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Original Research Article

Comparism of Bioactive Components in Hydroethanol Extract of *Corchorus olitorius* and *Amaranthus hybridus* Leaves

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ABSTRACT

The importance of dietary vegetables cannot be overemphasised. This study aimed to screen the bioactive compounds present in *C. olitorius* and *A. hybridus* hydroethanol (30:70%v/v) extract. A gas chromatography-mass spectrometer (GC-MS) was used for characterising the phytocomponents. *A. hybridus* had 9 compounds compared to *C. olitorius*, which had 15 compounds. Neophytadiene, hexadecenoic acid methyl ester, 9-octadecenoic acid methyl ester, phytol, and methyl stearate were present in both extracts. *C. olitorius* alone contained bicyclo (3,1,1) heptane, 2,6,6 trimethyl, 2-tridecanone, pyridine-3-carboxamide,4-dimethylamino-N-(2,4-difluorophenyl), octadecenal and octadecane 1-etheyloxyl while 2-pentadecanone, 6, 10, 14-trimethyl-, dodecanoic acid, ethyl 9-hexadecenoate and 3, 7, 11, 15-Tetramethyl-2-hexadece-1-ol were present in *A. hybridus* only. The three main compounds present in *C. olitorius* were hexadecenoic acid (21.99%), octadecane 1-ethenyloxy (19.51%) and hexadecanal (18.08%). In *A. hybridus*, hexadecenoic acid (43.75%), methyl stearate (23.43%), and phytol (10.90%) were the most common compounds. The bioactive constituents identified have numerous applications such as antimicrobial, flavour enhancing, anti-tumour, anti-inflammatory, chemo signalling, antidiarrheal, hepatoprotective, antioxidant, cytotoxic, and insecticidal properties.

Keywords: Gas Chromatography-Mass Spectroscopy analysis, phytochemicals, bioactivity, hexadecenoic acid, methyl stearate, medicinal potential.

Introduction

Dietary vegetables have been an important part of the nutrition of African countries since time immemorial. In the past, the incidence of non-communicable chronic diseases such as diabetes, cancer, and metabolic syndromes was very low among Africans, but the departure to Western lifestyles and diets has contributed to an increase in such diseases. Plant-based foods are rich in bioactive compounds known as phytochemicals, also known as secondary plant metabolites, which have been reported to promote health and well-being. These phytochemicals have served as drug targets for research for years. One of the prominent research areas is in the field of cancer therapy to enhance the success of chemotherapy and reduce resistance to chemotherapy through targeting cancer stem cells, inducing apoptosis, cell cycle arrest, inhibition of metastasis, and cancer progression inhibition, to mention just a few. [1–4] Research has revealed that phytochemicals can be used to sensitise cancerous cells, thereby enhancing therapy, which means these compounds can be used for drug development. [5] The use of herbs and natural remedies to treat or manage diseases is known as ethnomedicine, and this has been the primary mode of treatment in developing countries. it involves the use of decoctions, pastes, or infusions.

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This treatment modality depends on the phytochemical constituents of herbs (leaves, roots, stems, bark, seeds, flowers, etc.). The diversity of compounds present in plants serves as a subject for the discovery of innovative drugs. These compounds have been reported to possess antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial, and wound healing properties, as well as the added advantage of fewer side effects, which can be used as alternatives to synthetic drugs.[6,7,8] *Corchorus olitorius* (Linn.), commonly known as jute, is widely distributed in the tropical regions of Asia and Africa. They serve as weeds or dietary vegetables based on location. The leaves are used for soup preparation in Nigeria due to their rich protein and mineral contents and for weaning infants because their slimy nature helps with the easy passage of bolus. Some ethnomedicinal uses of the plant include the treatment of pain, chronic cystitis, purgative, etc. [9,10] *Amaranthus hybridus* L. is also known as African spinach. It is one of the Amaranthus species known to possess allelopathic potential. *A. hybridus* is used to prepare soup or salad in parts of Africa. Ethnobotanical uses of *A. hybridus* include treating diarrhoea, excessive menstruation, and intestinal bleeding. [11,12] Combining gas chromatography (GC), an ideal separation technique, and mass spectroscopy (MS), one of the best identification techniques, is constantly used in phytochemistry research to identify compounds. We employed GC-MS to separate and identify the bioactive constituents present in the hydroethanol crude extract of *C. olitorius* and *A. hybridus* leaves to obtain scientific knowledge on the constituents responsible for the therapeutic effects associated with these vegetables in ethnomedicine. To our knowledge, this is the first time the constituents of hydroethanol extracts of *C. olitorius* and *A. hybridus* leaves are being assessed, considering that most herbal preparations use ethanol, water or a combination of both solvents.

