Fatty acid profiles of *Phaseolus* species

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**Abstract**

The aim of this study was to identify and profile the fatty acids present in the *Phaseolus* species using an online Osiris server software. *Phaseolus* species (pinto beans, lima beans and kidney beans) were bought in King’s market, Akure, Ondo State, Nigeria and were air-dried and ground. The Association of Official Analytical Chemists protocol were used for proximate; mineral analysis was done using atomic absorption spectrophotometer; extraction of oil was done using Soxhlet apparatus and the extracts were characterized using gas chromatography mass spectrophotometer and identified compounds were screened for their chemical properties using online Osiris server. The oil extract for *pinto beans* revealed fatty acids in increasing order of percentage quality: Myristic acid, octadecenoic acid, stearic acid and palmitic acid. Identified fatty acids in kidney beans were in decreasing order of palmitic acid, linoleic acid, lauric acid, myristic acid and capric acid. Lima beans had highest palmitic acid and arachidic acid the lowest. However, from the results of all the *Phaseolus* species, linoleic acid was found only in kidney beans with quality of 11.87%. The identified fatty acids showed high toxicity properties and they exhibited negative drug-likeness. The chemistry of the identified compounds all showed that they exhibited various chemical properties. In conclusion, this study had revealed the presence of fatty acids in the selected food crops and their various chemical profiles have been discovered.

1. **Introduction**

The nutritional benefits of food crops are of immense benefits to humankind and it has been well documented the various physiological functions performs in the body by consumption of various classes of food ranging from leguminous, cereals and pulses, vegetable inclusive. Among the metabolites responsible for many health benefits are the fatty acids which can be classified as essential and non-essential fatty acids. Health benefits of both essential and non-essential fatty acids and their various presences in food crops have been enumerated. *Phaseolus* species have been researched and found to be nutritionally rich both in protein, crude fibre, crude fat and fatty acids such as linoleic acid, stearic acid and host of others. Graham and Ranalli (1997) has reported that there are over four hundred species of Beans world-wide. The lipid content of *Phaseolus vulgaris* L. is low but rich in proteins, vitamins, minerals and carbohydrate. (Graham and Ranalli, 1997; Shimelis and Rakshit, 2005; Costa et al., 2006; Anton et al., 2008; Montoya et al., 2008; Gathu and Njage, 2012). Arvanitoyannis et al. (2007) had reported that Nutritional quality is related to the composition of the bean and that beans can be a source of proteins, vitamins (thiamine, riboflavin, niacin, vitamin B6 and folic acid), dietary fibre (14-19%) (Particularly soluble fibre), minerals (Ca, Fe, Cu, Zn, P, K and Mg) and unsaturated fatty acids. Furthermore, Blair et al. (2013) and Jha et al. (2015) had reported that common beans provide minerals, fibre, thiamine, folate, and phytochemicals with analgesic and neuroprotective properties.

Moreover, *Phaseolus* species are attracting the attention of scientists as they are considered to be a nutraceutical food as a result of a variety of phytochemicals with potent health benefits and these include unsaturated fatty acids, trypsin inhibitors and many other beneficial compounds (Guzmán-Maldonado et al., 1998). Queiroz (2005) had reported the relevance of biological activities for *Phaseolus vulgaris* which include fibre and phytic acid from common beans like enhancement of the bifidogenic effect, antioxidant (Wu et al., 2004; Heimler et al., 2005), antimutagenic
(Cardador-Martínez, Castaño-Tostado, and Loarca-Piña 2002; Cardador-Martínez, Loarca-Piña, and Oomahn 2002). The objective of the study was to identify the fatty acids present in the different Phaseolus species, viz. pinto beans, lima beans, and kidney bean and profile the chemical properties of the identified compounds using Osiris online server.

2. Materials and methods

2.1 Sample collection

The varieties of Phaseolus species viz. pinto beans, lima beans, and kidney bean were purchased in a local market in Akure in Ondo State, Nigeria in April 2020.

2.2 Sample preparation

The dried samples were powdered by using a laboratory scale grinder (Sumeet CM/L 2128945) and sifted through 300 μm sieve to obtain the flour. The flour samples were sealed and packed in airtight containers for further analysis (Alves et al., 2002).

2.3 Extraction of oil from the flour

The conventional method of extraction involves the use of Soxhlet apparatus. This method of extraction was designed to extract lipid. Approximately, 1 g of moisture-free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Weighed, cleaned and dried receiving beaker was filled with petroleum ether and fitted into the apparatus. Water and heater were turned on to start extraction. After six siphoning, ether was allowed to evaporate and beaker disconnected before last siphoning. The extract was transferred into a clean glass dish with ether washing and the extract was concentrated using water bath. The dish was then placed in an oven at 105°C for 2 hrs and cooled in a desiccator.

