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# GC-MS analysis of chemical components seed oil of *Raphanus sativus L.*

## Abstract

GC-MS analysis were conducted on *Raphanus sativus* and observed that extracts of seeds analyzed with gas-chromatography mass spectrometry obtained the number of compounds and molecular weight of the compounds present. The isolated essential oils from seeds of *Raphanus sativus* were also analyzed by (GC-MS). Seeds oil of *Raphanus sativus* were found richer in compounds such as Oleic Acid, Erucic Acid representing 30.011%, 5.464% and 16.411% of the total oil, respectively. Seeds oil of *Raphanus sativus* was constituted of hydrocarbons, alcohols, and phenols. The essential oil from seeds of *Raphanus sativus* was characterized by the presence of alcohols and esters. Docosanoic Acid and Octadecanoic Acid predominated in essential oils, constituting 0.850% and 2.944%.

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## Introduction

Radish (*Raphanus sativus* L.) belongs to family Brassicaceae. It is a highly precious food crop, mostly an ingredient of salads in Asian countries during winter. It has been used as a medicinal plant from a long time in plant era. It has laxative effects on the intestine and acts as an appetizer,[1] used for curing liver dysfunction and poor digestion, acts as antioxidant, anti-tumorigenic, antimutagenic, anti-diabetic and anti-proliferative.[1–20] It is also very well known for its use in the treatment of bronchitis and diarrhea.[2,3] Different parts of radish fruit, including roots, seeds, and leaves have multi-nutritional medicinal properties. The radish seeds have been used to treat asthma and other chest complaints. Radish oil is known to have many different health benefits, similar to those attributed to the radishes commonly eaten in salads. It is important to note that this typeof oil is only extracted from radish seeds, not from the roots. Domings et al.,[4] reported that the seed oil was used to produce biodiesel and the condition for the ethanolysis of seed oil was optimized applying the response surface methodology and compared physicochemical properties and thermal behavior between fodder radish crude oil and biodiesel. They found that fodder radish biodiesel can meet physicochemical property specification although its acid number requires attention.[4] Oilseed radish has also been observed providing additional benefits of soil compaction reduction, soil aeration, weed suppression, and nitrogen trapping.[13,16] With these benefits in mind, oilseed radish is commonly referred to as a green manure crop. The seed of the oilseed radish contains 40% oil by weight. A high value oil makes this crop a good candidate for biodiesel production.[8] The current study demonstrated the total alkaloids and GC-MS analysis of *Raphanus sativus* seed oil for determination and identification of their volatile compounds. Therefore the aim of the present study was to investigate the integrated utilization of radish seeds efficient soxhlet extraction apparatus and identify the chemical components and their relative content in seeds oil.

## Materials and methods

### Study area

A study was conducted at NTHRI, Shinkiari, Mansehra during 2016 and work done in vitro, both well equipped Hort/ Quality control laboratories were utilized for complete results and analysis.

### Sampling and analysis

Seeds of Var;NRAC-16 obtained from the Gene Bank of National Agric. Res. Center (PARC)., Islamabad, for conducting of research studies . Samples prepared were grounded using pulverizer and passed through 60-seive mash for the accuracy of the results.

### Oil extraction

Proximate analyses of radish seed moisture, ash content were determined using the methods described.[1] Where as oil (Soxhlet) contents was determined by method described.[18] All determinations were done in triplicate.

### Moisture content

Drying to constant weight in an oven at 110$^0$C for 24h applied to determine moisture content of grounded seeds. One gm sample placed into pre-heated oven and dried at 110$^0$C for 24h. Hereafter samples exits from oven, and placed in a desiccators to cool and reweighing. The moisture content of the samples calculated by applying formula as under[6] $Moisture\ (\%) = Sample\ wt\ (g) - Dried\ sample\ wt\ (g)\ wt\ (g) \times 100$

### Ash content

The ash content of radish seeds determined through dry ash. Approximately one gm of sample placed in a porcelain crucible in a muffle furnace at 600°C for 3h. After preparation of ash, samples pull out from the furnace, cool down to room temperature in a desiccators and then reweighing. The ash content calculated as under formula:

$$Ash\ (\%) = Ash\ wt\ (g)\ wt\ (g) \times 100$$

## Oil extraction

In case of soxhlet extraction, 10gm sample of radish seeds were weighed and placed in an extraction thimble, using 300 mL petroleum ether. Oil was extracted continuously for 8 h with 60-80°C.[18] After extraction, the solvent was evaporated and the extract was dried at103°C to remove residual solvent, cooled for 30 min in a desiccators and weighed. All determinations were done in triplicate. Remaining pomade was again subjected to soxhlet for further extraction process. For oil extraction the dried radish seeds grounded & converted into fine powder using an electric grinder, which proper fixed cutter/ chopper for solvent extraction, 150 gm of ground seeds placed into a cellulose paper cone and extracted using light petroleum ether (b.p 40–60$^0$C) in a 5-l Soxhlet extractor for 8 h.[9] Radish oil was then recovered by evaporating off the solvent using rotary evaporator and residual solvent removed by drying in an oven at 60$^0$C for 1h. Residual moisture in the oil removed by gentle heating gradually.[19] The oil obtained from both extractions was stored at 20$^0$C until it was analyzed.