Materials and Methods

Plant collection and identification

Fresh *C. olitorius* and *A. hybridus* leaves were purchased from the local market in Ota, Ogun State (6º 70' 59.99" N and 3º 22' 59.99" E) in June 2021 and authenticated at the Forestry Research Institute of Nigeria (FRIN) with voucher numbers FHI.113948 for *C. olitorius* and FHI.113947 for *A. hybridus.* The specimens were deposited at the forest herbarium, Ibadan.

Plant extraction

The leaves were picked, air-dried, pulverised and extracted by maceration using bulk ethanol (96% purity) and distilled water in a ratio of 70:30 (volume/volume). Four hundred grammes each of *C. olitorius* and *A. hybridus* were weighed into separate plastic buckets and the solvent was added in a ratio of 4 ml of solvent to 1 g of powdered leaves. After 72 hours, the content of each bucket was filtered separately and the filtrate was concentrated using a rotary evaporator (BUCHI, Switzerland) and lyophilised using Lyotrap (LTE Scientific Ltd, Great Britain). Lyophilised hydroethanol extracts of *C. olitorius* and *A. hybridus* were analysed.

Identification of bioactive constituents using Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was performed using an Agilent 5977B GC/MSD system coupled with an Agilent 8860 auto-sampler (Agilent Technologies, United States of America). The ionization energy was set at 70 eV in the electron ionization system, while the carrier gas was 99.99% Helium gas at a 1 ml/min flow rate. The injection volume and temperatures were 1 µl and 300ºC, respectively, while the ion source temperature was 250ºC and 100ºC for the oven, which gradually increased to 280ºC at the rate of 20ºC/min. Scanning was done at an interval of 0.5 s for fragments ranging between 45 to 450 Da with a solvent delay of 0 to 3 minutes.

Results and Discussion

Medicinal plants are used in the treatment and management of diseases such as diabetes, cancer, and inflammatory disorders due to the presence of bioactive compounds that act singly, synergistically, or additively. These bioactive compounds may possess anti-inflammatory, anti-oxidative, anti-proliferative, or antimicrobial effects. [13,14] A very good technique for the identification of hydrocarbons, alcohols, esters, volatile compounds, and acids is the GC-MS. The components were identified based on the retention time and matching with the National Institute of Standards and Technology (NIST) library. *C. olitorius* had 15 compounds (Table 1), while *A. hybridus* had 9 compounds (Table 2). Figures 1 and 2 show the total ion chromatogram (TIC) of *A. hybridus* and *C. olitorius*, respectively.

