Journal of Advances in Science and Engineering 8 (2023) 1 – 17

Journal of Advances in Science and Engineering (JASE)

Journal homepage: www.sciengtexopen.org/index.php/jase

# Comparative assessment of the phytochemicals of the leaves and seeds of pigeon pea *(Cajanus cajan (L.) Huth)* plant

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ARTICLE INFO

*Article history:*
Received 22 October 2022

Received in revised form
7 January 2023

Accepted 28 January 2023

Available online
31 January 2023

*Keywords:*
Comparative assessment
Leaves
Phytochemicals
Pigeon pea
Seeds

ABSTRACT

The pigeon pea plant is one of the plants cultivated in the semi-arid tropics; it is widely cultivated in Nigeria. It is utilized as food and serves medicinal purposes. This study was carried out with the aim of assessing the phytochemicals. Pigeon pea leaves and seeds were purchased from farmers in Uromi, Edo state, and Rumugbo phase-2, Obia Akpor Local government area of Port Harcourt, Nigeria. The phytochemical components of both the fresh and dried leaves and seeds were determined using the standard method and the results obtained from this study showed that in both the fresh and dried leaves of pigeon peas alkaloids, flavonoids, terpenoids, steroids, phytosteroids, saponins, tannins, phenol, anthraquinones, quinones, xanthoproteins, and phlobatannin were detected while the acidic compound was absent. While in the fresh and dried seeds of pigeon pea, results obtained revealed that alkaloids, flavonoids, terpenoids, steroids, phytosteroids, saponins, anthraquinones, and quinones were present while tannins, phenol, xanthoproteins, phlobatannin, and acidic compound were absent. Phytochemical compounds of the ethanolic extract of the fresh and dried leaves and seeds of pigeon peas were determined using Gas Chromatography - Mass Spectroscopy and a total of 30, 13, 32, and 38 bioactive constituents were detected in the fresh leaves, dried leaves, fresh seeds, and dried seeds respectively using their retention times (RT) and mass-to-charge ratios (m/z).

## 1. Introduction

The pigeon pea plant belongs to the kingdom Plantae, phylum Magnoliopsida, Class Magnoliopsida, Subclass Rosidae, Order Fabales, Family Fabaceae, Sub Family Faboideae, Genus *Cajanus* and has its species as *cajan* [1]. The name pigeon pea (*Cajanus cajan*) was coined in Barbados where the pigeon pea seeds were used as feeds for pigeons. It is a legume crop rich in protein that is mainly cultivated in the semi-arid and sub-tropical regions of the world. It is the sixth most cultivated crop in global pulse production after beans, chickpeas, broad beans, and lentils and it is the third most cultivated crop in Asia [2]. The pigeon pea is a woody perennial shrub, which can adapt to different types of soil, rainfall, and adverse temperature. It is a deep-rooted plant that can grow up to 2 m in the soil; it can reach up to a height of 4 m and can fix nitrogen to the soil through a symbiotic association with Bradyrhizobium species thereby improving the soil's fertility. Its leaves are spirally arranged, pinnately trifoliate and lanceolate to oblong in shape. The stem branching pattern may vary from bush type. Pigeon pea flowers are usually yellow in colour with some variation; the flowers are zygomorphic, borne on a terminal or auxiliary racemes. The fruit of pigeon pea seeds varies in colours, it may be in shades of white, brown, or red and may be round or lens shape. Pigeon pea seed has been shown to provide essential amino acids such as lysine, tyrosine and arginine whereas cysteine and methionine are relatively low and the main stored protein is globulin protein [3]. Pigeon pea is enriched with 20–22% proteins and amino acids [4]. Pigeon pea dried seeds can be stored for a long period of time over 6 months, which makes it an important replacement for other food and serves as an alternative food during the shortage of cowpea and maize during the lean season which is May to June [5].

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https://doi.org/10.37121/jase.v8i1.205

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Pigeon pea in Nigeria is one of the minor crops cultivated by subsistence and income-generating farmers and majorly for household consumption unlike other plant products such as maize, cassava, cowpea, yam, sorghum, rice, and oil palm which are mainly cultivated for commercial purposes. The dried seeds of pigeon peas are boiled with groundnut oil or palm oil and consumed with garri or with maize flour (pap) oil [6]. Research [2] stated the importance of using the by-products of pigeon plant seeds as a source of producing edible food rich in protein. The pigeon pea seeds have been used with cassava flour to produce protein-rich products in bakeries and pigeon pea flour can be added to cereals to produce products like biscuits, cookies, and bread in order to increase their nutritional value [7]. In India pigeon pea is one of the largest imported and produced crops; the fresh seeds are used as green peas in a salad or eaten as vegetables. Pigeon pea leaves can be used as a cover crop to compete with weeds, it can also serve as green manure to replenish soil nutrients and be used as forage rich in protein for livestock. The woody stems of pigeon peas are used to make thatched roofs, baskets, and charcoal, and the decoctions from the leaves and stem are used to treat sore throat, cough, intestinal worms, skin irritations, and sores [9].

Pigeon peas stems and leaves have been reportedly used for the treatment of malaria, dizziness, measles, and eye infection, and this is based on different ethnical groups and localities. The juice from pigeon peas has been used for the treatment of various skin diseases and even mouth diseases in the past. This reveals the potential use of this plant in skincare research and development and has made it attractive in cosmetic applications [9]. Pigeon pea plant can be used medically or industrially beside been used as food [10]. It has also been reportedly used as an antioxidant, antistatic, antibacterial, anticancer, and antitumor [11]. The pigeon pea plant has been reportedly used as a medicine that heals wounds and sore, serves as an astringent, a medicine that cures diseases of the lungs and chest and it works as an antihelminthic that expels internal worms [12]. Consequently, research [6] has described the various ways in which the pigeon pea plant can be used for the treatment of various ailments as follows:

- *Malaria* –Pulverized leaves of a pigeon pea plant are mixed with either lemon juice or citronella leaves or both have been used. In some cases, a decoction of pigeon pea leaves and acacia leaves has been used for the treatment of malaria.
- *Ulcer* - Decoction of the leaves has also been used in the treatment of ulcers.
- *Measles* (children) - Pulverized leaves are mixed with water and consumed or used in bathing the infected child.
- *Fever* - Pulverized fresh leaves are mixed with water, filtered, and added to the vine or fermented water, this is consumed or used as bath water.
- *Snakebite* - Pulverized fresh leaves are applied to the affected site.
- *Eye infections* - Pulverized fresh leaves are mixed with water, filtered and the filtrate is dropped in the affected eye.
- *Dizziness* – Pulverized fresh leaves are mixed with water, filtered, and consumed.

Fewer research works have been actively carried out on the identification of secondary metabolites of the leaves and the seeds of pigeon peas and are underutilized in Nigeria probably due to the lack of knowledge of their nutritional value, hence the need to conduct this study.

## 2. Materials and Methods

Pigeon pea leaves and seeds were both purchased from farmers from Rumugbo phase-2, Obia Akpor LGA, Port Harcourt and Uromi LGA Edo state. All samples were screened, washed and transported to the Laboratory, Biochemistry, Kaduna State University for analysis. The authenticity of the seeds and leaves was carried out in the Biology Department, at Kaduna State University.

### *2.1. Preparation of Leaves and Seeds*

Fresh leaves were separated from the plant and rinsed thoroughly in clean running tap water. All the washed leaves were dried in the laboratory oven at 60 ℃ overnight, while the dried leaves were additionally air dried. Using an Electric Blender, both leaves were then ground into a fine powder. The powder was sieved, each separately, through a 250 µm mesh sieve to ensure homogeneity after which each was stored in different labelled air-tight containers before analysis.

The fresh seeds were carefully screened to remove all dirty and bad ones. Samples were dried in the laboratory oven at 60 ℃ overnight, the dried seeds were also screened to remove dirties and bad ones and were then ground respectively into a fine powder using an Electric Blender. The powder was sieved through a 250 µm mesh sieve to ensure homogeneity after which each was stored in air-tight containers before analysis.

### *2.2. Preparation of Aqueous Extract*

Fresh and dried ground plant leaves and seeds were extracted using distilled water. The aqueous extract was prepared for three days by soaking 20 g of the sample respectively in 450 ml of distilled water.

The solution was filtered using Whatman No. 1 filter paper; the filtrates were concentrated by evaporation at a low temperature (45℃) using a water bath. The concentrated extracts were stored in air-tight bottles in the refrigerator until when needed [13].

### *2.3. Preparation of Ethanolic Extract*

Ethanolic extract of leaves and seeds both fresh and dried samples were prepared by macerating 80 g of the sample respectively with absolute ethanol (450 ml) for 24 h. Thereafter each solution was filtered separately using Whatman No. 1 filter paper. Using a water bath, the filtrates were concentrated by evaporation at a low temperature (45 ℃). The concentrated extracts were stored in air-tight bottles in the refrigerator until when needed [13].

### *2.4. Experimental Design*

#### *2.4.1 Preliminary Phytochemical Analysis*

Phytochemical analysis was carried out using the method described by [14]. The pulverized samples were analysed in order to determine secondary metabolites. Three millilitres (3 ml) of each sample was measured into each test tube and preliminary screenings of samples were carried out for the following phytochemical components according to standard methods [14]. Alkaloids, Phytosteroids, Steroids, Phenol, Tannin, Terpenoid, Xanthroprotein, Saponins, Flavonoids, Anthraquinone, Quinone, Phlobatanin, Acidic compound.

*Alkaloids (Meyer's Test)* - Three millilitres (3 ml) of Meyer's reagent was added to 3 ml of the extract (aqueous and ethanolic). A pale precipitate indicates the presence of Alkaloids.

