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Bioactive Compounds in Ethanolic Extract of *Vigna radiata* (L.) Seed

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ABSTRACT

In the present study, the identification and quantification of bioactive compounds in matured *Vigna radiata* (L.) (Mung bean) seeds (VrS) was investigated. Vr seeds were processed into flour and subjected to ethanolic extraction using a soxhlet apparatus. The ethanolic extract was analyzed using Gas Chromatography – Mass Spectrometry (GC–MS) to identify and quantify the bioactive compounds content, which generated 20 observable peaks. The spectrum obtained when matched with NIST spectral database revealed a profile of 16 bioactive compounds. Linoelaidic acid was identified as the major bioactive compound in the sample, accounting for 30.68% of the total relative abundance. Other bioactive compounds identified with significant relative abundance are 10-Octadecenoic acid, methyl ester, Octadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, 6-Octadecenoic acid, (Z)- and Oleic acid while the remaining bioactive compounds were eluted at relative abundance less than 3%. These bioactive compounds have numerous therapeutic properties and can be incorporated as active ingredients in the formulation of nutraceuticals, pharmaceuticals and cosmeceuticals.

Keywords: Bioactive compounds, ethanolic extract, GC-MS, *Vigna radiata (L.)* seed.

INTRODUCTION

The use of food as a source of medicine has become a trending fact in the world of today. It is a concept known as nutraceuticals. This approach focuses on the utilization of whole foods, food extracts, or specific bioactive compounds from the edible part of plants to prevent, treat, or manage various health conditions. This was facilitated by researchers who isolated and identified various biochemical compounds from plants called phytochemicals that have potential health benefits generally called their therapeutic activities. These phytochemicals are plants' secondary metabolites with the primary function of chemical defense against insects, environmental stress and microorganisms [1].

The therapeutic activity, a medicinal property of plant, can be predicted by the identification of its phytochemical constituents [2]. Therefore, for proper utilization of the therapeutic activity of a plant in the treatment of any disease, there is great need to identify its phytochemical composition which can differ in terms of quantities regardless that the plants are of the same species but grown in different environmental conditions [3].

Vigna radiata (L.) seed, a well researched and documented bean seed with various reported therapeutic activities and general uses, is utilized worldwide, yet lesser known in Nigeria [4]. It is a leguminous crop from fabaceae [4]family of plants and a functional food that contains balanced nutrients, including high content of digestible protein, dietary fiber, minerals, vitamins, and significant amounts of bioactive compounds [5]. It is one of the most important edible legume crops, grown on more than 6 million hectares worldwide (about 8.5% of the global pulse area) and consumed by most households in Asia. This is due to its characteristics of relatively drought-tolerant, low-input crop, and short growth cycle of about 60 days [6]. The mung bean seeds are small, ovoid in shape, green in colour with husk but yellow when dehusked. They are grown widely for use as human food (as dry beans or fresh sprouts), but can be used as green manure for crops and as forage for livestock [7]. The seeds are sprouted for fresh use or canned for shipment to restaurants where they are used mostly in 'salad' making.

Many studies have recorded the anti-inflammatory, antioxidative, anticancer, anti-diabetic [8-10] and other therapeutic activities of *Vigna radiata* whole raw seed thereby bequeathing the name 'wonder crop' [11], but the actual bioactive molecules responsible for these therapeutic activities still vary as a result of the influence of environmental factors which affect the type and quantity of the phytochemical content. Wang *et al.,*[12] and Sreerama *et al*.,[13] reported different values of 18.6% and 22.4% for oleic acid content of mungbean seed oil and the seed content respectively with the same antioxidant and anti-inflammatory activities of the bioactive compound.

Therefore, in view of the variations in values recorded for bioactive compounds in *Vigna radiata* seeds by various researchers, it becomes imperative to conduct investigation using GC-MS to analyze the actual phytochemical content of the ethanolic extract from matured Nigerian *Vigna radiata* (mung bean) whole raw seed, planted and harvested in Nigeria where the plant is newly introduced. The findings of this research, is of importance, as it will facilitate an understanding of the bioactive compounds present in the Nigerian cultivated *Vigna radiata* seed. This, may lead to increased acceptability, cultivation, availability and general utilization of the *Vigna radiata* seeds through which these bioactive compounds can be isolated and utilized especially by pharmaceuticals, nutraceauticals and cosmeceuticals for the formulation of novel potent products that can be beneficial in the treatment of many health defects.