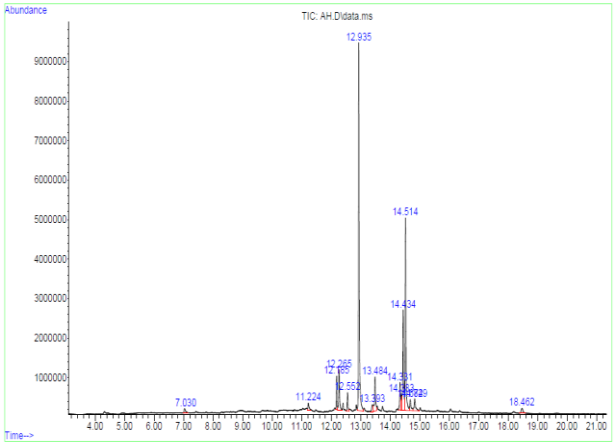

Figure 1: GC-MS chromatogram of hydroethanol extract of *A. hybridus* leaves

The three major compounds (Table 1) present in the hydroethanol extract of *C. olitorius* are hexadecenoic acid, also known as palmitic acid (21.99%), octadecane 1-(ethenyloxy)- (19.51%) and hexadecanal (18.08%). Fatty acids have been reported to possess antifungal and antibacterial effects; Hexadecenoic acid, a fatty acid, has anti-inflammatory, antimicrobial, antihypertensive, and antidiarrheal activity and is also reported to inhibit growth and induce apoptosis in the human gastric cancer cell. [15–17]Octadecane 1-ethenyloxy- is a vinyl ether with an antisepsis effect, [18] while hexadecanal is a volatile long-chain aliphatic aldehyde with chemo signalling activity in mammals. [19]

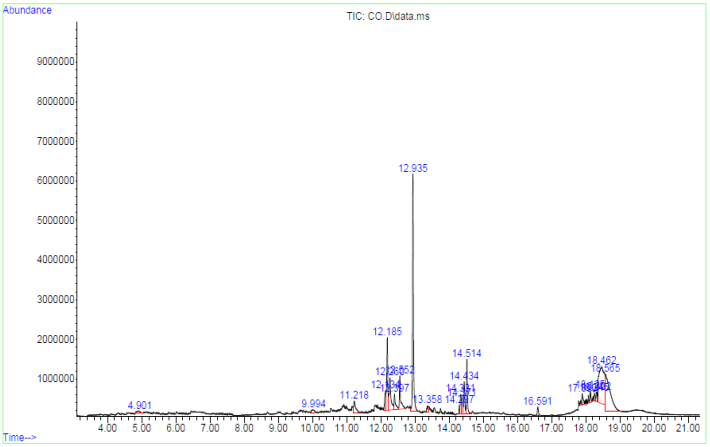

Figure 2: GC-MS chromatogram of hydroethanol extract of *C. olitorius* leaves

In *A. hybridus* (Table 2), hexadecenoic acid (43.75%), methyl stearate (23.73%), and phytol (10.90%) are the major bioactive constituents. Methyl stearate is a fatty acid with diverse biological effects in microorganisms and mammals. It inhibits different strains of pathogenic fungi and bacteria and possesses antioxidant activity. [20–22] Phytol is a precursor for Vitamin K and E. It is a diterpene alcohol with anti-inflammatory, antioxidant, antidiabetic, hypolipidemic, and anticancer properties. [23,24]

The five bioactive compounds common to *C olitorius* and *A. hybridus* (Figure 3) are neophytadiene, hexadecenoic acid, 9-octadecenoic acid, phytol, and methyl stearate. Neophytadiene possesses anti-inflammatory, cardioprotective, and antioxidant properties by inhibiting the production of nitric oxide and inflammatory cytokines like interleukin 6 (IL-6), interleukin 10 (IL-10) and tumor necrosis factor-alpha (TNF-α) during *in vitro* and *in vivo* experimentation. [25]Selmy et al. [26] reported the potential anticancer activity of neophytadiene due to its high affinity for Human LRH-1 and Human A2a receptor, which have multiple signalling pathways that play essential roles in cancer cell viability. Hexadecenoic acid and 9-octadecenoic acid possess antioxidant, anticancer, hepatoprotective, and antimicrobial properties. [27,28,29]

Bicyclo (3,1,1) heptane, 2,6,6 trimethyl-, 2-Tridecanone, pyridine-3-carboxamide, 4-dimethylamino-N-(2,4-difluorophenyl)-, octadecenal and octadecane 1-ethyloxyl were present only in *C. olitorius* (Figure 4).