*Steroids/Phytosteroids (Lieberman Burchard Reaction)* - To 3 ml of extract (aqueous and ethanolic), 1 ml of chloroform and a few drops of Concentrated Sulphuric acid were added inside the test tube; a reddish-brown coloured precipitate was observed at the bottom of the test tube which indicates the presence of Steroid/Pytosteroids

*Phenol and Tannin (Ferric Chloride Test)* - Few drops of 10 % Ferric Chloride were added to 3 ml of extracts (aqueous and ethanolic), and a blue or green colour precipitate indicates the presence of Phenol and Tannin

*Terpenoids (Salkowski Test)* - To 3 ml of extract (aqueous and ethanolic), 1 ml of chloroform, and a few drops of Conc. Sulphuric acid was carefully added inside the tube, and a reddish-brown coloured precipitate indicates the presence of Terpenoid

*Flavonoids* - To 3 ml of the extract (aqueous and ethanolic), 1 ml of 10 % ammonia, and 1 ml of concentrated. sulphuric acid was added. The disappearance of the yellow colour indicates the presence of Flavonoids [14].

*Anthraquinones (Borntrager's Test)* - 1 ml of Benzene and 1 ml of 10 % ammonia were added to 3 ml of extract (aqueous and ethanolic). The formation of pink, red or violet colour in the lower phase of ammonia indicates the presence of Anthraquinones.

*Quinones* - To 3 ml of the extract (aqueous and ethanolic), 1 ml of concentrated sulphuric acid was added. A red-to-blue colouration indicates the presence of Quinones.

*Saponins (Froth Test)* - Three millilitres (3 ml) of extract (aqueous and ethanolic), was added to 2 ml of distilled water in a test tube. The solution was vigorously shaken and observed for stable froth persistence.

*Acidic compounds* - One pinch of sodium bicarbonate was added to 3 ml of extract (aqueous and ethanolic). Effervescence indicates the presence of acidic compounds

*Phlobatanin* - Three millilitres (3 ml) of the extract (aqueous and ethanolic), was boiled with diluted HCl, deposition of a reddish precipitate indicates the presence of phlobatannins

*Xanthroprotein* - To 3 ml of the extract (aqueous and ethanolic) add a few drops of nitric acid and ammonia were added. A reddish-brown precipitate indicates the presence of xanthoproteins

#### 2.4.2 *Determination of Phytochemical Compounds using Gas Chromatography-Mass Spectroscopy (GC-MS)*

The phytochemical compounds of the ethanolic extract of the fresh and dried leaves and seeds of pigeon peas were determined using GC-MS analysis. This was performed using a GC-MS of Hewlettt-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with Agilent 7890A/5975 C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d.) with Helium (He) was used as the carrier gas with a split ratio of 1:5. Oven temperature was 80 ℃ (2 min) to 280 ℃ at 1-40 ℃/min, detector temperature 250-280 ℃, and carrier gas He (0.9 ml/min). 2.0 µl of respective diluted samples were manually injected in the splitless mode, with a split ratio and with a mass scan of 50-600 amu. The total running time of GC-MS is 40 min; the relative percentage of each extract constituent was expressed as a percentage with peak area normalization [15]. Interpretation on the mass spectrum of GC-MS was carried out using the database of the National Institute of Standards and Technology (NIST) and Wiley library for mass spectra, having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight (MW), and structure of the components of the test materials were ascertained [15]. In Fig. 1, the map showing the study area is presented.

**Table 1.** Phytochemical compounds detected from the aqueous extract of the leaves and seeds of the pigeon pea (*Cajanus Cajan*) plant.

| Phytochemicals | Fresh leaves | Dried Leaves | Fresh seeds | Dried Seeds |
|---|---|---|---|---|
| Alkaloids | + | + | + | + |
| Flavonoids | + | + | + | + |
| Terpenoids | + | + | + | + |
| Steroids | + | + | + | + |
| Phytosteroids | + | + | + | + |
| Saponins | + | + | + | + |
| Tannins | + | + | - | - |
| Phenol | + | + | - | - |
| Anthraquinones | + | + | + | + |
| Quinones | + | + | + | + |
| Xanthoproteins | + | + | - | - |
| Phlobatannin | + | + | - | - |
| Acidic compound | - | - | - | - |

Key: "+" = Present; "-" = Absent

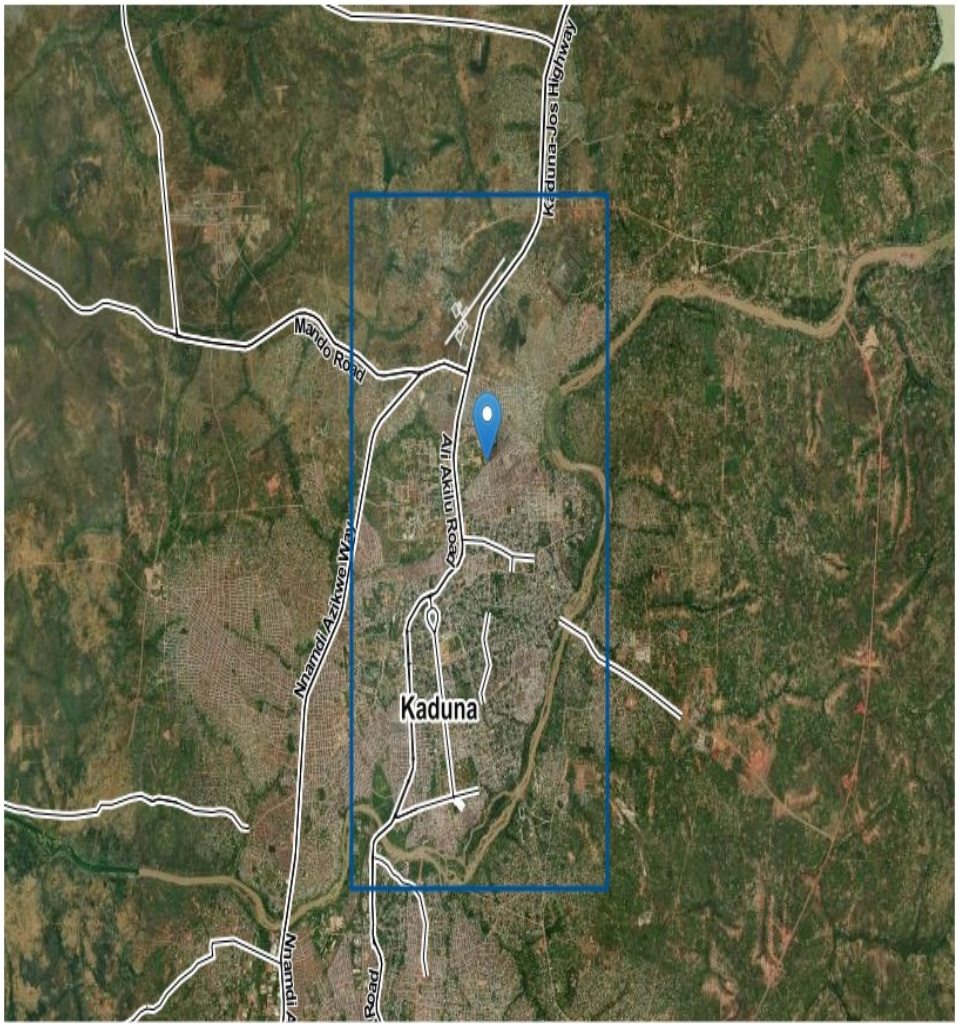


**Fig. 1** Map of Kaduna North Local Government Area.

## 3. Results

The results of the phytochemical compounds detected from the aqueous and ethanolic extracts of the leaves and seeds of the pigeon pea (*Cajanus Cajan*) plant are presented in Tables 1 – 2 whereas the phytochemical constituents identified from ethanolic extracts of the fresh and dried leaves and seeds of pigeon pea plant using GC-MS are shown in Tables 3 – 6.

**Table 2.** Phytochemical compounds detected from the ethanolic extract of the leaves and seeds of the pigeon pea (*Cajanus Cajan*) plant.

| Phytochemicals | Fresh leaves | Dried Leaves | Fresh seeds | Dried Seeds |
|---|---|---|---|---|
| Alkaloids | + | + | + | + |
| Flavonoids | + | + | + | + |
| Terpenoids | + | + | + | + |
| Steroids | + | + | + | + |
| Phytosteroids | + | + | + | + |
| Saponins | + | + | + | + |
| Tannins | + | + | - | - |
| Phenol | + | + | - | - |
| Anthraquinones | + | + | + | + |
| Quinones | + | + | + | + |
| Xanthoproteins | + | + | - | - |
| Phlobatannin | + | + | - | - |
| Acidic compound | - | - | - | - |

Key: "+" = Present; "-" = Absent

**Table 3.** Phytochemical constituents identified from ethanolic extracts of the fresh leaves of pigeon pea plant GC-MS.