## GC-MS analysis

The extracts of the seeds analyzed with gas-chromatography mass spectrometry to obtain the number of compounds and molecular weight of the compounds present in the extracts. The software already adopted to handle mass spectra and chromatograms a Turbo-Mass ver-5.2.[21–23]

# Results and discussion

The GC/MS analyses was performed in vitro on an Agilent 6,850 Ser. II apparatus, fitted with a fused silica DB-5 capillary column (30m × 0.25mm i.d., 0.33µm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization energy voltage 70 eV; electron multiplier voltage energy 2000 V. Mass spectra were scanned in the range 40–500amu, scan time 5 scans/s. The results obtained narrated in Table 1. Most constituents were identified by gas chromatography by comparison of their Kovats retention indices (Ri) with either those of the literature or with those of authentic compounds available in our laboratories (Figures 1-15) accordingly. The Kovats retention indices were determined in relation to a homologous series of *n*-alkanes ($C_{10}$–$C_{35}$) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature and a homemade library in the system. The components relative concentrations were obtained by peak area normalization.

*Raphanus sativus* seeds contain a high concentration of oil content. In traditional chinese therapeutics, radish seed oil is used in digestive and intestinal problems (Ahangari and Sargolzaei, 2012). The petroleum ether extracted seeds oil from *Raphanus sativus* were found to be rich in compounds like Oleic Acid (1), Oleic acid (2) and Erucic Acid representing 30.011%, 5.464% and 16.411% of the total oil, respectively. According to Seiichiro et al (1984) major fatty acid compound found in radish seed oil was erucic acid (30-33%). Due to toxicological health effects of erucic acid, radish seed oil is recommended to be used in other industries rather than food industry. Seeds of *Raphanus sativus* oil was constituted of hydrocarbons, alcohols and phenols. The essential oil from seeds of *Raphanus sativus* was characterized by the presence of alcohols and esters. Docosanoic Acid and Octadecanoic Acid predominated in essential oils, constituting 0.850% and 2.944%, respectively. It was observed that molecular weight of the seed of the radish was minimum 256 with n-Hexadecanoic acid at RT minutes 16.804, however the maximum 416 were recorded with Tocopherol at Rt minutes 25.597 respectively. It is further stated that the range of MW below 300 to under 500 shows the presence of different constituent in the extracted oil. The total percent of the oil extracted at minimum level was 0.850 with C22H44O2 while the maximum were 20.874 with C29H50O were noted, however it may be differ among each other according to the compounds and the varietal seed used.

**Table 1** GC-MS analysis of seeds of Radish (*Raphanus sativus* L.)

| S. No. | RT Min. | Percent of Total | Name of Compound | Molecular Formula | Molecular Weight |
|---|---|---|---|---|---|
| 1. | 20.270 | 5.464 | Oleic Acid (1) | $C_{18}H_{34}O_2$ | 282 |
| 2. | 16.804 | 4.746 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 |
| 3. | 18.537 | 30.011 | Oleic Acid (2) | $C_{18}H_{34}O_2$ | 282 |
| 4. | 18.707 | 2.805 | Octadecanoic Acid | $C_{18}H_{36}O_2$ | 284 |
| 5. | 21.936 | 16.411 | Erucic Acid | $C_{22}H_{42}O_2$ | 338 |
| 6. | 22.080 | 0.850 | Docosanoic Acid | $C_{22}H_{44}O_2$ | 340 |
| 7. | 23.074 | 2.944 | Octadecanoic Acid | $C_{21}H_{40}O_4$ | 356 |
| 8. | 23.074 | 2.944 | Ethyl Ester | $C_{21}H_{40}O_4$ | 356 |
| 9. | 25.597 | 5.959 | Tocopherol | $C_{28}H_{48}O_2$ | 416 |
| 10. | 26.234 | 0.968 | Cholesterol | $C_{27}H_{46}O$ | 386 |
| 11. | 26.591 | 2.534 | Ergosta | $C_{28}H_{46}O$ | 398 |
| 12. | 27.169 | 6.435 | Campesterol | $C_{28}H_{48}O$ | 400 |
| 13. | 28.052 | 20.874 | Stigmasterol, 22,23-dihydro | $C_{29}H_{50}O$ | 414 |

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**Figure 1** Powdered form of Radish Seed.