2.4 Characterization of the oil extract

The analyses of the compounds in the active extracts were run on a GC–MS system (Agilent Varian GC: 4800/3000). The fused-silica MS capillary column (30 m 0.25 mm ID, film thickness of 0.25 mm) was directly coupled to an Agilent Varian. The oven temperature was programmed (35°C for 5 mins, then 35–300°C at 10°C/min) and subsequently, held isothermal for 20 mins. The injector port; was 250°C, the transfer line: 290°C, splitless. The volume injected: 0.2 mL and the column flow rate was 1 mL/min of 1 mg/mL solution (diluted in chloroform). The peaks of components in gas chromatography were subjected to mass-spectral analysis. The MS operate with an EI-source at –70 eV; the solvent delay was 9 min. Scan time 1.5s; acquisition rate 10 spectra/second; mass range 50–1000 amu; detector voltage 1800 V, and Ion source temperature: 250°C. Data were recorded in TIC mode. The software adopted to handle the mass spectra and chromatograms was Agilent chemstation software. The constituents were identified after comparing with available data in the GC–MS library in the literature. The GC–MS mass spectrum data were analysed using M nova 11.0.1 and the database of National Institute Standard and Technology (NIST) was used to interpret analysed data. Comparison of the mass spectrum of the unidentified components was carried out against the mass spectrum of already known components available in the NIST library. The name, molecular weight and peak area percentage of unknown compounds were evaluated by the software as observed from the chromatogram.

3. Results and discussion

Figure 1 shows the chromatogram of pinto beans and forty-seven peaks revealed the presence of various compounds at the different running time; each peak corresponds to the identified compound and its relative abundance. Table 1 shows four identified fatty acid compounds and their properties which include varying running time, molecular formula and peak numbers, the highest percentage quality was palmitic acid (9.90%) and the least fatty acid was myristic acid (0.12%) quality. Figure 2 shows the chromatogram of lima beans at running time of 29 mins on the x-axis and relative abundance in y-axis. Table 2 shows some of the identified compounds and their properties, among is palmitic acid with highest % quality of 16.70% and Arachidic acid having 0.34% quality.

Figure 1. Chromatogram of pinto beans

Figure 2. Chromatogram of lima beans
irritability effect. However, all the compounds had exhibited no toxicity such as mutagenic, tumorigenic and chromatograms, it is worthy to note that Linoleic acid properties of the identified fatty acids in the three compounds such as Palmitic acid (14.48%), Linoleic minutes and Table 3 reveals some of the identified were identified within the running time of twenty

| Peak No | Compound name  | Retention time | % quality | CAS             | Molecular formula   |
|---------|----------------|----------------|-----------|-----------------|---------------------|
| 11      | Myristic acid  | 10.867         | 0.38      | 544-63-8        | C16H32O2            |
| 20      | Palmitic acid  | 14.067         | 16.70     | 57-10-3         | C16H32O2            |
| 25      | Stearic acid   | 18.634         | 5.39      | 57-11-4         | C16H32O2            |
| 32      | Arachidic acid | 21.633         | 0.34      | 506-30-9        | C20H40O2            |

Table 2. Retention time, % quality, CAS and molecular formula of identified compounds in the chromatogram of lima beans

| Peak No | Compound name  | Retention time | % quality | CAS            | Molecular formula   |
|---------|----------------|----------------|-----------|----------------|---------------------|
| 2       | Capric acid    | 7.208          | 0.07      | 334-48-5       | C10H20O2           |
| 6       | Lauric acid    | 9.014          | 3.15      | 143-07-7       | C16H32O2           |
| 7       | Palmitic acid  | 13.966         | 14.48     | 57-10-3        | C16H32O2           |
| 8       | Myristic acid  | 10.881         | 1.86      | 544-63-8       | C18H36O2           |
| 14      | Linoleic acid  | 18.057         | 11.87     | 60-33-3        | C18H32O2           |
| 15      | Stearic acid   | 18.558         | 3.46      | 57-11-4        | C18H36O2           |

Table 3. Compound names and the properties identified chromatogram of kidney

| Peak No | Compound name | Retention time | % quality | CAS | Molecular formula |
|---------|---------------|----------------|-----------|-----|-------------------|
| 2       | Capric acid   | 7.208          | 0.07      | 334-48-5 | C10H20O2         |
| 6       | Lauric acid   | 9.014          | 3.15      | 143-07-7 | C16H32O2         |
| 7       | Palmitic acid | 13.966         | 14.48     | 57-10-3  | C16H32O2         |
| 8       | Myristic acid | 10.881         | 1.86      | 544-63-8 | C18H36O2         |
| 14      | Linoleic acid | 18.057         | 11.87     | 60-33-3  | C18H32O2         |
| 15      | Stearic acid  | 18.558         | 3.46      | 57-11-4  | C18H36O2         |