| S/no | Retention time (min) | Peak area (%) | Compound | Molecular weight | Molecular formula |
|---|---|---|---|---|---|
| 1 | 8.386 | 0.42 | Phenol | 94.11g/mol, | $C_6H_6O$ |
| 2 | 18.5136 | 1.5959 | Benzenepropanol | 150.22 g/mol | $C_{10}H_{14}O$ |
| 3 | 28.7249 | 0.4769 | Heptacosane | 380.74 g/mol | $C_{27}H_{56}$ |
| 4 | 30.1891 | 0.7068 | Octadecane | 254.494 g/mol | $C_{18}H_{38}$ |
| 5 | 31.2619 | 0.4459 | Tridecane | 184.37 g/mol | $C_{13}H_{28}$ |
| 6 | 31.3405 | 26.5121 | Hexadecanoic acid (Methyl palmitate) | 270.45 g/mol | $C_{17}H_{34}O_2$ |
| 7 | 31.9318 | 0.992 | Hexadecanoic acid (Ethyl palmitate) | 284.5 g/mol | $C_{18}H_{36}O_2$ |
| 8 | 32.1108 | 0.6246 | Methoxyacetic acid, 2-tetradecyl ester | 286.4 g/mol | $C_{17}H_{34}O_3$ |
| 9 | 32.6447 | 3.6219 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 294.4721 | $C_{19}H_{34}O_2$ |
| 10 | 32.7069 | 32.8484 | 13-Octadecenoic acid, methyl ester | 296.5 g/mol | $C_{19}H_{36}O_2$ |
| 11 | 32.8036 | 1.0078 | Methylguanidine | 73.1 g/mol | $C_2H_7N_3$ |
| 12 | 32.8379 | 1.5057 | Phytol | 128.1705 g/mol | $C_{20}H_{40}O$ |
| 13 | 32.9023 | 10.169 | Methyl stearate | 298.50 | $CH_3(CH_2)_{16}CO_2CH_3$ |
| 14 | 33.5347 | 0.5179 | Eicosane | 282.5475 g/mol | $C_{20}H_{4200}$ |
| 15 | 33.9137 | 1.0552 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13, 15, 15-hexadecamethyl- | 577.2g/mol, | $C_{16}H_{48}O_7Si_8$, |
| 16 | 33.9694 | 1.2998 | Methyl(3oxodecahydroquinoxalin-2-yl) acetate | 226.27 g/mol | $C_{11}H_{18}N_2O_3$ |
| 17 | 34.2625 | 0.6973 | Octadecane,3-ethyl-5-(2-ethylbutyl)- | 366.7 g/mol | $C_{26}H_{54}$ |
| 20 | 34.3458 | 0.9725 | Eicosanoic acid, methyl ester | 326.5570 | $C_{21}H_{42}O_2$ |
| 21 | 34.413 | 0.82 | 2-Mercaptoethanol | 78.13 g/mol | $C_2H_6OS$ |
| 22 | 34.7366 | 1.3193 | 5-Methyl-2-phenyl-1H-indole | 207.27 g/mol | $C_{15}H_{13}N$ |
| 24 | 35.054 | 0.6531 | Di-n-decylsulfone | 346.61100g/mol | $C_{20}H_{42}O_2S$ |
| 25 | 35.1728 | 0.4201 | Decamethyltetrasiloxane | 310.68 g/mol | $C_{10}H_{30}O_3Si_4$ |
| 27 | 35.9595 | 0.9615 | 6,6-Diethylhoctadecane | 310.6 g/mol | $C_{22}H_{46}$ |
| 28 | 36.1143 | 3.5655 | Bis(2-ethylhexyl) phthalate | 390.564 g/mol | $C_{24}H_{38}O_4$ |
| 29 | 36.8479 | 3.1597 | Quinoline-2-carboxylic acid | 173.17 g/mol | $C_{10}H_7NO_2$ |
| 30 | 37.0303 | 0.707 | Methyl1-nitro-9,10-dioxoanthracene-2-carboxylate | 311.24 g/mol | $C_{16}H_9NO_6$ |

**Table 4.** Phytochemical constituents identified from ethanolic extracts of the dried leaves of pigeon pea plant using GC-MS.

| S/ no | Retention time (min) | Peak area (%) | Compound | Molecular weight | Molecular formula |
|---|---|---|---|---|---|
| 1 | 8.3785 | 2.4841 | Phenol, Phosphonic acid | 192.11g/mol | $C_6H_9O_5P$ |
| 2 | 18.5167 | 9.4844 | Phenol, 4-cyclohexyl-phenol | 176.25g/mol | $C_{12}H_{16}O$ |
| 3 | 31.337 | 8.8989 | Hexadecanoic acid (Palmitic Acid) | 256.4 g/mol | $C_{16}H_{32}O_2$ |
| 4 | 32.6453 | 3.6046 | 9,12-Octadecadienoic acid, | 280.4 g/mol | $C_{18}H_{32}O_2$ |
| 5 | 32.6707 | 1.3029 | [1,2,4] Triazolo [1,5-a] pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester | 207.19 g/mol | $C_8H_9N_5O_2$ |
| 6 | 32.7058 | 9.9878 | 9-Octadecenoic acid, methyl ester, (E)- | 282.47 g/mol | $C_{18}H_{34}O_2$ |
| 7 | 32.8385 | 2.7986 | 1,2-Bis(trimethylsilyl)benzene | 222.47 g/mol | $C_{12}H_{22}Si_2$ |
| 8 | 32.9015 | 4.3599 | Heptadecanoic acid (Margarinic acid) | 270.5 g/mol | $C_{17}H_{34}O_2$ |
| 9 | 33.1279 | 2.4966 | Hexanoic acid (Caproic acid) | 116.1583 g/mol | $C_6H_{12}O_2$ |
| 10 | 33.1845 | 4.7137 | (t-Butyl-dimethylsilyl)[2-methyl-2-(4-methyl-pent-3-enyl)-cyclopropyl]-methanol | 282.5 g/mol | $C_{17}H_{34}OSi$ |
| 11 | 34.8359 | 3.2141 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl- | 577.2g/mol | $C_{16}H_{48}O_7Si_8$ |
| 12 | 36.1176 | 14.9381 | Bis (2-ethylhexyl) phthalate | 390.564g/mol | $C_{24}H_{38}O_4$ |
| 13 | 36.8443 | 9.1187 | Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl- | 207.23g/mol | $C_{11}H_{13}NO_3$ |

**Table 5.** Phytochemical constituents identified from ethanolic extracts of the fresh seeds of the pigeon pea plant using GC-MS.

| S/no | Retention time (min) | Peak area (%) | Compound | Molecular weight | Molecular formula |
|---|---|---|---|---|---|
| 1 | 18.507 | 0.1634 | Benzene, 1-isocyanato-4-methyl- | 78.11g/mol | $C_6H_6$ |
| 2 | 31.1082 | 0.4723 | (Z)-9-Hexadecenoic acid, methyl ester | 254.41 g/mol | $C_{16}H_{30}O_2$ |
| 4 | 31.4318 | 0.113 | Myristic acid | 228.37 g/mol | $C_{14}H_{28}O_2$ |
| 5 | 31.6665 | 0.3619 | Undecanoic acid | 186.29 g/mol | $C_{11}H_{22}O_2$ |
| 8 | 31.9309 | 0.9238 | Ethyl palmitate | 284.5 g/mol | $C_{18}H_{36}O_2$ |
| 9 | 32.1795 | 0.1988 | Hexadecanehydrazide (Palmitic Acid Hydrazide) | 270.45 g/mol | $C_{16}H_{34}N_2O$ |
| 11 | 32.5014 | 0.1851 | Oleic Acid | 282.5 g/mol | $C_{18}H_{34}O_2$ |
| 13 | 32.6538 | 27.7527 | 9,12-Octadecadienoic acid | 280.4 g/mol | $C_{18}H_{32}O_2$ |
| 14 | 32.7176 | 43.291 | 13-Octadecenoic acid, methyl ester | 296.5 g/mol | $C_{19}H_{36}O_2$ |
| 15 | 32.9052 | 6.6418 | Methyl stearate | 298.5 g/mol | $C_{19}H_{38}O_2$ |
| 16 | 32.9758 | 2.682 | Vaccenic acid | 282.5 g/mol | $C_{18}H34O_2$ |
| 17 | 33.1252 | 0.8089 | Ethyl linoleate | 308.5 g/mol | $C_{20}H_{36}O_2$ |
| 18 | 33.1813 | 0.6029 | Ethyl Oleate | 310.5 g/mol | $C_{20}H_{38}O_2$ |
| 19 | 33.3762 | 0.1613 | Pentanoic acid (ethyl esteEthyl valerater) | 130.18 g/mol | $C_7H_{14}O_2$ |
| 20 | 33.6085 | 0.1384 | Squalene | 410.73 g/mol | $C_{30}H_{50}$ |
| 21 | 33.9157 | 0.1027 | Diethyl 3-chloro-2-hydroxypropylmalonate | 252.69198 g/mol | $C_{10}H_{17}ClO_5$ |
| 22 | 33.9601 | 0.1889 | N-Benzylidene-m-nitroaniline | 226.23 g/mol | $C_{13}H_{10}N_2O_2$ |
| 23 | 34.1457 | 0.258 | 1-Cyclohexylnonene | 208.38 g/mol | $C_{15}H_{28}$ |
| 24 | 34.3475 | 0.8058 | Eicosanoic acid, methyl ester | 326.5570 | $C_{21}H_{42}O_2$ |
| 25 | 34.4147 | 0.1122 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl- | 577.2g/mol, | $C_{16}H_{48}O_7Si_8$ |
| 27 | 34.7382 | 0.1336 | 5-hydroxy-7-methoxyflavanone | 270.28 g/mol | $C_{16}H_{14}O_4$ |
| 28 | 35.6614 | 0.1648 | Trisiloxane | 124.32 g/mol | $H_8O_2Si_3$ |
| 29 | 35.7107 | 0.1525 | Fumaric acid, heptyl tridecyl ester | 396.6 g/mol | $C_{24}H_{44}O_4$ |
| 30 | 36.1 | 0.8425 | Bis(2-ethylhexyl) phthalate | 390.564 g/mol | $C_{24}H_{38}O_4$ |
| 31 | 36.8425 | 0.1988 | trans-3-Methoxy-4-propoxy-beta-methyl-beta-nitrostyrene | 251.28 g/mol | $C_{13}H_{17}NO_4$ |
| 32 | 37.2332 | 0.1861 | 2,5-Dihydroxybenzoic acid | 154.12 g/mol | $C_7H_6O_4$ |