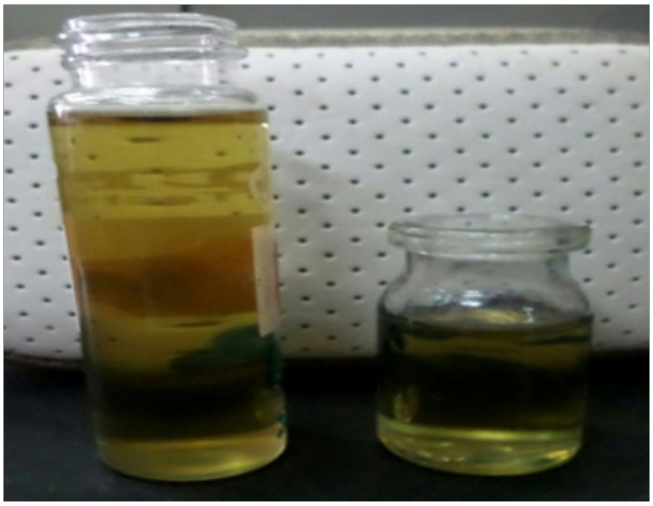

**Figure 2** Radish Seed Oil.

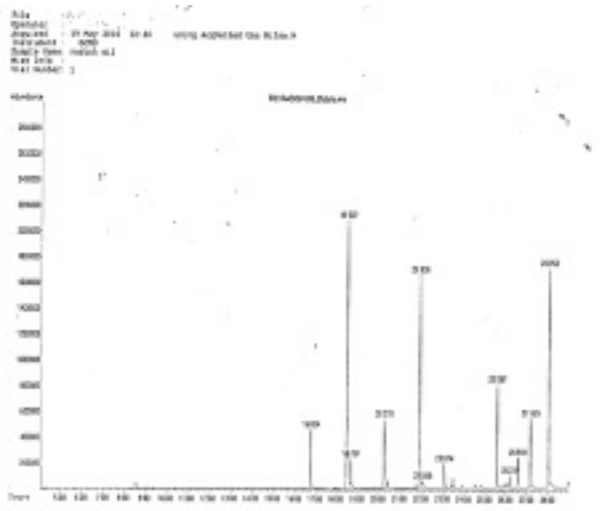

**Figure 3** GC-MS analysis of Radish seed oil.

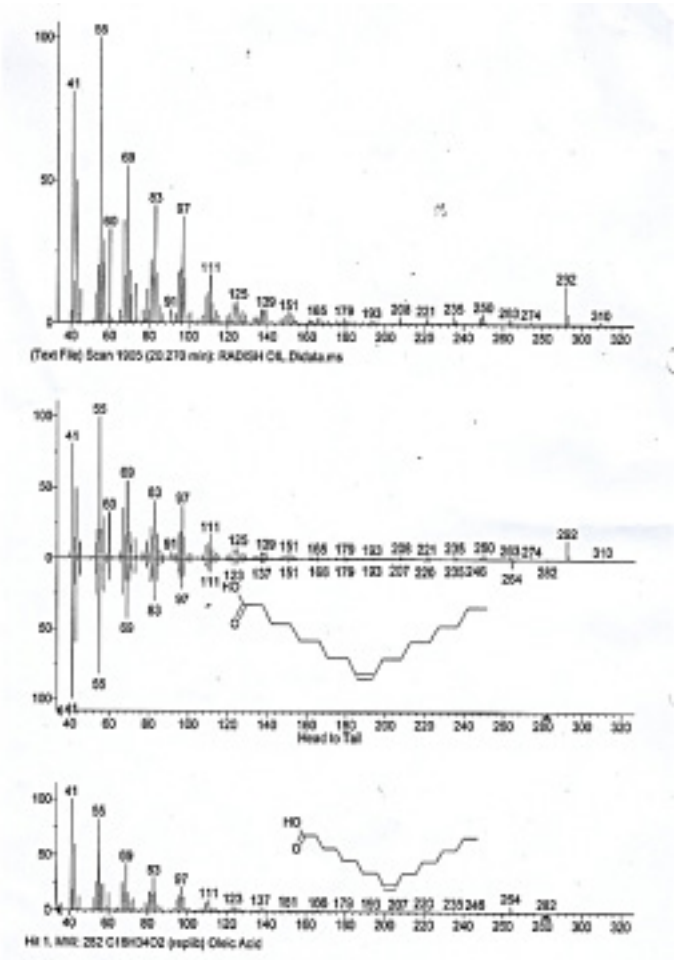

**Figure 4** GC-MS spectrum of Oleic Acid.

```
Data Path :
Data File : RADISH OIL.D
Acq On    : 19 May 2016  12:26
Operator  :
Sample    : radish oil

Signal    : TIC: RADISH OIL.D\data.ms

Peak  R.T.  first  max  last  PK   peak      corr.     corr.    % of
 #    min    scan  scan  scan  TY  height     area     % max.   total
--  ------ ------ ---- ---- --- -------- -------- ------ -------
1  16.804  1485 1497 1526 rBV   474367  1110022  15.81%  4.746%
2  18.537  1681 1701 1717 rBV  2099417  7019829 100.00% 30.011%
3  18.707  1717 1721 1754 rVB   238919   655992   9.34%  2.805%
