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GC-MS Compound Identification in *Phaseolus vulgaris* - A Low-Cost Cataract Prevention Food

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Abstract *Phaseolus vulgaris* is one of the most widely used traditional remedies for diabetes mellitus. Over a thousand medicinal plants have been found to contain anti-diabetic chemicals, the bulk of which have long been used in traditional medicine. The pharmacological significance of eating *Phaseolus vulgaris* in diabetics has been intensively explored in order to identify the several bioactive components responsible for their diabetes-controlling benefits. Researchers have previously linked the eating of P. vulgaris as a possible protective factor against cataract surgery based on their findings on the evaluation of beans and other vegetables. This study was driven by the need to find more bioactive chemicals that are active in the management of diabetes and the prevention of cataract, which is one of the disease's consequences. In Ado-Ekiti, Nigeria, Phaseolus vulgaris is a leguminous crop that is widely consumed. *Phaseolus vulgaris* was purchased from two different sources, pulverized with a Sumeet CM/L 2128945 grinder, and extracted with methanol and ethanol. The extracts were concentrated. The bioactive components in the extracts were identified using GC-MS. The ethanolic extract includes twelve bioactive compounds, while the methanolic extract contains ten. The ethanol extract contains neophytadiene, β-amyrin, and squalene, among other chemicals. Neophytadiene, 1,2-benzisothiazol-3-amine, TBDMS derivative, and 1-Methyl-3-phenylindole were found in methanol extract. Both extracts contain bioactive chemicals that have been shown to have anti-diabetic and cataract-prevention properties in humans. Finally, the discovery of these various beneficial components lends credence to scientific proof that *Phaseolus vulgaris* can be consumed by diabetics.

Keywords Extraction, GCMS, Bioactive Compounds, Diabetes

1. Introduction

For nearly a century, scientists have been intrigued by the prospect of discovering a blood glucose-lowering agent in the plant kingdom [1]. Scientific interest in the anti-diabetic potential of *Phaseolus vulgaris* pods has not waned and has even increased in the last decade. Recent research findings are inconsistent, as have previous investigations. Mexican researchers discovered significant antihyperglycemic effects of a *Phaseolus* preparation in 1991, [1,2] stated that Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by high blood glucose levels. Worryingly, it is spreading rapidly in Sub-Saharan Africa. According to the literature, the major risk factors for overweight are westernization, sedentary lifestyle, metabolic syndrome and nutritional transition [3-5]. Diabetic retinopathy (DR), cataract, glaucoma, macular edema, age-related macular degeneration (AMD), and optic nerve atrophy are the leading causes of blindness in diabetic patients. Red kidney beans (*Phaseolus vulgaris L*) are a popular legume crop in Nigeria and other parts of the world. This plant contains a variety of bioactive compounds as well as nutritional components like proteins, resistant starch, dietary fibre and fat [6-8]. Red kidney *Phaseolus vulgaris* have a red seed coat, which indicates that they may be a good source of polyphenols, as coloured beans are frequently found to contain polyphenols. Several previous studies have shown that these *Phaseolus vulgaris* contain anti-diabetic polyphenol compounds [9-14] and saponins [15,16]. Furthermore, scholars have reported on the beneficial effects of nutritious foods and *Phaseolus vulgaris* has been investigated as a reservoir of nutritious values, with protein contents ranging between 20-25 percent, complex carbohydrate (50-60 percent), and a good source of vitamins and minerals as reported by [17,18] and some folate and fibre contents [19]. There are about 20 leguminous species that are used in substantial amounts as dry grains for human nutrition. [20,21]. Grain legumes are commonly referred to as "poor man's meat" by [22], underlining their importance for consumption in third-world countries where protein energy deficit is a major worry for couples who have been together for quite some time [23]. Beans (*Phaseolus vulgaris*) are members of the Leguminosae, *Phaseoleae* and *Papilionoideae* subfamilies [24,25]. According to [26-27], *Phaseolus vulgaris* are domesticated and originated in America. According to [28], previous substances found with promise for decreasing lipid processes in *Phaseolus vulgaris* include -sitosterol, Stigmasterol, and Campesterol. Furthermore, according to [29], foods are currently being studied for additional physiological effects [29]. The goal of this study is to add to the existing knowledge about the bioactive chemicals found in *Phaseolus vulgaris*.