**Table 6.** Phytochemical constituents identified from ethanolic extracts of the dried seeds of pigeon pea plant using GC-MS.

| S/ no | Retention time (min) | Peak area (%) | Compound | Molecular weight | Molecular formula |
|---|---|---|---|---|---|
| 1 | 18.512 | 0.7048 | Benzyl isocyanate | 133.15 g/mol | $C_8H_7NO$ |
| 2 | 20.7541 | 1.0644 | Methyl l-phenylalaninate | 179.22 g/mol | $C_{10}H_{13}NO_2$ |
| 3 | 24.6622 | 0.3539 | alpha.-L-Galactopyranoside, methyl 6-deoxy- | 394.72 g/mol | $C_{16}H_{38}O_5Si_3$ |
| 4 | 24.7138 | 0.1339 | beta-D-Ribopyranose | 150.13 g/mol | $C_5H_{10}O_5$ |
| 5 | 30.1894 | 0.4677 | Hexacosane | 366.71 g/mol | $C_{26}H_{54}$ |
| 6 | 31.2595 | 0.7882 | 7-Hexylicosane | 366.7 g/mol | $C_{26}H_{54}$ |
| 7 | 31.3392 | 8.8636 | Methyl palmitate | 270.45 g/mol | $C_{17}H_{34}O_2$ |
| 8 | 31.6811 | 1.4142 | 1-(4-Bromobutyl)-2-piperidinone | 234.13 g/mol | $C_9H_{16}BrNO$ |
| 9 | 31.7297 | 0.8644 | Oleic Acid | 282.5 g/mol | $C_{18}H_{34}O_2$ |
| 10 | 31.7822 | 0.5064 | n-Hexadecanoic acid(Palmitic acid) | 256.4 g/mol | $C_{16}H_{32}O_2$ |
| 11 | 31.8393 | 1.2412 | Estra-1,3,5(10)-trien-17.beta.-ol | 334.4 g/mol | $C_{23}H_{26}O_2$ |
| 12 | 31.9313 | 4.0862 | Ethyl palmitate | 284.5 g/mol | $C_{18}H_{36}O_2$ |
| 13 | 32.0458 | 1.7634 | Undec-10-ynoic acid, dodecyl ester | 350.6 g/mol | $C_{23}H_{42}O_2$ |
| 14 | 32.1112 | 2.06 | Tetratetracontane | 619.2 g/mol | $C_{44}H_{90}$ |
| 15 | 32.1772 | 1.564 | Propyl tetradecyl ether | 256.5 g/mol | $C_{17}H_{36}O$ |
| 16 | 32.6445 | 12.6864 | 9,15-Octadecadienoic acid, methyl ester | 294.5 g/mol | $C_{19}H_{34}O_2$ |
| 17 | 32.7052 | 6.2561 | 11-Octadecenoic acid methyl ester | 296.5 g/mol | $C_{19}H_{36}O_2$ |
| 18 | 32.8366 | 4.3603 | Oxalic acid, cyclobutyl tetradecyl ester | 340.5 g/mol | $C_{20}H_{36}O_4$ |
| 19 | 32.9065 | 5.8619 | Heptadecanoic acid, 16-methyl-, methyl ester ( Methyl isostearate) | 298.5 g/mol | $C_{19}H_{38}O_2$ |
| 20 | 32.9481 | 5.3891 | 9-Octadecenal | 266.5 g/mol | $C_{18}H_{34}O$ |
| 21 | 33.125 | 5.0923 | 2-Chloroethyl linoleate | 342.9 g/mol | $C_{20}H_{35}ClO_2$ |
| 22 | 33.3766 | 1.5745 | 2,3,4-Tri-O-methyl-d-glucose | 222.24 g/mol | $C_9H_{18}O_6$ |
| 23 | 33.5369 | 2.148 | Docosane | 310.6 g/mol | $C_{22}H_{46}$ |
| 24 | 33.8619 | 2.4675 | (Z,E)-7,11-Hexadecadien-1-yl acetate | 280.4 g/mol | $C_{18}H_{32}O_2$ |
| 25 | 33.9641 | 1.37 | 1,2-Benzenedicarboxylic acid, mono(dimethylcyclohexyl) ester- | 276.33 g/mol | $C_{16}H_{20}O_4$ |
| 26 | 34.1718 | 1.5267 | trans-1,2-Diethoxycyclohexane | 112.21 g/mol | $C_8H_{16}$ |
| 27 | 34.2614 | 2.337 | Tricosane | 324.6 g/mol | $C_{23}H_{48}$ |
| 28 | 34.3549 | 0.9381 | 2,2-Dimethyl-3-hexanol | 130.23 g/mol | $C_8H_{18}O$ |
| 29 | 34.7243 | 4.0705 | 5-hydroxy-7-methoxyflavanone | 270.28 g/mol | $C_{16}H_{14}O_4$ |
| 30 | 35.055 | 1.6121 | Eicosane | 282.5475 g/mol | $C_{20}H_{42}$ |
| 31 | 35.1672 | 0.7813 | Cyclohexane, 1,1'-(2-propyl-1,3-propanediyl)bis- | 222.41 g/mol | $C_{16}H_{30}$ |
| 32 | 35.3548 | 1.678 | Rotoxamine | 290.79 g/mol | $C_{16}H_{19}ClN_2O$ |
| 33 | 35.7156 | 2.2401 | 15-Hydroxypentadecanoic acid | 258.4 g/mol | $C_{15}H_{30}O_3$ |
| 34 | 35.7954 | 0.9136 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl- | 577.2 g/mol | $C_{16}H_{48}O_7Si_8$ |
| 35 | 35.9626 | 1.3762 | Methoxyacetic acid, 2-tetradecyl ester | 286.4 g/mol | $C_{17}H_{34}O_3$ |
| 36 | 36.1181 | 1.8706 | Diisooctyl phthalate | 390.6 g/mol | $C_{24}H_{38}O_4$ |
| 37 | 36.845 | 1.8123 | 8-Hydroxy-4-oxo-2,3-dihydro-1H-quinoline-2-carboxylic acid | 207.18 g/mol | $C_{10}H_9NO_4$ |
| 38 | 37.0371 | 0.9101 | 1-Chlorononadecane | 302.966g/mol | $C_{19}H_{39}Cl$ |

The chromatogram of some phytochemical constituents identified from ethanolic extracts of the fresh leaves (Figs. 2 – 7) and dried leaves (Figs. 8 – 13) of the pigeon pea plant using GC-MS are presented, while the phytochemical constituents identified from ethanolic extracts of the fresh seeds (Figs. 14 – 19) and dried seeds (Figs. 20 – 25) of the pigeon pea plant using GC-MS are presented.