4  20.270  1894 1905 1921 rBV   553080  1277973  18.21%  5.464%
5  21.936  2091 2101 2115 rVV  1666112  3838580  54.68% 16.411%

6  22.080  2115 2118 2136 rVB4   54276   198869   2.83%  0.850%
7  23.074  2227 2235 2251 rBV3 197212   688654   9.81%  2.944%
8  25.597  2525 2532 2543 rBV   809117  1393795  19.86%  5.959%
9  26.234  2601 2607 2616 rVB2  101584   226315   3.22%  0.968%
0  26.591  2642 2649 2669 rVB   242814   592644   8.44%  2.534%

1  27.169  2709 2717 2728 rBV2  542709  1505227  21.44%  6.435%
2  28.052  2809 2821 2831 rBV  1716880  4882580  69.55% 20.874%

                    Sum of corrected areas:    23390480
```

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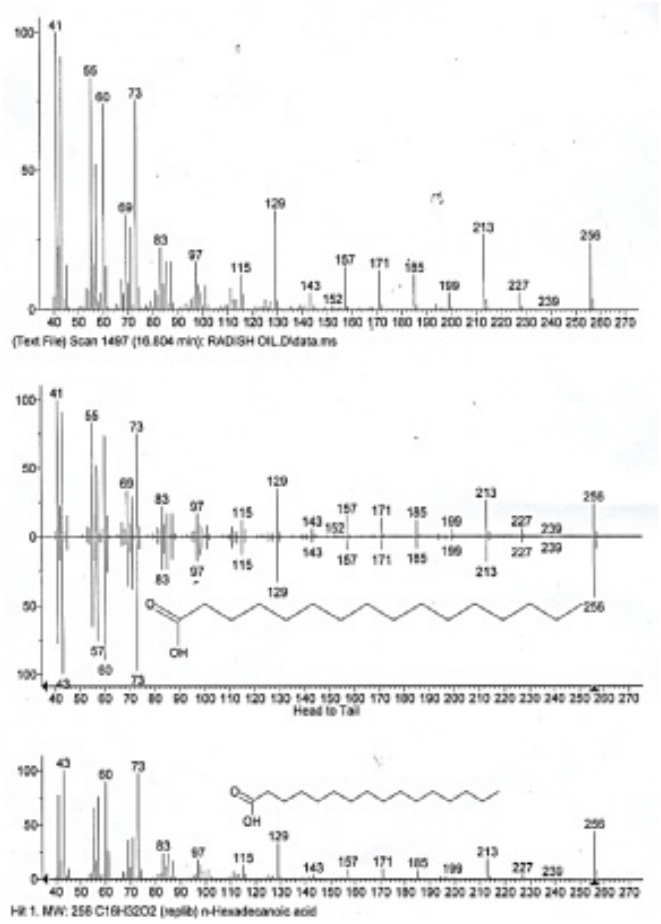