2. Methodology

Sample Collection and Preparation

The *Phaseolus vulgaris* were purchased on the 5th November, 2021 in two different locations in Ekiti state, Nigeria, the samples were powdered and extractions were performed by soaking 50 g of each sample in 300 ml of ethanol and methanol for five days, filtered, and the extracts were concentrated using a rotary evaporator at 35°C.

Characterization of the Crude Extract

The chemicals in the extracts were identified using a Turbo-Mass GC System with an Elite-5 capillary column (30 m long, 0.25 mm inner diameter, 0.25 m film thickness, and a maximum temperature of 350°C) couple with a Perkin Elmer Clarus 600C MS. A continuous flow rate of 1 mL/min of helium acted as the gas carrier. The injection, transfer, and ion source temperatures were all 280 degrees Celsius, and the ionization energy was 70 electron volts. From 40 °C (hold for 2 minutes) to 280 °C, the oven temperature was increased at a rate of 5 °C/min (hold for 10 min). The extracts were solubilized in ethyl acetate and filtered using a Corning 0.45 m syringe filter. A volume of 1 µL of crude extracts with a split ratio of 1:20 was injected. The data were collected by collecting mass spectra ranging from 50 to 550 m/z. Gas chromatography retention time and mass spectra that matched those of NIST library 2014 standards were used to identify chemical compounds found in the extracts.

3. Results

Figure 1 shows a chromatogram of ethanolic extract with twelve peaks identified within a time range of fourteen to twenty-seven minutes and at varying abundance as shown on the y-axis. The identities of the identified compounds with various properties such as peak number, retention time, percent quality, CAS number, and compound names were revealed in Table 1. It is worth noting that beta-amyrin had the highest percent quality of 24.146, followed by Hexadecanoic acid, methyl ester with 18.539 percent quality. The quality of Neophytadiene was 16.551 percent. The lowest quality was 1.316 percent for 1,2-Benzenediol,3,5-bis (1,1-dimethyl ethyl). Quantitatively, the ethanolic extract was highly rich in beta-Amyrin (241.46 mg/g), Neophytadiene (196.68 mg/g) at retention times of 13.472 and 16.242 minutes, Hexadecanoic acid, methyl ester (185.39 mg/g).