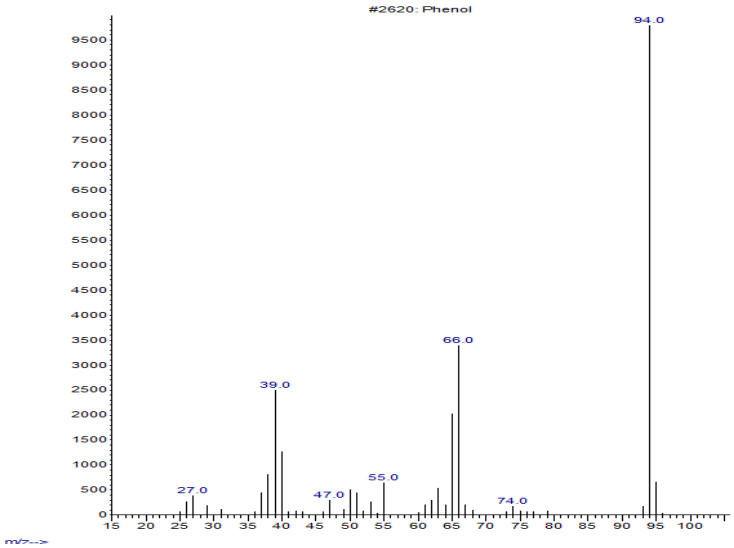


**Fig. 2** Phenol

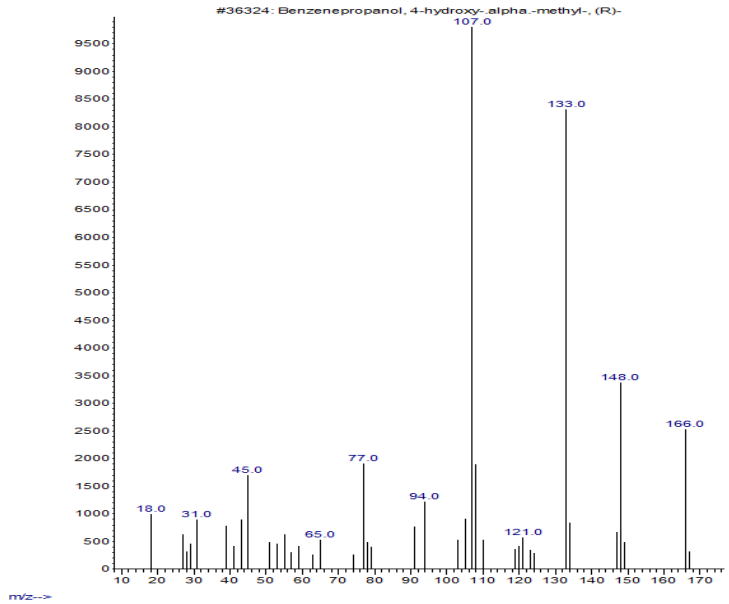


**Fig. 3** Benzenepropanol, 4-hydroxy-alpha-methyl- (R)

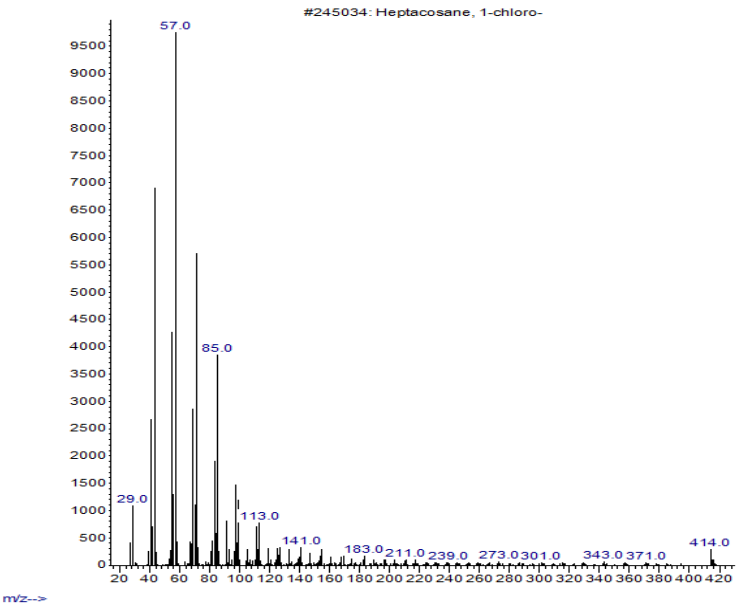


**Fig. 4** Heptacosane, 1-chloro

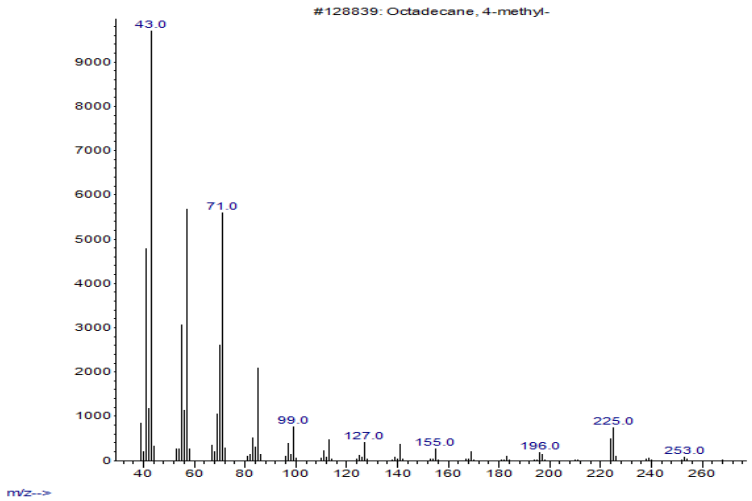


**Fig. 5** Octadecane, 4-methyl-

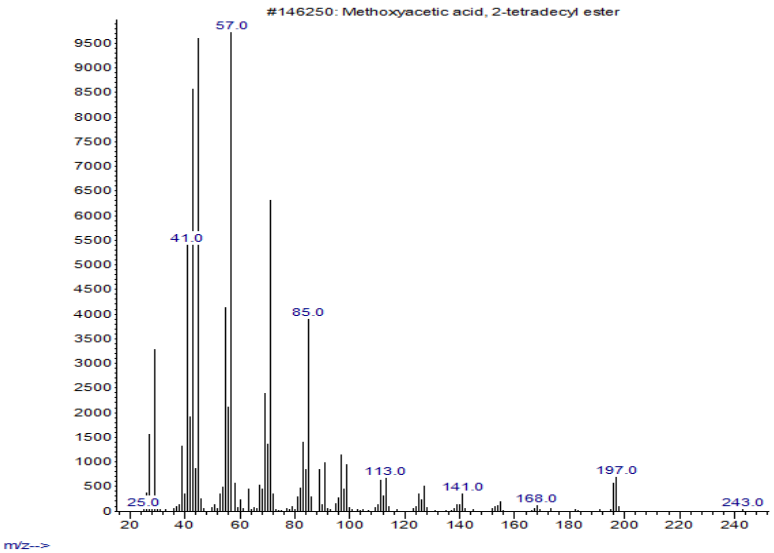


**Fig. 6** Methoxyacethic acid, 2-tetradecyl ester

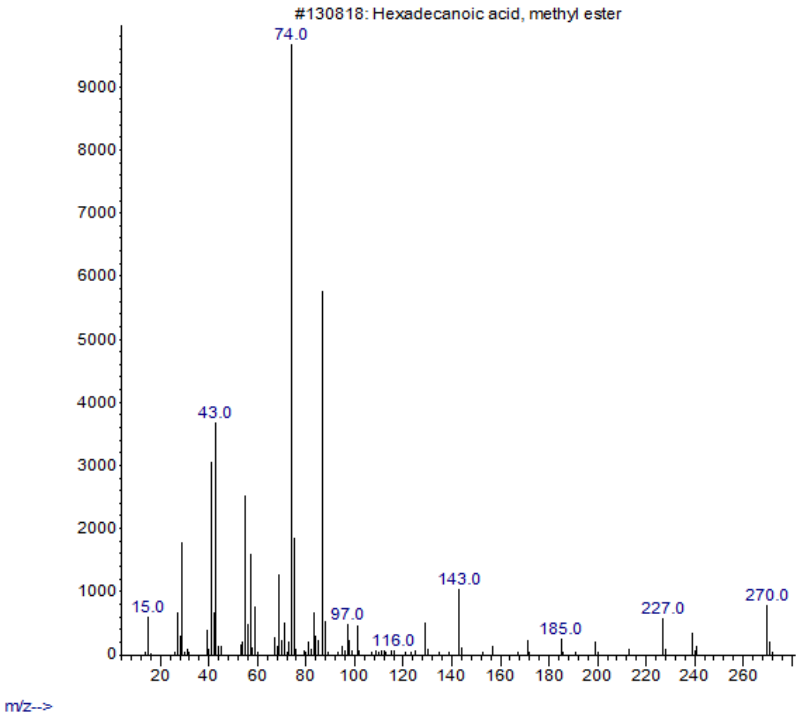


**Fig. 7** Hexadecanoic acid, methyl ester

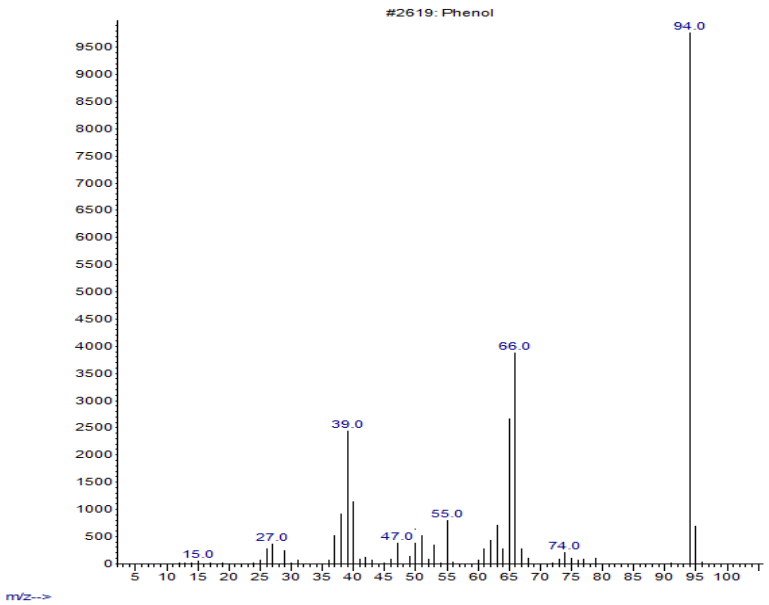


**Fig. 8** Phenol

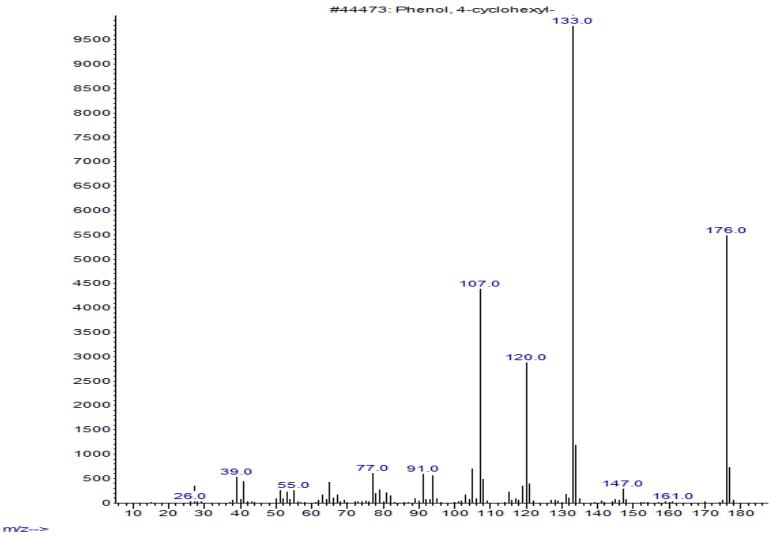


**Fig. 9** Phenol, 4-cyclohexyl-

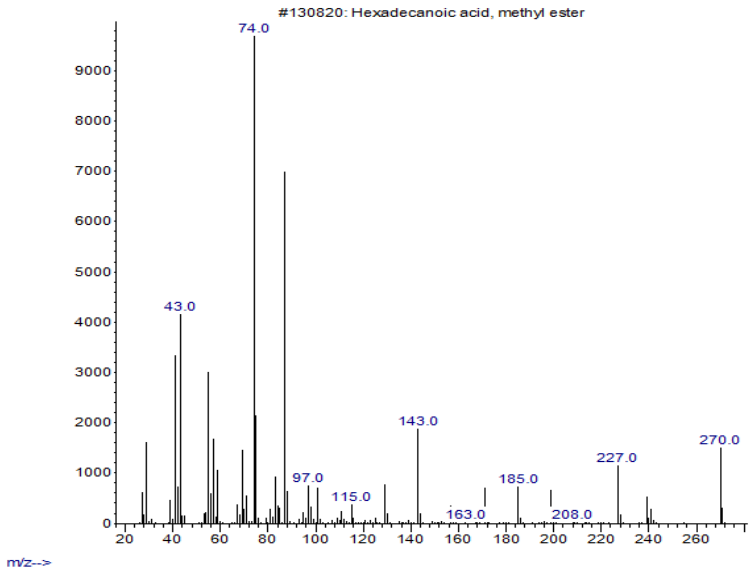


**Fig. 10** Hexadecanoic acid, methyl ester

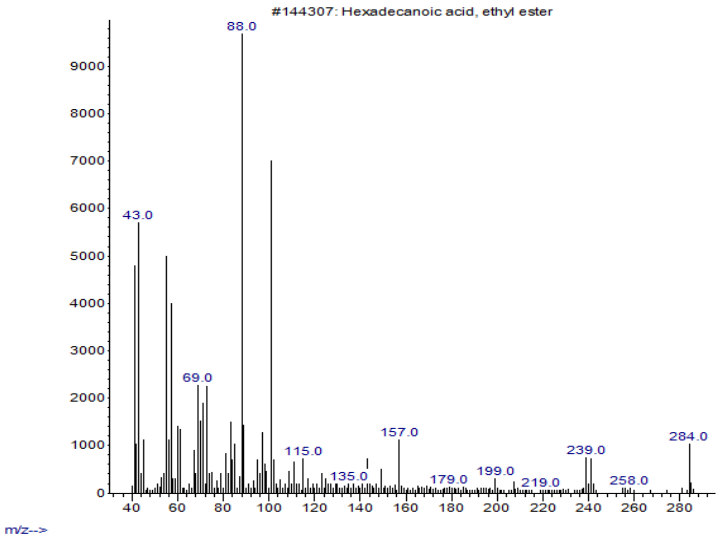


**Fig. 11** Hexadecanoic acid, ethyl ester

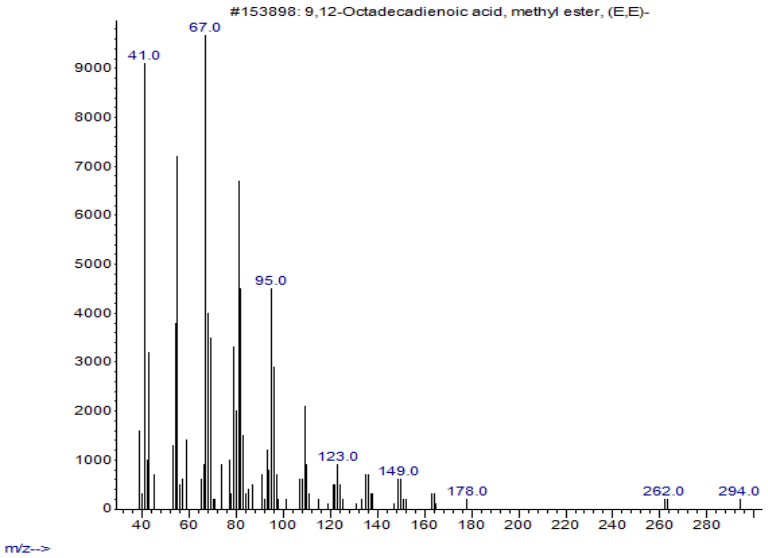


**Fig. 12** Octadecadienoic acid, methyl ester, (E,E)

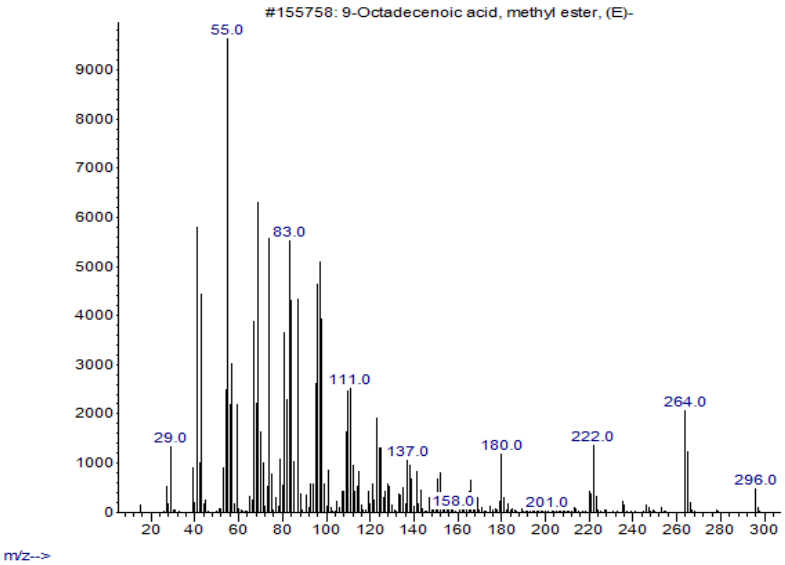


**Fig. 13** 9-Octadecedienoic acid, methyl ester, (E)-

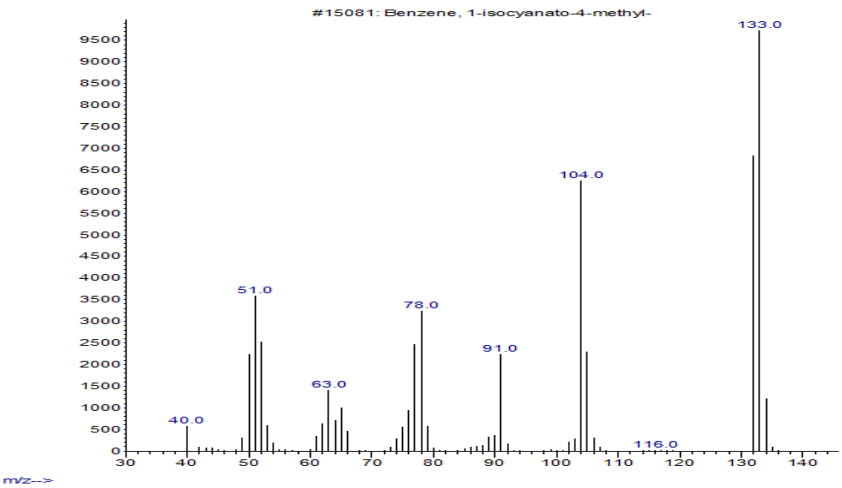


**Fig. 14** Benzene, 1-isocyanato-4-methyl

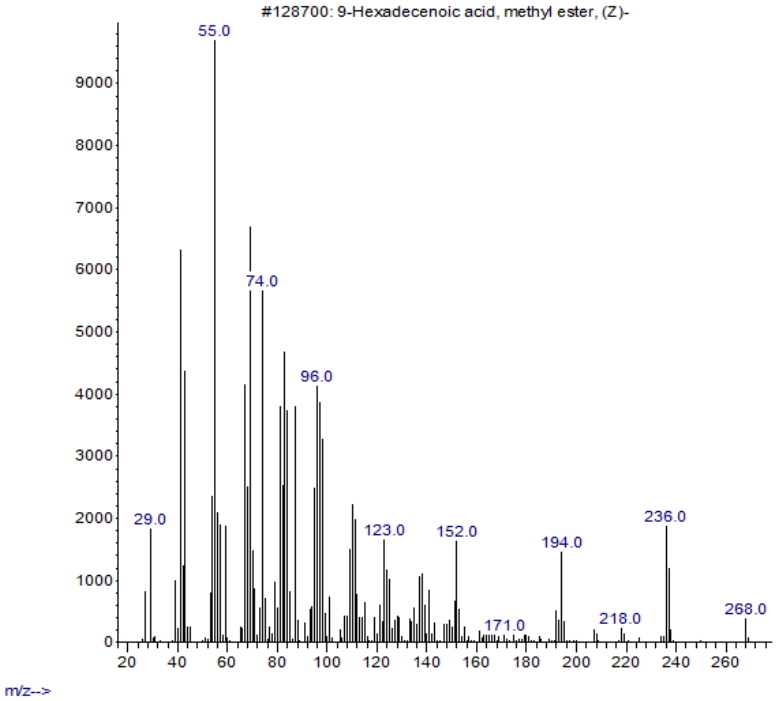


**Fig. 15** 9-Hexadecenoic acid, methyl ester, (Z)-

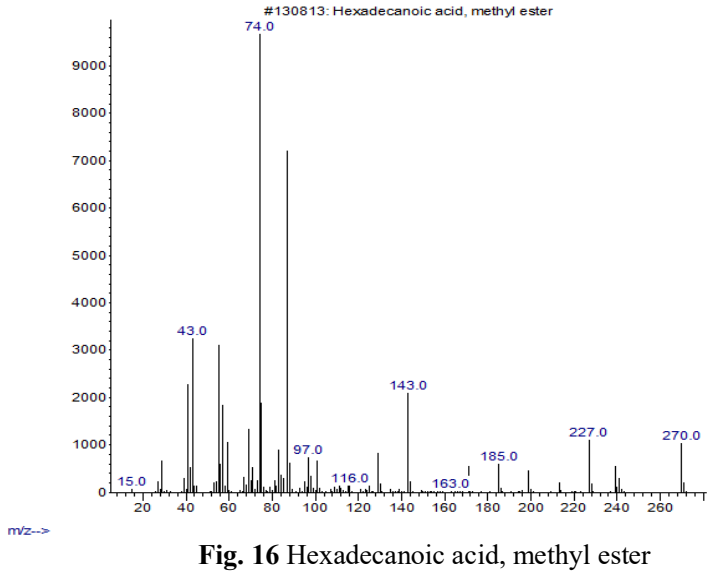


**Fig. 16** Hexadecanoic acid, methyl ester

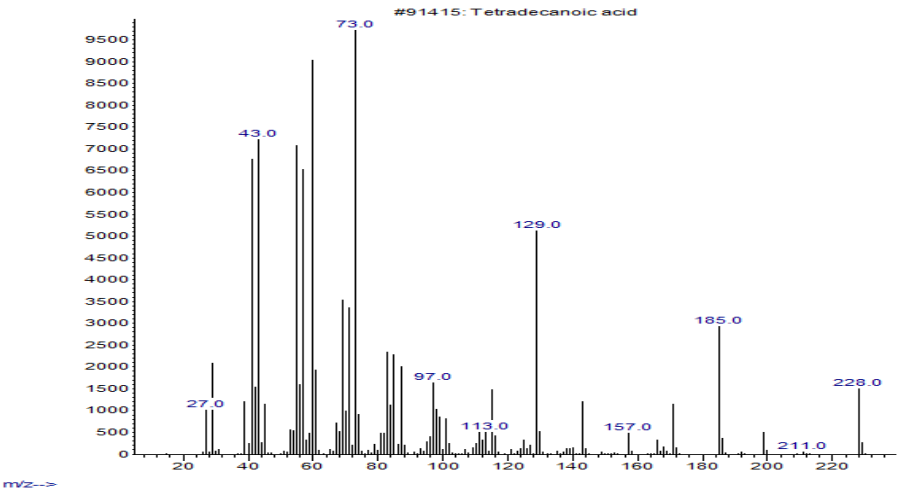


**Fig. 17** Tetradecanoic acid

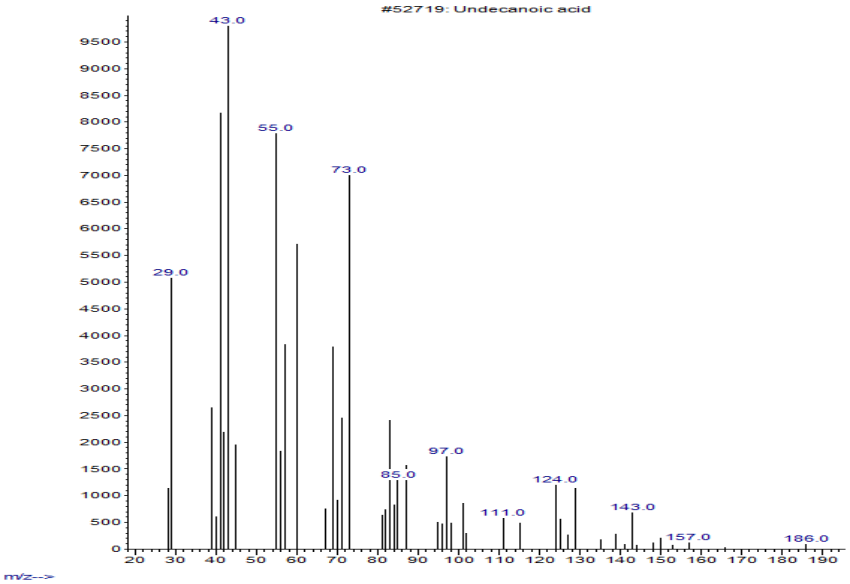


**Fig. 18** Undecanoic acid

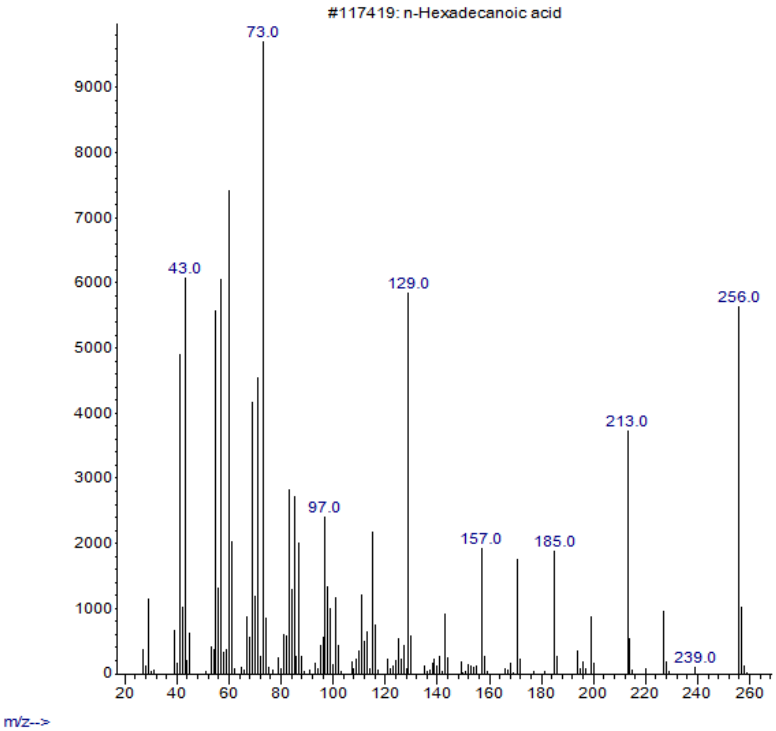


**Fig. 19** n-Hexadecanoic acid

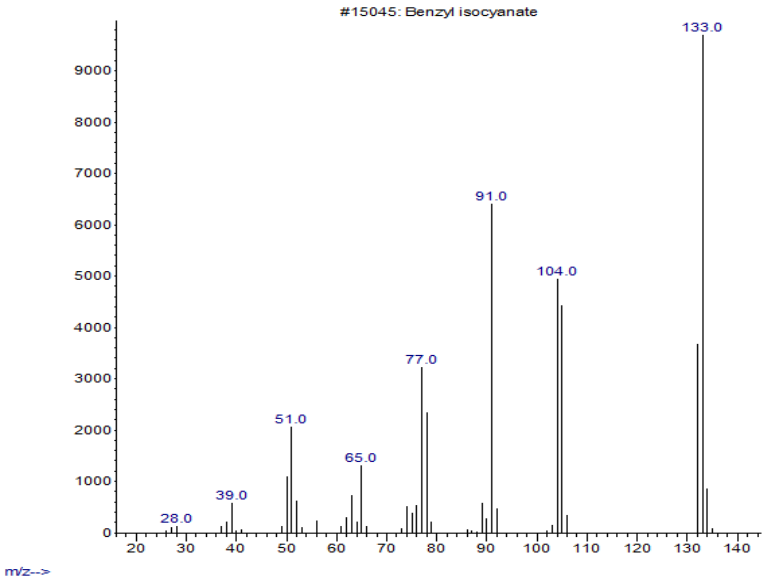


**Fig. 20** Benzene Isocyanate

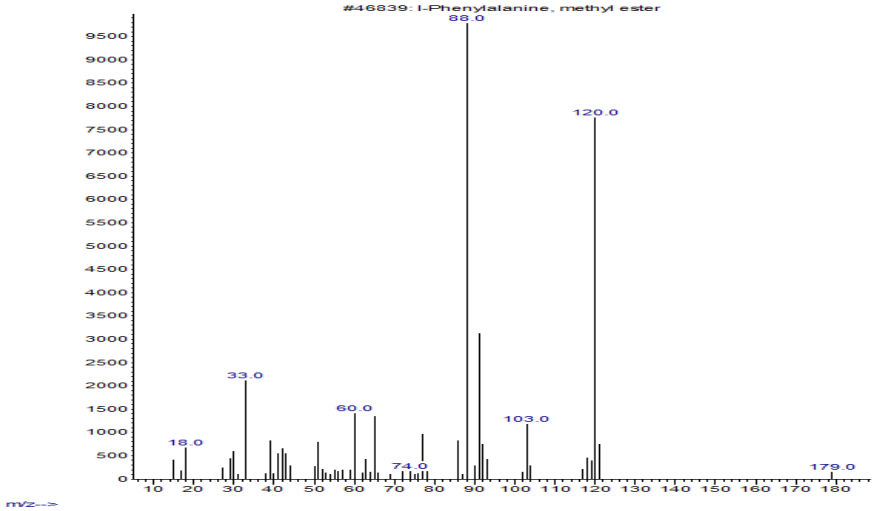


**Fig. 21** I-Phenylalanine, methyl ester

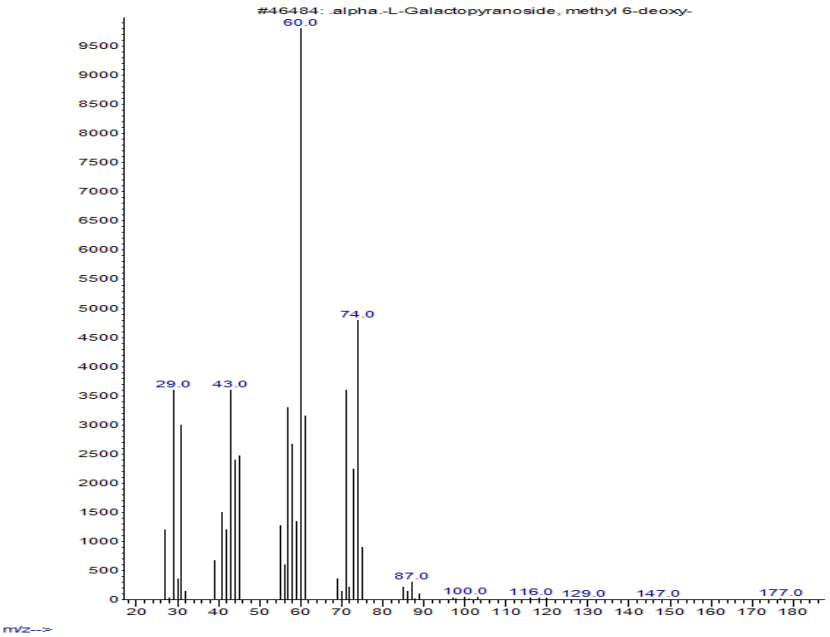