**Figure 5** GC-MS spectrum of n- Hexadecanoic acid.

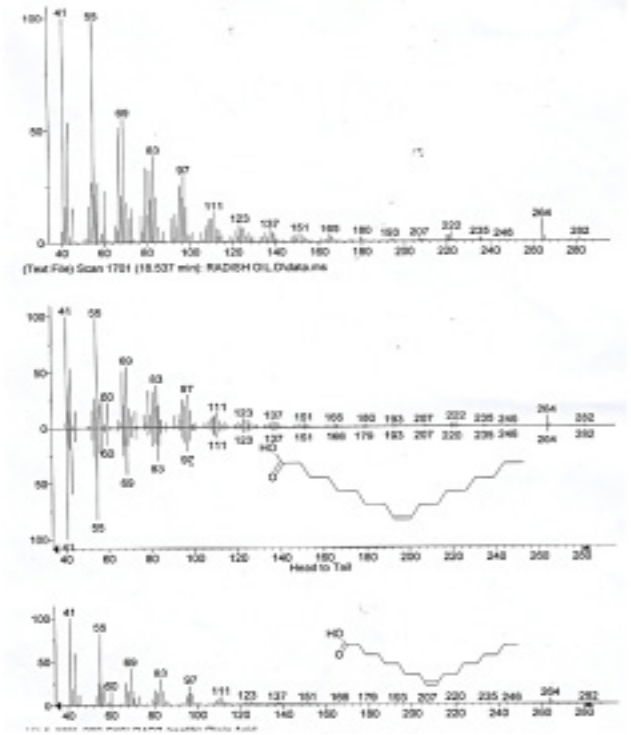

**Figure 6** GC-MS spectrum of Oleic Acid.

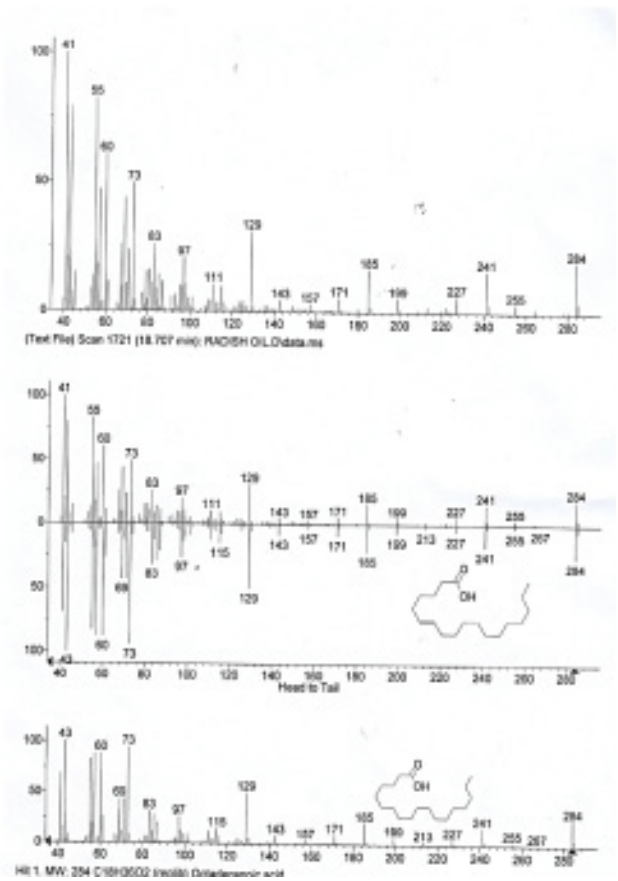

**Figure 7** GC-MS spectrum of Octadecanoic Acid.

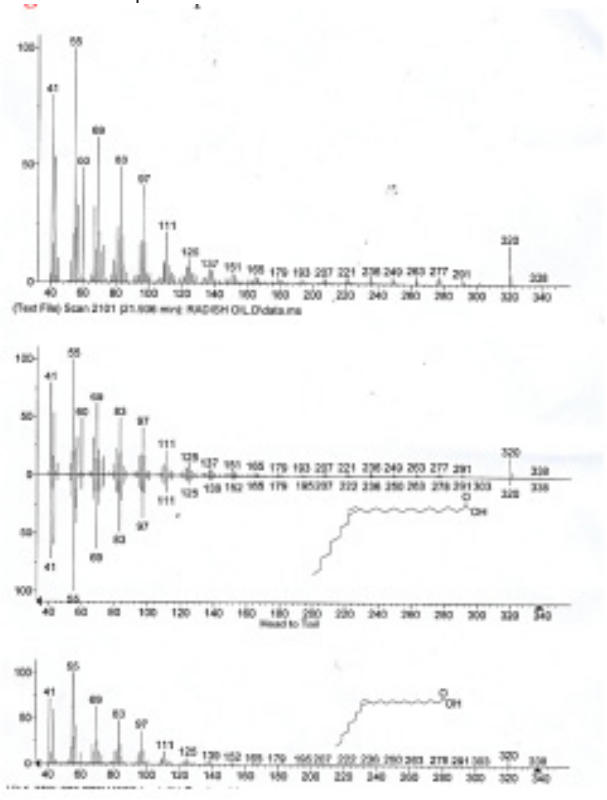

**Figure 8** GC-MS spectrum of Erucic Acid.

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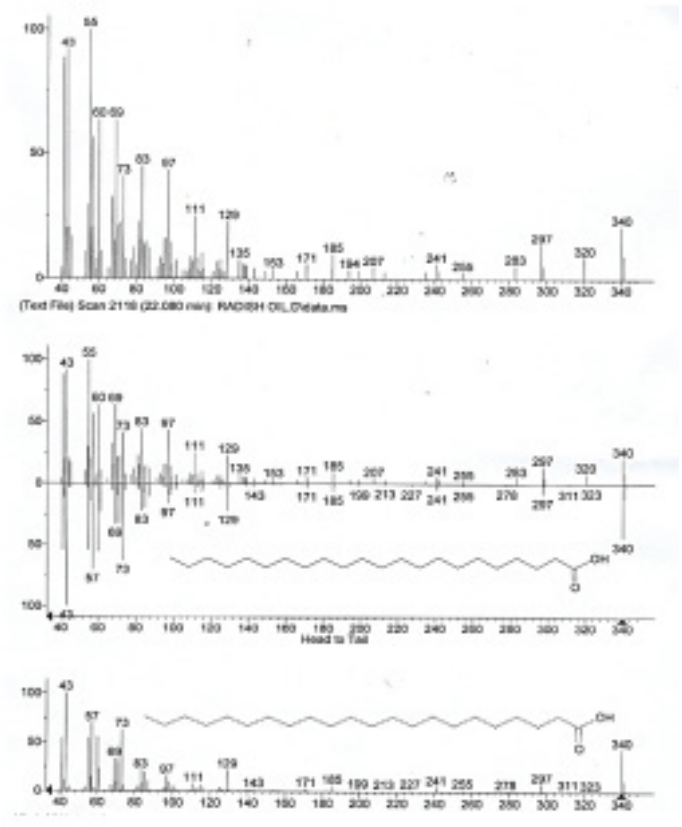