Bicyclo (3,1,1) heptane, 2,6,6 trimethyl- is an aromatic bicyclic synonymous with pinane and dihydropinene [13]with antimicrobial activity. Dihydropinene is among the major components of turpentine oil and has been reported to possess anti-inflammatory, immunomodulatory, antibacterial, antitumor, and antioxidant properties. When added to squalene emulsions, it increases stability, prolonged shelf-life, and effectiveness. Squalene emulsions are used as drug carriers to enhance delivery and reduce hyper-inflammatory responses in chemotherapy. [30–32] 2-Tridecanone is a methyl ketone that aids plants like *Lycopersicon hirsutum f. glabratum* (wild tomatoes) in resisting arthropods because it affects insect development. It acts as a non-alkaloid insecticide. [33, 34]

It can be used as a natural pesticide to protect vegetables from damage by insects and spider mites because it can selectively inhibit, repulse, and, in some cases, kill non-adapted organisms that feed on the plants. Chatzivasileiadis et al. [35]reported that a single or double contact of a spider mite with the tips of the glandular trichomes of wild tomatoes is enough to cause 50% death daily because the trichomes have high concentrations of 2-Tridecanone. 2-Tridecanone is also present in foods like allium species, cheese, lamb, malt, rice, and banana. The safety assessment of 2-Tridecanone was based on seven human health endpoints, and all of them were cleared. The endpoints included genotoxicity, repeated dose toxicity, reproductive toxicity, skin sensitisation, photo irritation/photo allergenicity, local respiratory toxicity, and environmental toxicity.[36,37] Pyridine-3-carboxamide,4-dimethylamino-N-(2,4-difluorophenyl)- is a heterocyclic compound. It is a derivative of Pyridine-3-carboxamide (nicotinamide). Different derivatives of Pyridine-3-carboxamide have various pharmacological effects, such as selective inhibition and cytotoxicity. [34] Pyridine compounds are also known to have a pleasant smell. [39] Pyridine-3-carboxamide,4-dimethylamino-N-(2,4-difluorophenyl)- is reported to possess anti-inflammatory actions because it blocks the inflammatory actions of iodides. [40]

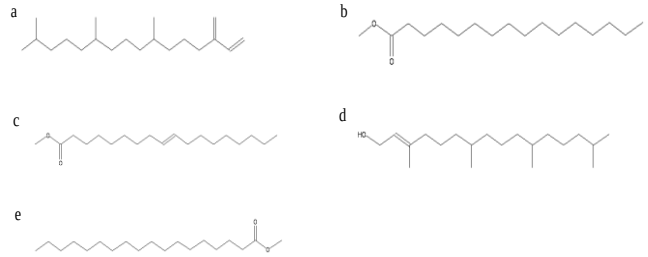

Figure 3: Compounds identified in *C. olitorius* and *A. hybridus*.
a) Neophytadiene, b) Hexadecanoic acid, c) 9-Octadecenoic acid, d) Phytol, e) Methyl stearate

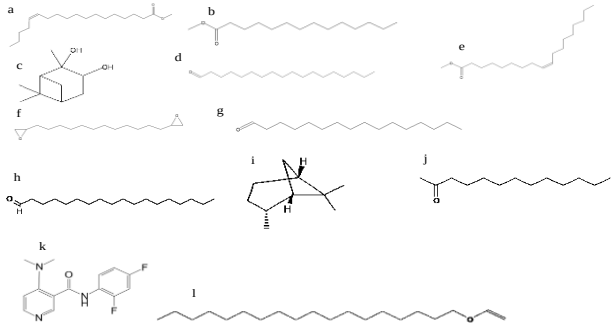

Figure 4: Structure of compounds present in *C. olitorius* only
a) cis-13-Octadecenoic acid, methyl ester; b) Methyl tetradecanoate; c) 2,4-Diaminophenol; d) Octadecanal; e) 9-Octadecenoic acid (*Z*)-, methyl ester; f) 1,2-15,16-Diepoxyhexadecane; g) Hexadecanal; h) Octadecanal; i) Bicyclo (3,1,1) heptane,2,6,6 trimethyl-; g) 2-Tridecanone; k) pyridine-3-carboxamide,4-dimethylamino-N-(2,4-difluorophenyl)-; l)Octadecane,1-(ethenyloxy)-

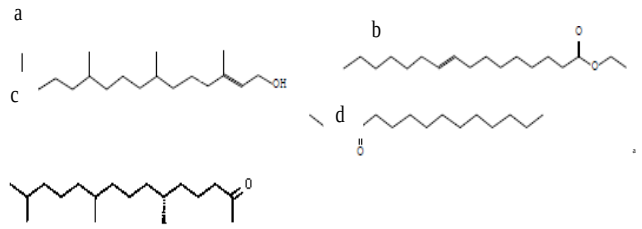

Figure 5: Structure of bioactive compounds present in *A. hybridus* only.
a) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; b) Ethyl 9-hexadecenoate; c) Dodecanoic acid, ethyl ester; d) 2-pentadecanone, 6, 10,14-rimethyl-