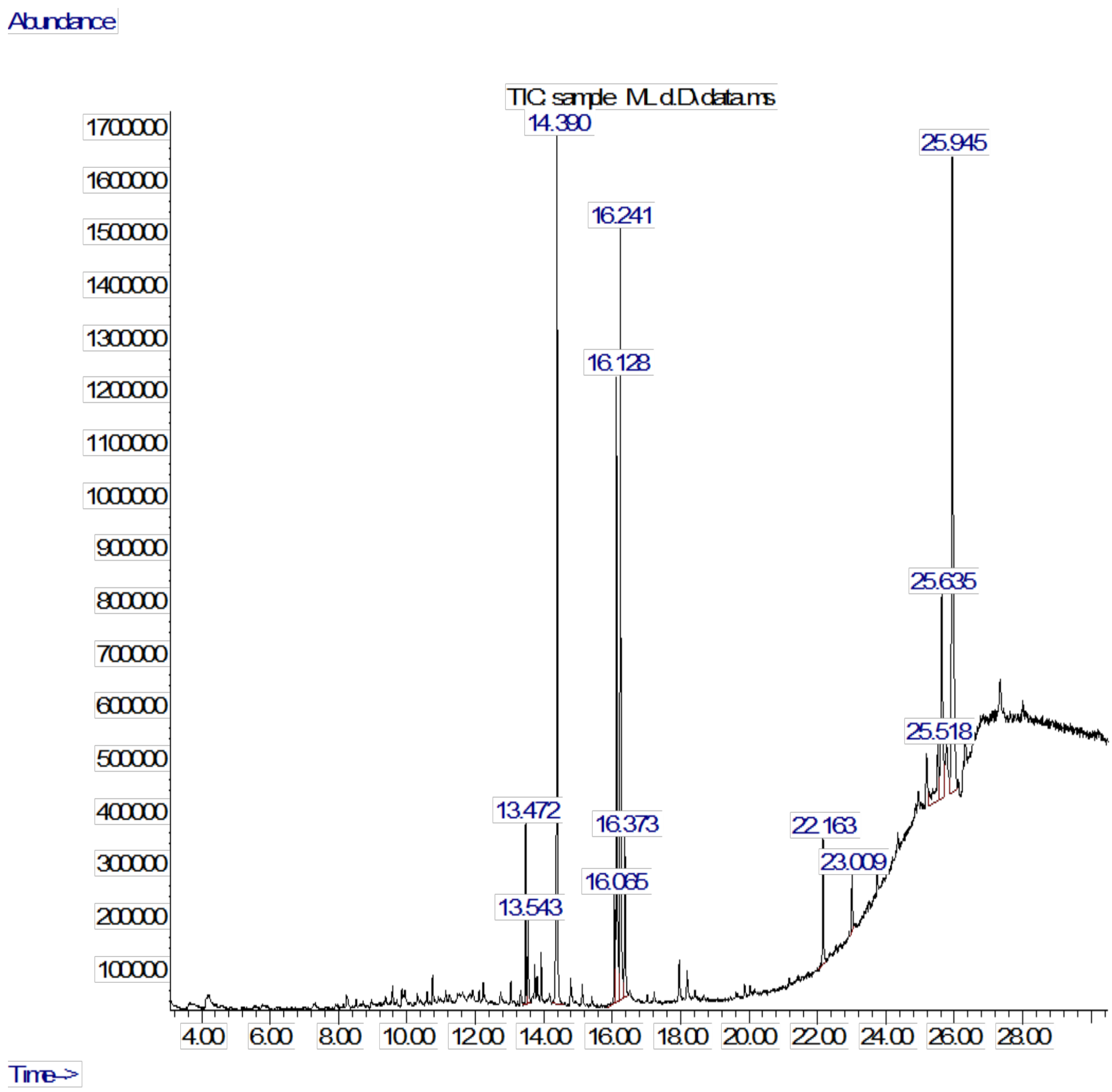

Figure 1. Chromatogram of ethanolic crude extract of *Phaseolus vulgaris* sample (A)

Table 1. Detected peaks in the chromatogram of *Phaseolus vulgaris* ethanolic crude extract

Peak Number	Retention Times (minutes)	Compound name	CAS NO	quality (%)	Quantity (mg/g)
1	13.472	Neophytadiene	138502 000504-96-1	3.117	31.17
2	13.541	2-Pentadecanone, 6,10,14-trimethyl	128826 000502-69-2	1.697	16.97
3	14.388	Hexadecanoic acid, methyl ester	130813 000112-39-0	18.539	185.39
4	16.064	8,11-Octadecadienoic acid, methyl ester	153872 056599-58-7	2.261	22.61
5	16.127	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	152040 000301-00-8	14.106	141.06
6	16.242	Neophytadiene	138502 000504-96-1	16.551	165.51
7	16.373	Methyl stearate	157884 000112-61-8	3.748	37.48
8	22.164	Squalene	243216 000111-02-4	2.833	28.33
9	23.011	1,2-Benzenediol,3,5-bis(1,1-dimet hylethyl)-	85510 001020-31-1	1.316	13.16
10	25.517	Cyclotrisiloxane, hexamethyl-	85992 000541-05-9	3.524	35.24
11	25.637	Tris(tert-butyldimethylsilyloxy)arsane	260810 1000366-57-5	8.162	81.62
12	25.946	beta-Amyrin	249544 000559-70-6	24.146	241.46