**Figure 9** GC-MS spectrum of Docosanoic Acid.

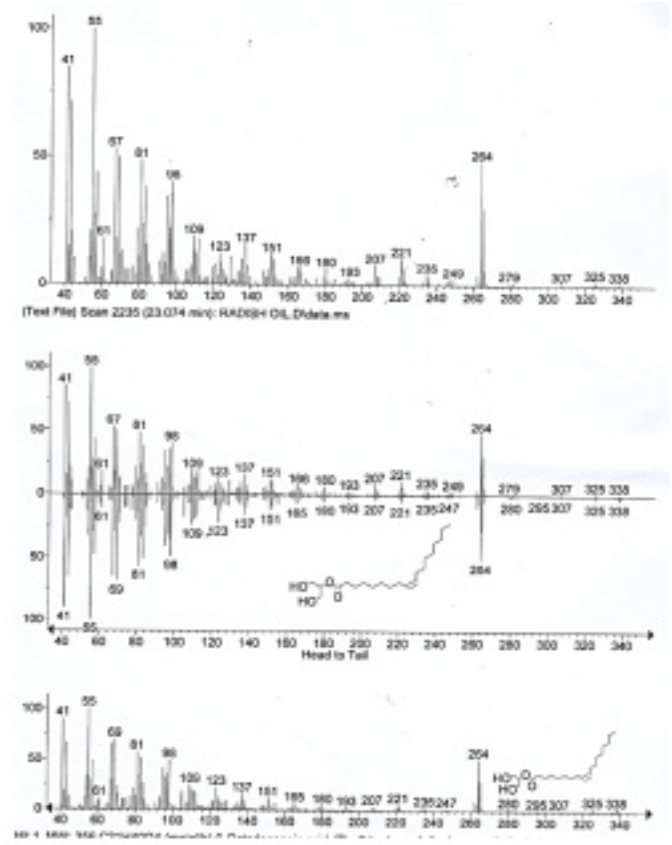

**Figure 10** GC-MS spectrum of ethyl ester.

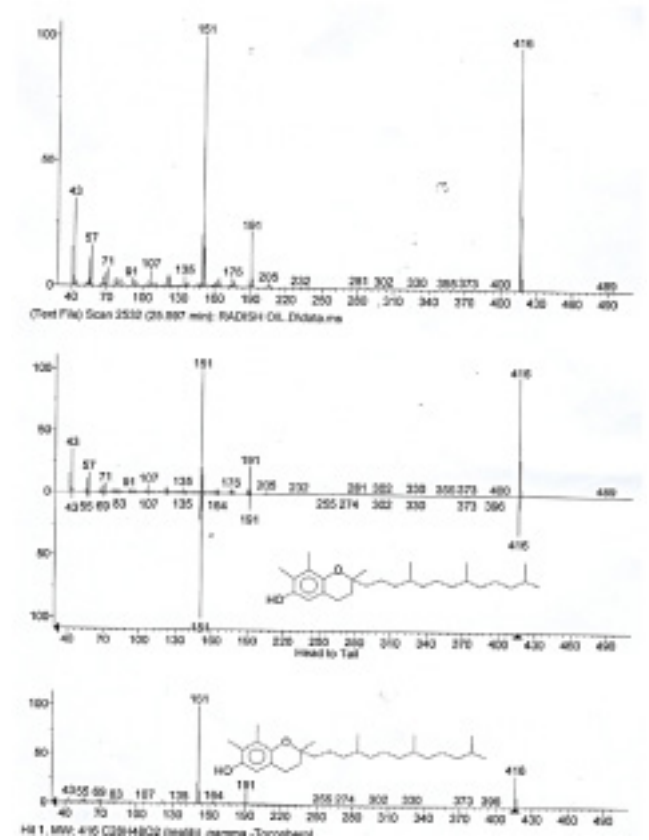

