



Chemical composition of oils of *Azadirachta indica* A. Juss and *Ricinus communis* Linn seed in Marigat, Baringo County, Kenya

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Abstract

All parts of *A. indica* (neem) and *R. communis* (castor) plants have mostly been used as natural remedies in the control and treatment of several ailments, control of pests and insects, animal feeds and production of industrial products globally. The seed oils of *A. indica* and *R. communis* are known to have antidiabetic, anti-helminthic, antifertility, antioxidant, antibacterial, anti-inflammatory, anti-cancer, insecticidal and mosquitocidal activity. This study reports for the first time the chemical composition of *A. indica* and *R. communis* seed oils from Marigat, Baringo County, Kenya. Seed oils of *A. indica* and *R. communis* were extracted from mature dried seeds through cold pressing and boiling respectively and chemical composition determined using Gas Chromatography (GC)-Mass Spectrometry (MS). The constituents of both seed oils were dominated by saturated and unsaturated fatty acids, cyclic esters and methyl esters. The predominant constituents of *R. communis* were (Z)-6-Octadecenoic acid (37.33%), Ricinoleic acid (30.22%) and 13-Hexyloxacyclotridec-10-en-2-one (26.67%) while those of *A. indica* were 2-hexyl-1-decanol (30.97%), Octadecanoic acid (29.69%) and Oxalic acid, 6-ethyloct-3-yl ethyl ester (15.55%). Oils contained Hexadecanoic acid and Octadecanoic acid which are used in the manufacture of several products such as candles, soaps, lotions, perfumes and cosmetics. Octadecenoic acid is important in control of human diseases and Ricinoleic acid in production of alkyd resins for surface coating and biofuel. From the results, *A. indica* and *R. communis* seed oils constituents have potential in the agricultural, industrial, cosmetics and pharmaceutical sectors but require further fractionation to isolate the bioactive compounds.

Keywords: *Azadirachta indica*; chemical constituents; GC-MS, *Ricinus communis*; seed oil

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Introduction

Natural seed oils from plants are in high demand for the development of new products in therapeutics, agriculture, cosmetics and industrial sector compared to synthetic products. Synthetic products have several limitations such as high cost, toxicity to non-targeted species, poor absorption, develop resistance, low bio-availability, pollutants to the environment and adverse side effects (Abdul et al., 2018). The use of plant-based products in therapeutics, agriculture, cosmetics and

industrial sector as alternative to synthetic-based products could be a better option because they are biodegradable, inexpensive, have less side effects and are readily available (Mkenda et al., 2015). Some of these seed oil plants are *Azadirachta indica* A., *Ricinus communis* L., *Celosia argentea* L., *Aesculus indica* C., *Sisymbrium irio* L., *Abies pindrow* R., *Ulmus wallichiana* P., *Nigella sativa*, *Cuminum cyminum* L., *Cassia abbreviate*, *Moringa oleifera* Z., *Annona squamosa* L. and *Pangium edule* R. which have been shown to

contain polyunsaturated, monounsaturated and saturated fatty acids but vary in contents due to different environmental conditions (Martín *et al.*, 2010; Jena and Gupta, 2012; Dangarembizi *et al.*, 2015; Ayu *et al.*, 2017; Maizuwo *et al.*, 2017; Dubey *et al.*, 2018; Thilakarathna *et al.*, 2018; Zahid *et al.*, 2018; Nengroo and Rauf, 2019).

Azadirachta indica A.Juss (neem) tree is also called "Wonder Tree" in most parts of the world because of its numerous useful characteristics such as biological and pharmacological properties (Atawodi and Atawodi, 2009). Neem tree is an evergreen or deciduous, fast growing plant that may reach a height of 25 meters and thrives primarily in tropical climates that have annual rainfall of 400-800mm (Schmutterer, 1990). *Azadirachta indica*, a native of India, grows in nutrient-poor soils in arid habitats and has tremendous potential for human use.

Ricinus communis (Euphorbiaceae) commonly known as castor oil plant is a soft wooden perennial shrub, indigenous to tropical Africa, but now cultivated and often wild in most tropical and many temperate countries. The ecological requirements are deep well drained sandy loams with pH ranging from 5-6.5, altitude of 0-1800m above sea level, temperature of 15-39°C and annual rainfall of 500-600mm. In Kenya, it grows over a wide range of altitudes and habitats, preferring humus-rich and disturbed ground, especially along streams and roadsides (Mburu, 2006).