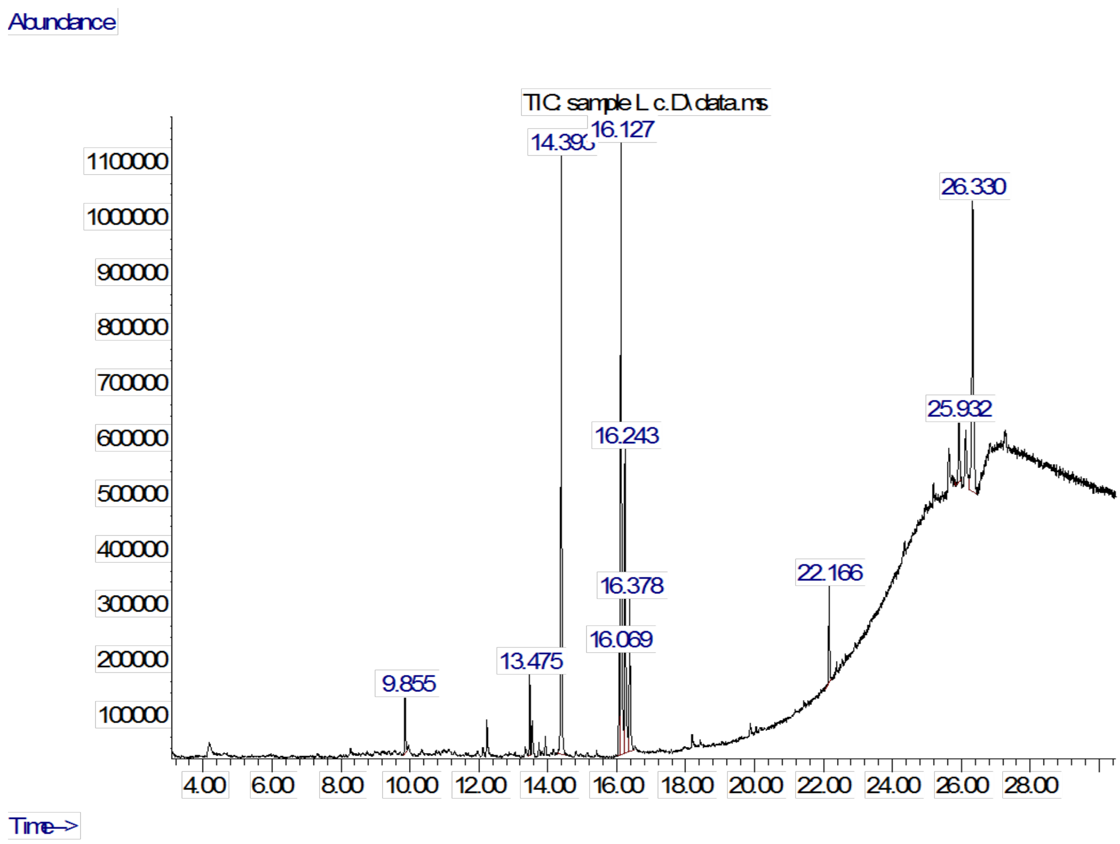

Figure 2. Chromatogram of methanolic crude extract *Phaseolus vulgaris* sample (B)

Table 2. Peaks on the chromatogram of a methanolic crude extract of *Phaseolus vulgaris* were detected, indicating the existence of bioactive chemicals with various pharmacological roles in the human body system, such as Neophytadiene

Peak Number	Retention Times (minutes)	Compound name	CAS NO	Quality (%)	Quantity (mg/g)
1	9.856	Dodecanoic acid, methyl ester	78067 000111-82-0	2.291	22.91
2	13.478	Bicyclo[3.1.1]heptane, 2,6,6-trime thyl-, (1.alpha.,2.beta.,5.alpha.)	17424 006876-13-7	2.343	23.43
3	14.394	Hexadecanoic acid, methyl ester	130818 000112-39-0	21.682	216.82
4	16.070	10,13-Octadecadienoic acid, methyl ester	153881 056554-62-2	3.344	33.44
5	16.127	9-Octadecanoic acid, methyl ester	155721 002462-84-2	24.985	249.85
6	16.242	Neophytadiene	138502 000504-96-1	13.011	130.11
7	16.379	Heptadecanoic acid, 16-methyl-, methyl ester	157951 005129-61-3	6.641	66.41
8	22.164	1,2-Benzisothiazol-3-amine, TBDMS derivative	124363 1000332-57-2	3.506	35.06
9	25.935	1,4-Phthalazinedione, 2,3-dihydro- 6-nitro	71758 003682-19-7	3.419	34.19
10	26.330	1-Methyl-3-phenylindole	71645 030020-98-5	18.778	187.78

Figure 2 depicted a chromatogram of methanolic extract, with ten distinct peaks on the x-axis ranging from ten minutes to less than twenty-eight minutes and abundance on the y-axis. The identities of the peaks numbers were presented in Table 2, which included the compound names, retention time, CAS number, and percent quality. The highest quality compound was 9-octadecenoic acid, methyl ester at 24.985 percent, followed by Hexadecanoic acid at 21.682 percent, Neophytadiene at 13.011 percent, and Dodecanoic acid, methyl ester at 2.291 percent.