**Figure 11** GC-MS spectrum of Tocopherol.

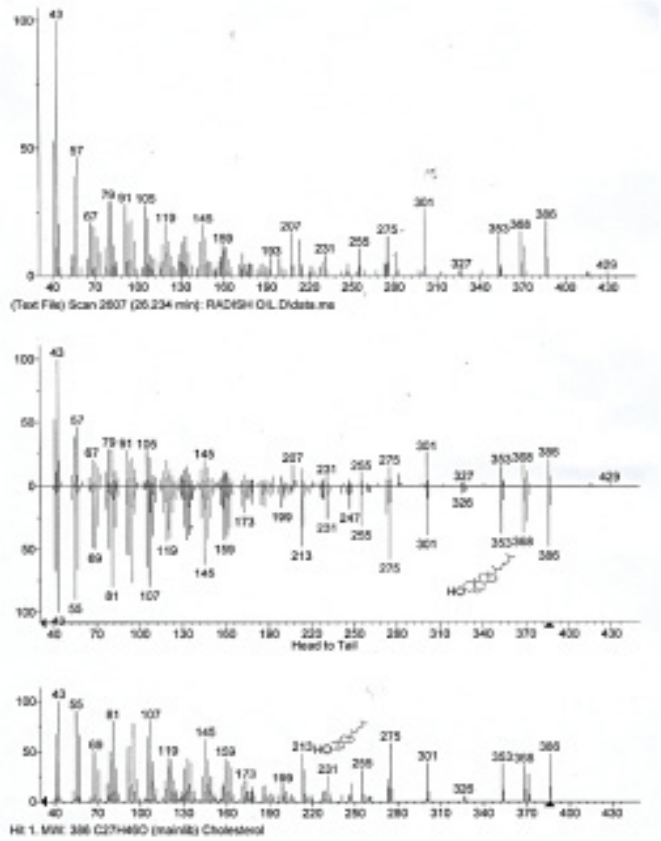

**Figure 12** GC-MS spectrum of cholesterol.

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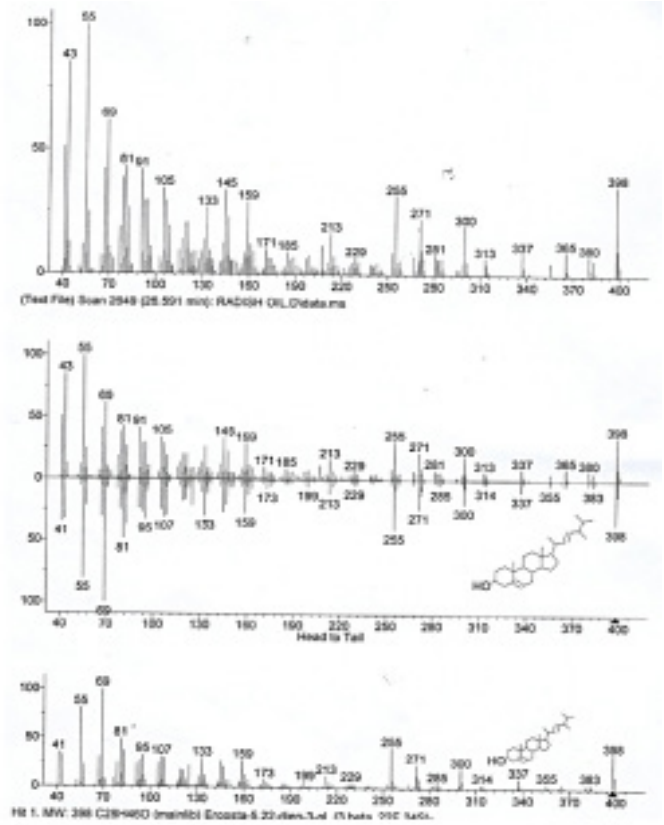