Table 4. Toxicological properties of the fatty acids

| Compound names | Drug likeness | Mutagenic | Tumorigenic | Irritability |
|----------------|---------------|-----------|-------------|--------------|
| Stearic acid   | -25.216       | High      | High        | High         |
| Myristic acid  | -25.216       | High      | None        | High         |
| Palmitic acid  | -25.216       | None      | High        | High         |
| Lauric acid    | -25.216       | High      | High        | High         |
| Linoleic acid  | -25.561       | None      | None        | None         |

Table 5. Chemical properties of the identified fatty acids

| Compound Names | Non-rotatable bond count | Meta atom count | Ring closure count | Rotatable bond count | Aromatic Ring count | Aromatic atom count | SP atom count | Aromatic atom count | Aromatic atom count | Aromatic atom count | Aromatic atom count | Aromatic atom count |
|----------------|--------------------------|-----------------|-------------------|---------------------|---------------------|---------------------|---------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Stearic acid   | 2                        | 0               | 16                | 0                   | 0                   | 0                   | 18            | 0                   | 0                   | 0                   | 0                   |
| Myristic acid  | 2                        | 0               | 16                | 0                   | 0                   | 0                   | 14            | 0                   | 0                   | 0                   | 0                   |
| Palmitic acid  | 2                        | 0               | 14                | 0                   | 0                   | 0                   | 16            | 0                   | 0                   | 0                   | 0                   |
| Lauric acid    | 2                        | 0               | 10                | 0                   | 0                   | 0                   | 12            | 0                   | 0                   | 0                   | 0                   |
| Linoleic acid  | 2                        | 0               | 14                | 0                   | 0                   | 0                   | 14            | 0                   | 0                   | 0                   | 0                   |

Source: Sander et al. (2015).

Furthermore, Figure 3 shows the chromatogram of kidney beans -Phaseolus vulgaris- and thirty-two peaks were identified within the running time of twenty-nine minutes and Table 3 reveals some of the identified compounds such as Palmitic acid (14.48%), Linoleic acid (11.87% quality).

Table 4 shows the drug-likeness and toxicological properties of the identified fatty acids in the three chromatograms, it is worthy to note that Linoleic acid exhibited no toxicity such as mutagenic, tumorigenic and irritability effect. However, all the compounds had negative drug-likeness, which is an indication that they are not pure drugs but can be drug components.

Figure 3. Chromatogram of kidney bean
4. Conclusion

The profiles of the identified fatty acids have been revealed; for pinto beans - palmitic acid: 9.90% quality, lima beans - palmitic acid: 16.70% quality, kidney beans - palmitic acid: 14.48% quality. It is worthy to mention that the fatty acids identified all showed negative values for drug likeness such as stearic acid (-25.216) and linoleic acid (-25.561) which is an indication that they are not drugs and also their toxicities have been evaluated. It is significant to note that the identified fatty acids all have the same electronegativity atom count which means that they possess the power to attract bonding pair of electrons and their Sp$^3$ varied.

Table 6. Classification of the identified fatty acids into saturated fatty acid, monounsaturated fatty acids (MUFA) contain one double bond and polyunsaturated fatty acids (PUFA) contain more than one double bond

| Compound name | Class of fatty acid |
|---------------|---------------------|
| Stearic acid  | saturated fatty acid |
| Myristic acid | saturated fatty acid |
| Palmitic acid | saturated fatty acid |
| Lauric acid   | saturated fatty acid |
| Linoleic acid | polyunsaturated fatty acids |
| Capric acid   | saturated fatty acid |
| Arachidic acid| polyunsaturated fatty acids |

The health benefits of linoleic acid had been well discussed in learned journals. Linoleic acid is said to be a principal essential fatty acid and gained the attention of nutritionists over many years. Whelan and Fritsche (2013), had reported the various health benefits of this fatty acid to include the source of energy, that it can be esterified to form neutral and polar lipids such as phospholipids, triacylglycerols, and cholesterol esters. Moreover, PUFA identified in the kidney beans was linoleic acid and its functions in the human body have been exemplified being an essential fatty acid which cannot be synthesized in the human system; hence it is supplemented through consumption of food crops rich in it (Whelan and Fritsche, 2013). In another development, Myristic acid was identified all the Phaseolus species and the pharmacological functions of this saturated fatty acid include improvement on long-chain omega-3 fatty acids stage in plasma phospholipids and have a positive impact on cardiovascular health parameters in humans (Dabadie et al., 2005). Also, myristic acid is directly involved in post-translational protein changes and mechanisms that control important metabolic processes in the human body (Legrand and Rioux, 2015; Ruiz-Núñez et al., 2016). Moreover, Lauric Acid has been found to possess a lot of health advantages such as in treating viral infections which includes swine flu, avian flu, fever blisters, cold sores, gonorrhea, chlamydia.

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