MATERIALS AND METHODS

Materials and reagents

All the reagents and solvents were of analytical grades and were bought from Sigma Aldrich (U S A). These included ethanol, hexane, anhydrous sodium sulphate and pyridine. Helium gas was used as carrier gas. The materials used included soxhlet apparatus, hotplate, electric grinder, refrigerator, digital scale, Buchi Rotavapor R-210, Apel 3000 UV model spectrophotometer and Agilent Technologies GC systems with GC-220 model (Varian, Santa Clara, CA, USA)

Sample Collection and Preparation

Matured *Vigna radiata* (L.). black pods were selectively harvested from the farm of National Biotechnology and Research Centre, Abagana, Anambra State, Nigeria, identified and confirmed at the National Bioresources Development and Research Centre Laboratory at Abagana, Anambra State, Nigeria. The pods were air dried for 3 days inside a well ventilated cheese-cloth bag to avoid spontaneous splitting and scattering of the seeds by heat and subsequently mashed with minimal pressure to separate the pods from the seed. Winnowing method of separation was used to separate the chaff from the seeds and the bad roughly shaped multi-coloured seeds were sorted out. The good pure green seeds were rinsed with distilled water, air dried for 3 weeks and later milled into fine powder by using high power blender and grinder (QRSA, QBL-8008 pro2). The ground *Vigna radiata* (L.) seed flour was put in an air tight container and kept for further analysis.

Preparation of Plant Extracts

About 100 g of the test sample was placed in soxhlet apparatus and was added 500 ml of ethanol. This extraction process was allowed to operate for 12 hours at 64 ^{o}C. The extract was filtered through Whatman filter paper number 41 (110 mm). The concentration of the resulting solution of the extract was done in an evaporation dish to remove most of the solvent from the ethanolic seed-flour extract and the resulting concentrate of the viscous liquid was stored in a refrigerator for further use.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

About 1 g of the ethanolic extract of the test sample was weighed and transferred into a test tube and 25 ml of ethanol was added. The test tube content was allowed to react in a hotplate at 60^0C for 90 min. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successively with 20 ml of ethanol; 10 ml of cold water, 10 ml of hot water and 3 ml of hexane, which was all transferred to the funnel. The extract was combined and washed three times with 10 ml of 10% v/v ethanol aqueous solution. The solution obtained was dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000 ul of pyridine of which 200 ul was transferred to a vial for GC-MS analysis.

The GC–MS analysis of the bioactive compounds from the extract was done using Agilent Technologies GC systems with GC-220 model (Varian, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). Spectroscopic detection by the GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas at flow rate of 1 ml/min. The initial temperature was set at 50 –150 °C at increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. Relative quantity of the bioactive compounds present in the aqueous extract was expressed as percentage based on peak area (concentration of the compound) produced in the chromatogram.

Identification of Chemical Constituents

Bioactive compounds contained in the ethanolic extract was identified based on GC retention time and percentage area of the spectrum on HP-5MS column and matching of the spectrum with the spectra of the known components stored in the NIST library (D:\MassHunter\Library\NIST14.L).

RESULTS AND DISCUSSION

GC-MS Analysis of the Ethanolic Extract of Nigerian *Vigna radiata* Raw Seed Flour

Approximately 20 observable peaks with total peak area of 100% were revealed in the spectrum generated from the GC-MS analysis of ethanolic extract of *Vigna radiata* seed flour sample as shown in Figure 1**.**