**Fig. 22** alpha-L-Galactopyranoside, methyl 6-deoxy-

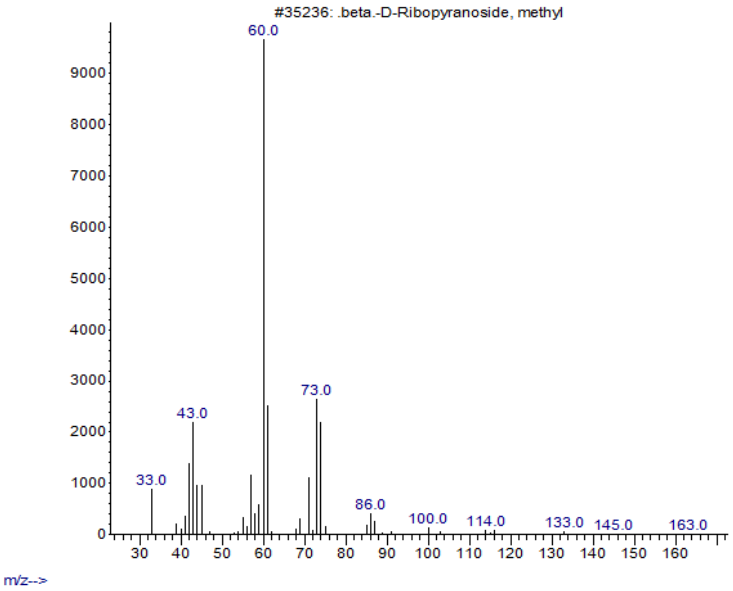


**Fig. 23** beta-D-Ribopyranoside, methyl

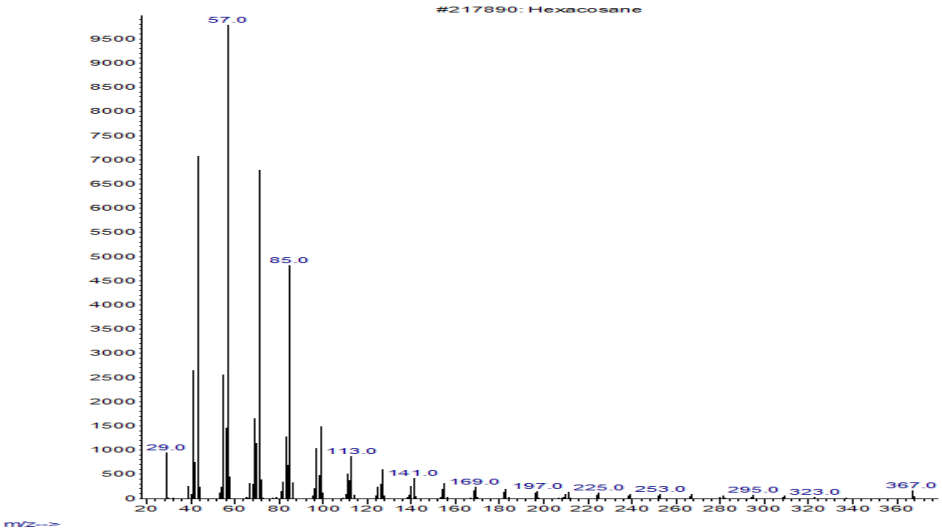


**Fig. 24** Hexacosane

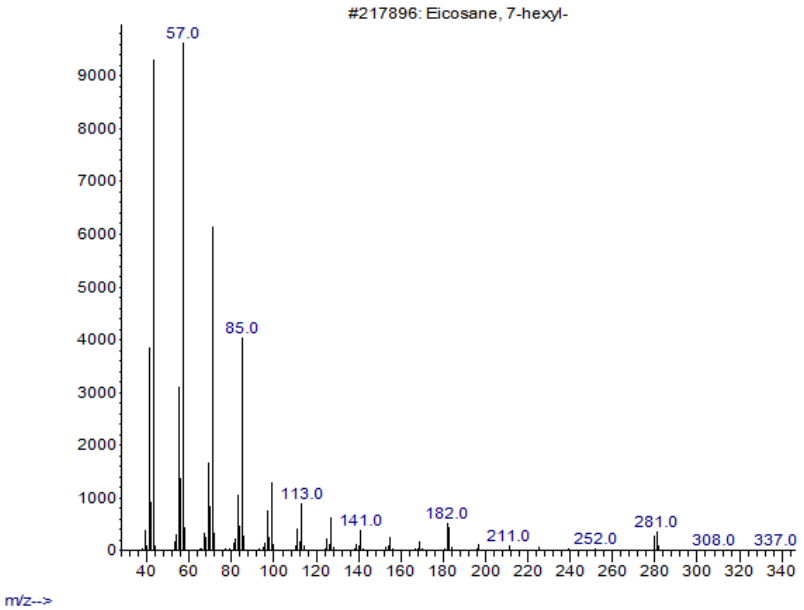


**Fig. 25** Eicosane, 7-hexyl-

## 4. Discussion

In this study, the high number of phytochemicals obtained in the leaves compared to the seeds is similar to the findings of [16] affirmed that studied aqueous and ethanolic extract of the leaves of pigeon pea and revealed the following phytochemicals constituents; Alkaloids, Flavonoids, Terpenoids, Steroids, Saponins, Tannins, Phenol, were present in the leaves of pigeon pea while in the aqueous and ethanolic extract of the seeds Alkaloids, Flavonoids, Steroids, Saponins, Tannins, Phenol, and anthraquinones were present. The presence of secondary metabolites shows that this plant can be used medically or industrially aside been used as food.

Research [10] reported that the presence of tannins in the extract indicates that it can be used as an astringent. The astringent activities of tannins are by precipitating proteins which will lead to safeguarding the underlying tissues thereby improving the healing of wounds.

The presence of phenols and flavonoids in the leaf extract indicated that they could be used as antioxidant, anticancer, anti-allergic, anti-inflammatory and gastroprotective properties [17]. Phenolic compounds are the best active antioxidant in plants such as foods, grains, vegetables and fruits [18]. Flavonoids are also known to be inducers for nitrogen-fixing bacteria, phytoalexin and phytoprotectors [19].