Table 1: Major compounds present in lyophilised hydroethanol extract of *C. olitorius* leaves

S/N	Hit Name	RT (min)	Quality	Mol Weight (amu)	% Composition
1	Methyl tetradecanoate	11.218	92	242.225	2.38
2	2,4-Diaminophenol	12.134	52	124.064	2.80
3	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)-	12.185	87	138.141	7.21
4	2-Tridecanone	12.26	50	198.198	5.45
5	Neophytadiene	12.397	96	278.297	2.26
6	Neophytadiene	12.552	84	278.297	4.05
7	Hexadecanoic acid, methyl ester	12.935	98	270.256	21.99
8	Pyridine-3-carboxamide, 4-dimethylamino-N-(2,4-difluorophenyl)-	13.358	59	277.103	1.04
9	cis-13-Octadecenoic acid, methyl ester	14.331	99	296.272	2.10
10	9-Octadecenoic acid (*Z*)-, methyl ester	14.371	97	296.272	1.75
11	Phytol	14.434	87	296.308	3.26
12	Methyl stearate	14.514	98	298.287	5.023
13	Neophytadiene	16.591	95	278.297	0.99
14	Octadecanal	17.89	93	268.277	1.01
15	1,2-15,16-Diepoxyhexadecane	18.125	90	254.225	1.09
16	Octadecane, 1-(ethenyloxy)-	18.462	86	296.308	19.51
17	Hexadecanal	18.565	83	240.245	18.08

Octadecenal is a component of the female sex pheromones in some moth species. Pheromones are chemical messengers secreted by females to attract males, and their components can vary from species to species with geographical variations. [41] Octadecenal is also present in coriander, which was found to deodorise almost completely the stench from the porcine large intestine. This means that octadecenal has a pleasant smell and antimicrobial properties, thereby preventing odour. The deodorisation mechanism for octadecenal may be due to the modification effect through the modulation of an offensive odour compound to a different odour compound. [42] Figure 5 shows the compounds present in *A. hybridus* only. They are 2-pentadecanone, 6, 10, 14,-trimethyl, 3,7,11,15-tetramethyl-2-hexadecen-1-ol which has anti-inflammatory and antimicrobial activities,[43] dodecanoic acid ethyl ester and ethyl-9-hexadecenoate.

Table 2: Major compounds present in lyophilised hydroethanol extract of *A. hybridus* leaves

S/N	Hit Name	RT (min)	Quality	Mol Weight (amu)	% Composition
1	Neophytadiene	12.185	91	278.297	3.44
2	2-Pentadecanone, 6,10,14-trimethyl-	12.265	90	268.277	5.76
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	12.552	64	296.308	2.04
4	Hexadecanoic acid, methyl ester	12.935	98	270.256	43.75
5	Dodecanoic acid, ethyl ester	13.484	90	228.209	4.50
6	9-Octadecenoic acid, methyl ester, (*E*)-	14.331	99	296.272	3.89
7	Phytol	14.434	68	296.308	10.90
8	Methyl stearate	14.514	98	298.287	23.43
9	Ethyl 9-hexadecenoate	14.829	90	282.256	2.29

Conclusion

Natural compounds are excellent sources for the discovery of drug compounds due to their numerous pharmacological properties. The GC-MS analysis of *C. olitorius* and *A. hybridus* revealed the bioactive constituents present in these leaves. The bioactive constituents have numerous applications such as antimicrobial, flavour enhancing, antitumor, anti-inflammatory, chemo signalling, antidiarrhoeal, hepatoprotective, antioxidant, cytotoxic, and insecticidal properties. From the result of this study, there is a need to isolate and purify the bioactive compounds present in both vegetables for further investigations to develop natural antitumor drug candidates and formulation studies for developing insecticides and deodorants.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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