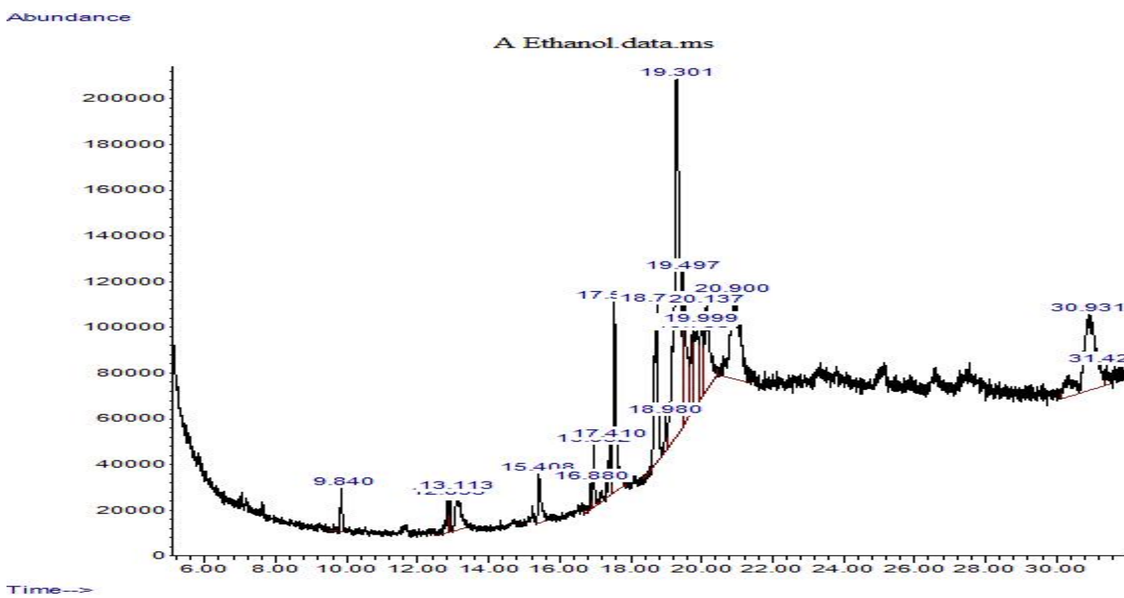

Figure 1 : Total Ion Chromatogram (TIC) of Ethanolic Extract of Raw *Vigna radiata* seed

The spectrum when matched with MassHunter\Library\NIST14.L spectral database, revealed the presence of 16 bioactive compounds. Linoelaidic acid, eluted two times, was identified as the major component with the highest peak on the spectrum, with first elution at RT 19.301 and second elution at RT 20.137 which yielded a relative abundance of 30.68%. Other bioactive compounds, which were eluted with appreciable quantities that revealed the importance of the seed sample, arranged in the order of decrease in their relative abundance are Oleic Acid - RT 30.931 (relative abundance 12.97%), 6-Octadecenoic acid, (Z)- RT 20.900 (relative abundance 10.94%), 9,12-Octadecadienoic acid (Z,Z)- (eluted three times with total relative abundance of 10.45%) and n-Hexadecanoic acid – (eluted two times with total relative abundance of 9.26%). The remaining bioactive compounds with relative abundance less than 3% were also noted. All the names of the bioactive compounds identified, with their retention times, percentage relative abundance, therapeutic activities and their general uses are detailed in Table 1.

Table 1: Bioactive compounds identified from GC-MS analysis of ethanolic extract of *Vigna radiata* seeds with their therapeutic activities.

Pk	RT	RA%	Bioactive compounds	Therapeutic activities / uses
1	9.840	1.07	5-Octadecene, (E)-	Antimicrobial and antifungal Properties. Used in cosmetics and pharmaceuticals as an emollient and solvent
2	12.828	0.98	Pyrimidine-4,6(3H,5H)-dione, 2-butylthio-	Antiviral and antibacterial Properties. Potential use in treating viral infections and bacterial diseases
3	12.893	0.81	Trichloroacetic acid, pentadecyl ester	Antimicrobial and antifungal properties Used in pharmaceuticals and cosmetics as a preservative
4	13.113	2.69	Dodecanoic acid	Antimicrobial and antifungal properties. Used in soaps, cosmetics, and pharmaceuticals as an emollient and surfactant
5	15.408	2.11	Tetradecanoic acid	Antimicrobial and antifungal properties Used in soaps, cosmetics, and pharmaceuticals as an emollient and surfactant
6	16.880	0.66	Carbonic acid, propyl 2,2,2-trichloroethyl ester	Anesthetic and analgesic properties - Potential use in pain management and anesthesia
7	16.952	1.44	Hexadecanoic acid, methyl ester	Emollient and surfactant properties. Used in cosmetics and pharmaceuticals as a skin moisturizer and solubilizer
8	17.410	2.14	n-Hexadecanoic acid	Antimicrobial and antifungal properties. Used in soaps, cosmetics, and pharmaceuticals as an emollient and surfactant
9	17.538	7.12	n-Hexadecanoic acid	Same as above