The presence of terpenoids indicated its potential in drug products used in the treatment of different illnesses, anti-malarial, artemisinin and its derivatives are terpenoids [20]. Saponins showed its potential in the production of detergent; in medicine, it is used in hypercholesterolaemia, used to reduce body weight, hyperglycaemia, and antioxidant. It has been found to kill cancer cells without causing harm to the normal cell (anti-cancer), and anti-inflammatory [20].

The differences in phytochemicals among the plants could be as result of geographical conditions, the extraction method or the type of solvent used during the plant extraction. The ethanolic extract of the fresh and dried leaves and seeds of pigeon peas was used for the GC–MS in this study. The investigation revealed different useful compounds having medicinal and nutritional value, some of which are Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, Diisooctyl phthalate, Ethyl palmitate 11-Octadecenoic acid methyl ester which are used as antimicrobial and antioxidant [21]. Eicosane is used as cosmetic, lubricants, plasticizer Squalene is used as an antioxidant, antistatic, antibacterial, anticancer, and antitumor according to [11]. 13-Octadecenoic acid, methyl ester, 9, 12-Octadecadienoic acid, methyl ester, (E,E) are used as anti-inflammatory, antiandrogenic, cancer, preventive, dermatitigenic, irritant, hypocholesterolemic, 5-alphareductase inhibitor, anemiagenic, insectifuge, flavour Oleic Acid is used as anti-inflammatory, anti-androgenic, anti-cancer, preservative and hypocholesterolemic [21]. Vaccenic acid is a trans-fatty acid which may regulate gluconeogenesis and Liver fat accumulation. Ethyl