4. Discussion

Phaseolus vulgaris, a leguminous plant, is a major recommended food for diabetic patients with type 2 diabetes. Diabetic patients have traditionally been advised to eat more beans than other carbohydrate foods, which could be due to the presence of various bioactive compounds discovered in the extracts. The presence of numerous components with varied retention durations was confirmed by the GC-MS spectrum, as shown in [Figure 1 and 2]. The mass spectrometer examines the molecules eluted at various periods to determine their type and structure. The big compound splits into little compounds, causing peaks with varying m/z ratios to appear. These mass spectra are the compound's fingerprint, which can be identified using the data library [30]. Table 1 shows the chromatograms of twelve compounds discovered in the ethanolic crude extract of *Phaseolus vulgaris* sample (A) and ten compounds detected in the methanolic extract of *Phaseolus vulgaris* sample (B). These chemicals have previously been discovered to exhibit a variety of pharmacological properties. Neophytadiene found in sample A (196.68 mg/g) and B extracts having (249.85 mg/g) has been found to have various medicinal properties, including an antidiabetic enzyme inhibitor as reported by [31], good analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compound as discussed by [32]**.** According to researchers, beta-Amyrin identified in the ethanolic extract of sample A has previously been discovered to have antidiabetic, anti-inflammatory, and anti-cancer characteristics [33], the extract was highly rich in the compound having 241.46 mg/g. The strong antimicrobial, antidiabetic, and anti-inflammatory activities of Haxadecanoic acid found in the extracts of the sample A have been reported by [34-36] and it is worth mentioning that the ethanolic extract is highly rich quantitively having 185.39 mg/g. 1-Methyl-3-phenylindole, a derivative of indole, has been found to have many degrees of biological usefulness such as anti-Alzheimer, these pharmacological properties have attracted the attention of scholars to the synthesis of indole derivatives, as reported by [37-40]. According to [41], octadecanoic acid (33.44 mg/g), which was discovered in the methanolic extract of sample B, exhibits anti-diabetic properties as alpha-glucosidase inhibitors. The TBDMS derivative 1,2-benzisothiazol-3-amine, which was also detected in the methanolic extract of sample B, was discovered to have many pharmacological activities [42]. Bean dietary supplements have been shown to be a low-cost preventative strategy for cataracts and other visual comorbidities linked with type 2 diabetes mellitus [43]. *Phaseolus vulgaris* has negligible fat and a little amount of saturated fat, as well as phytate and phenolic components (which work in the same way as glucose-lowering alpha-glucosidase or alpha-amylase inhibitors, such as metformin and acarbose) [44-49]. The absolute risk of cataract was observed to be greatly lowered with frequent intake of *P. vulgaris* (beans) among 500 type 2 diabetic individuals in Kinshasa, Africa as reported by [50]. In conjunction with prior studies on the high antioxidant content of beans, the effectiveness of bean consumption among diabetic patients has been proven to be worthy as a therapeutic tool for cataract prevention. Previously, *P. vulgaris'* antioxidant activity has been proven to benefit in blood glucose regulation in previous epidemiologic studies [51]. Furthermore, [51] indicated that only beans were independent and significantly possible protective factors against cataract surgery, based on their findings on the evaluation of beans and other vegetables, and advocated the intake of beans [52] and [53] had identified amyrin as anticataract agent.

5. Conclusions

Finally, because of its endowered bioactive compounds identified, the *P. vulgaris* may be a cost-effective dietary supplement to prevent or stop cataract formation in diabetic individuals.

Recommendation

However, further well-designed study is recommended to determine the amount (mg/kg) per body weight required for consumption, which could lead to new horizons in cataract pharmacology with fewer side effects and lower costs.

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