10	18.743	6.63	10-Octadecenoic acid, methyl ester	Emollient and surfactant properties. Used in cosmetics and pharmaceuticals as a skin moisturizer and solubilizer
11	18.980	0.88	Heptadecanoic acid,	Emollient and surfactant 16-methyl-, methyl ester properties. Used in cosmetics and pharmaceuticals as a skin moisturizer and solubilizer
12	19.301	24.92	Linoelaidic acid	Anti-inflammatory and antioxidant properties. cardiovascular health, anti-cancer, immune enhancer, skin and wound healing properties, anti-diabetic properties, ant-microbial properties.
13	19.497	7.73	Octadecanoic acid	Antimicrobial and antifungal properties. Used in soaps, cosmetics, and pharmaceuticals as an emollient and surfactant
14	19.753	2.98	9,12-Octadecadienoic acid (Z,Z)-	Anti-inflammatory and antioxidant properties.
15	19.869	4.56	9,12-Octadecadienoic acid (Z,Z)-	Same as above
16	19.999	2.91	9,12-Octadecadienoic acid (Z,Z)-	Same as above
17	20.137	5.76	Linoelaidic acid	Same as earlier
18	20.900	10.94	6-Octadecenoic acid, (Z)-	Anti-inflammatory, antioxidant and anticancer properties. Used in cosmetics for its moisturizing, emollient properties and potential use in treating skin conditions like psoriasis and atopic dermatitis
19	30.931	12.97	Oleic Acid	Emollient and moisturizing properties in cosmetics. Antimicrobial and anti-inflammatory properties.

20	31.429	0.72	Ethyl Oleate	Emollient and solvent properties in cosmetics and pharmaceuticals

Notes: PK= Peak; TR- Retention time; RA= Relative abundance

The results obtained from this study have shown that the plant can actually thrive in Nigerian soil having grown unto maturity on Nigerian soil until maturity and was used in this investigation. The seeds of this crop, through the result obtained in this GC-MS analysis, contain very important bioactive compounds reported to play vital roles in normal growth, development, and generation of new cells in the human body. Amare *et al*. [14], reported the antidiabetic activities of mung bean seed. The linoelaidic acid, a bioactive compound revealed to be contained in the bean seed used in this analysis, has been recorded among the safe eucosmetics by the NCBI [15] hence essential in cosmeceuticals, but mung bean (*Vigna radiata*) seed was not included among the sources of linoelaidic acid in food additives category listed in NCBI database. This was spotted as an omission since this research has confirmed the presence of linoelaidic acid as the bioactive compound among the 16 bioactive compounds identified through this GC-MS analysis conducted on the ethanolic extract of matured *Vigna radiata* raw seed sample and with the highest relative abundance (30.68%) . Linoelaidic acid, an isomer of linoleic acid, is essential for the maturation of the developing brain, retina and other organs both utero and in early life of infants [16], hence applicable as a raw material in neuraceuticals.

Oleic acid which also recorded appreciable relative abundance of 12.9% in this analysis has been attributed with anti-cancer, anti-inflammatory and wound healing therapeutic activities [17]. These bioactive compounds, with all other identified compounds have reported therapeutic activities (Table 1) which can be used as nutraceuticals, pharmaceuticals and cosmeceuticals important raw materials.

In view of these finding, matured Nigerian *Vigna radiata* seeds are considered as important bioresources.

CONCLUSIONS

The GC-MS analysis conducted on ethanolic extract of matured Nigerian *Vigna radiata* seed revealed a treasure trove of bioactive compounds with potentials for numerous applications. The acceptance of the mung bean (*Vigna radiata* L.) seed by the National Agricultural Seed Council in Nigeria for adoption and nationalization is recommended. This will lead to increased cultivation of the bean seed which will result in its abundance with subsequent introduction and availability in Nigerian markets. With this done, there will be overall abundance of the bean seeds which will help to bring down the inflated prices of other beans in the Nigerian market. The high cost of infant foods in the Nigerian markets has called for more research on the use of *Vigna radiata* (L.) seeds in the formulation of weaning foods for infants. This is important for the formation of a well developed infant brain which has suddenly become a neglected aspect of human development as a result of inflated standard of living in recent times. Natural products have emerged as global desirable products due to the reported after-effects of their synthetic counterparts. Therefore the isolation of linoelaidic acid from *Vigna radiata* seed and its subsequent usage in the formulation of soap, lotion, cream or gel in cosmeceauticals can replace its synthetic counterparts like Isopropyl myristate (IPM) which is a synthetic emollient used in cosmetic products. Diabetes may be controlled if there is frequent consumption of this bean seed because of the reported therapeutic activities of linoelaidic acid and oleic acid which are majorly found bioactive compounds revealed in the *Vigna radiata* (L.) seed that was used in this analysis. Hence a recommendation for comprehensive research on the isolation and incorporation of these bioactive compounds in the formulation of organic consumables, and further testing of the formulated products to ascertain their potency .

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