serves as an antioxidant, hemolytic, hypocholesterolemic, flavour nematicide, and antiandrogenic. Methyl stearate serves as an antioxidant; Phytol is used as an antinociceptive, antioxidant, anticancer, anti-inflammatory, antimicrobial, diuretic, and chemopreventive properties. Phenol, Phosphonic acid is used as an antioxidant, anticancer, anti-inflammation, antidiabetic, and skin care product [22].

## 5. Conclusion

In conclusion, the phytochemical compounds obtained from the aqueous and ethanolic extract of the fresh and dried leaves of the pigeon pea plant are Alkaloids, Flavonoids, Terpenoids, Steroids, Phytosteroids, Saponins, Tannins, Phenol, Anthraquinones, Quinones, Xanthoproteins and Phlobatannin while the Acidic compound was absent. However, in the aqueous and ethanolic extract of the fresh and dried seeds of the pigeon pea plant, it was observed that Alkaloids, Flavonoids, Terpenoids, Steroids, Phytosteroids, Saponins, Anthraquinones, Quinones were present while Tannins, Phenol, Xanthoproteins and Phlobatannin, Acidic compound were absent.

The phytochemical constituents identified from ethanolic extracts showed that the dried seeds of the pigeon pea plant had more bioactive compounds and it was followed by the fresh seeds and fresh leaves while the dried leaves showed the lowest number of bioactive compounds, some of which are useful in medicinal activities, cosmetic, lubricants, plasticizers, antioxidant, antistatic, antibacterial, anticancer, antitumor, anti-inflammatory, antiandrogenic, antidiabetic and skin care product.

## Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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