**Figure 13** GC-MS spectrum of Ergosta-5,22-dien-3-ol.

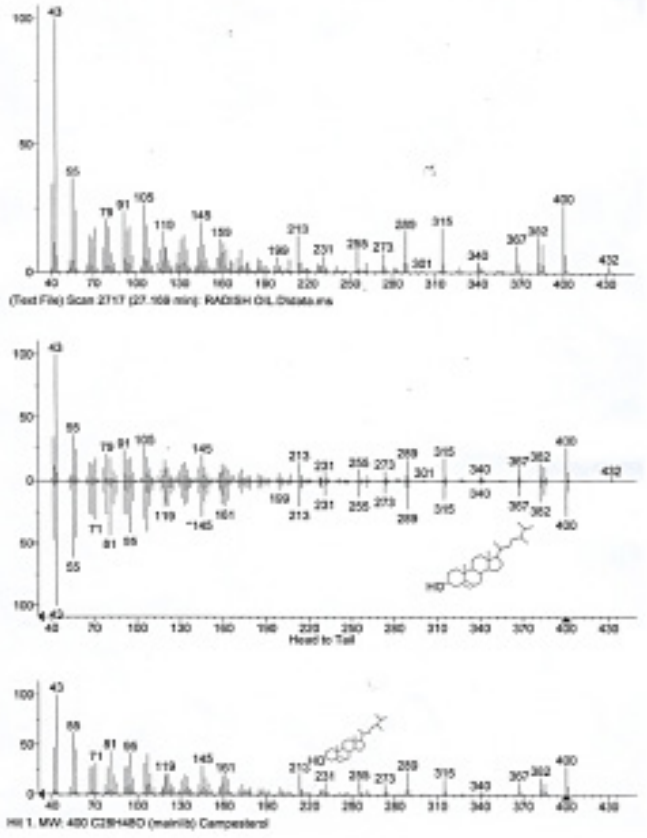

**Figure 14** GC-MS spectrum of Campesterol.

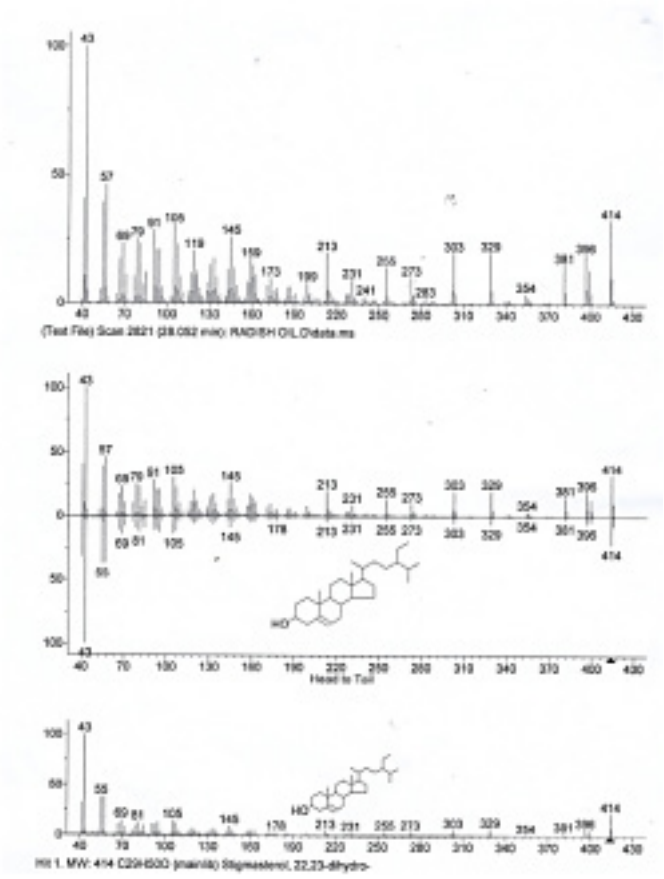

**Figure 15** GC-MS spectrum of stigma sterol.

## Conclusion

Seeds of *Raphanus sativus* oils were found to be rich in compounds like Oleic Acid, Erucic Acid representing 30.011%, 5.464% and 16.411% of the total oil, respectively. Seeds of *Raphanus sativus* oil was constituted of hydrocarbons, alcohols, and phenols. The essential oil from seeds of *Raphanus sativus* was characterized by the presence of alcohols and esters. Docosanoic Acid and Octadecanoic Acid predominated in essential oils, constituting 0.850% and 2.944%, respectively.

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## Conflicts of interests

The authors declares that there is no conflict of interest.

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