonanoyl chloride
 Dicotyl ether	 Oleic acid, hexyl ester

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REFERENCES

1. A. Bernhoft., Proceedings from a symposium held at The Norwegian Academy of Science and Letters, Oslo, 13 - 14 **2008.** Oslo: The Norwegian Academy of Science and Letters, 11-18.
2. A. Mann, M. Gbate, U. A. Nda., Niger State, Nigeria, **2003**, 276.
3. T. Bulus, S. E. Atawodi, M. Mamman., *Niger. J. Biochem. Mol. Bio.* **2008**, 23(1), 7-11.
4. WHO Report, World Health Organization, Geneva, WHO/EDM/TRM/ **2002**, 21, 19.
5. T. A. Odugbemi., University of Lagos Press, Nigeria, **2008**, 10-11.
6. G. H. Naik, K. I. Priyadarsini, J. G. Satav, M. M. Banavalikar, D. P. Sohani, M. K. Bivani., *Phytochem.* **2003,** 63, 97-104.
7. M. R. S. Zaidan, N. A. Rain, A. R. Badrul., *Trop. Biomed.* **2005**, 22, 165-170.
8. K. Sheeja, G. Kuttan. *Immunopharmarcol. Immunotoxicol.* **2007**, 29, 81-93.
9. P. K. Mukherjee, V. Kumar, P. J. Houghton. *Phytother. Res.* **2007,** 21, 1142-1445.
10. N. S. Gyang., B.Sc. Thesis, University of Jos, Nigeria.
11. B. K. Sahira, L. Cathrine., *Inter. J. Adva. Res. Chem. Sci.* **2015**, 2(4), 25-32.
12. A. Muthulakshmi, J. R.Margret, V. R. Mohan., *J. App. Pharm. Sci.* **2012**, 2(2), 69-74.
13. H. A., Ronald. Handbook of Instrumental Techniques for Analytical Chemistry. **1997**, 609-611.
14. B. Tepe, M. Sokmen, H. Akpulat, A. Sokmen., *F. Chem.* **2005**, 92, 89-92.
15. Y. Yabuki, Y. Mukaida, Y. Saito, K. Oshima, T. Takahashi, E. Muroi, Y. Uda., *F. Chem.* **2010**, 120(2), 343-348.
16. N. Bernaert, D. De Paepe, C. Bouten, H. De Clercq, D. Stewart, E.Van Bockstaele, B. Van Droogenbroeck., *F. Chem.* **2012,** 134(2), 669-677.
17. R. Pennington., In Bulletin of Agriculture and Horticulture Development Board, Harper Adam University College, Shorpshire, **2011**, 1-13.
18. D. Mnayer, A. S. Fabiano-Tixier, E. Petitcolas, T. Hamieh, N. Nehme, C. Ferrant., *F. Chemat. Mol.* **2014**, 19(12), 34-53.
19. E. O. Donatus, C. I. Ephraim., *Afr. J. Pharm. Pharmac.* **2009**, 3(5), 277–281.
20. H. O. Edeoga, D. E. Okwu, B. O. Mbaebie., *Afr. J. Biotec.* **2005**, 4(7), 685-688.
21. J. A. Duke. Dr. Duke's Phytochemical and Ethnobotanical Databases.
22. S. Banerjee, K. Mukherjee, S. Maulik., *Phytotherap. Res.* **2003**, 17, 97-106.
23. J. W. Kim, J. E. Huh, S. H. Kyung, K. H. Kyung., *F. Sci. Biotec.* **2004,** 13, 235-239.
24. A. Ghasemzadeh, H. Z. E. Jaafar, A. Rahmat., *J. Med. Pla. Res.* **2011,** 5(7), 1147-1154.
25. S. Kpoviessi, J. Bero, P. Agbani, F. Gbaguidi, B. Kpadonou-Kpoviessi, B. Sinsin, J. Quetin-Leclercq., *J. ethnopharmac.* **2014**, 151(1), 652-659.
26. S. Hoets, C. Stevigny, M. Herent, J. Quetin-Leclrcq. *Pl. Med.* **2005,** 72, 480-482.
27. G. Belakhdar, A. Benjouad, E. H. Abdennebi., *J. Mat. Env. Sci.* **2015**, 6(10), 2778-2783.