All parts (roots, stem, leaves, flowers, fruits and seeds) of *A. indica* and *R. communis* plants have been reported as natural remedies in control and treatment of several ailments and also play an important role in the agricultural sector as animal feeds and in the control of pests and insects due to their phytochemical constituents (Girish and Shankara, 2008; Tiwari *et al.*, 2014; Khan and Sanwal, 2015; Alzohairy, 2016). The seed oils of *A. indica* and *R. communis* have been used in traditional medicine for antidiabetic, anti-helminthic, birth control, antioxidant, antibacterial, anti-inflammatory, anti-cancer and mosquitocidal activity (Lord *et al.*, 2003; Roop *et al.*, 2005; Jombo and Enenebeaku, 2008; Khan and Sanwal, 2015; Abdul *et al.*, 2018). *Ricinus communis* oil also plays an important role in industrial applications. The *R. communis* seed oil is valuable due to the high content of ricinoleic acid (RA), which is used in a variety of applications in the chemical industry such as

coatings, tannings, ink, soaps and biodiesel production (Martín *et al.*, 2010). Refined and processed seed oil of *R. communis* can be safely used in pharmaceutical applications because it is free from glycoprotein ricin, which is poisonous to humans and animals (Patel *et al.*, 2016).

Sharma *et al.* (2013) reported the solvent extracted seed oil of *A. indica* to contained steroids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, flavonoids and tannins while *R. communis* had alkaloids, saponins, phenols, flavonoids and tannins. The active compounds available in the plant materials may vary considerably across geographical locations depending on soil and growth limitations (Brooker and Kleinig, 2006; Chéraif *et al.*, 2007; Tran and Hinds, 2013). Furthermore, different extracts from the same plant material using different solvents for extraction may give different responses.

Studies have been carried out on *A. indica* and *R. communis* seed oils for their medicinal value but little research on their chemical constituents which determine their biological effect. Therefore, the aim of the current study was to investigate the chemical constituents of *A. indica* and *R. communis* seed oils from Marigat, Baringo County, Kenya and elucidate their structures.

Materials and methods

Collection of A. indica and R. communis seeds

The ripe drupes of *A. indica* (Figure 1a) and capsules of *R. communis* (Figure 2a) were collected each from three plants chosen at random from Marigat, Baringo County, Kenya (47055°N, 35.9792°E). Five kilograms per sample of each plant seed were separately packaged in paper bags. The samples of both plants were authentically identified by a plant taxonomist in the Department of Biological Sciences, Egerton University, Kenya and voucher specimens (Kip1 and Kip 2) deposited in the department's herbarium.

Extraction of seed oils

The ripe drupes of *A. indica* were washed to remove dirt and other impurities. They were dried in the open sun until the casing dried. Then they were further dried in the oven at 60°C for 7hrs to a constant weight in order to reduce moisture content. Oil from the seeds (Figure 1b) was extracted using a cold pressing machine Kickstart Ram press, Model VE 963 OP

(Technology Exchange Lab, Inc. Cambridge Innovation Center, United State of America). The ram press uses a piston inside a cage to crush the seed and force out the oil (Bachmann,

2004). The extracted oil was stored in a sealed glass vial (Bijoux bottle) at 4 °C until use.



(a)



(b)

Figure 1: a) *Azadirachta indica* (Neem) fruiting stage; b) seeds

Extraction of *R. communis* oil from the seeds (Figure 2b) was done using traditional method according to Oluwole *et al.*, (2012). The process included shelling the pods, dehulling the seeds and winnowing to remove any unwanted

materials and boiling the dehulled seeds in water at 90°C for 10mins. The boiled seeds were spread in the sun to dry in order to reduce the moisture content. Dried seeds were ground using mortar and pestle to form a paste. The

paste was mixed with water in proportion of 500g of paste to 1 litre of water. The mixture was cooked and as the water evaporated, the oil

started gushing out and settled on the surface. The oil was scooped using a spoon into another container and then dried by heating.



(a)



(b)

Figure 2: a) *Ricinus communis* fruiting stage; b) seeds

GC-MS analysis of chemical constituents of seed oils

Analysis of the chemical constituents of *A. indica* and *R. communis* seed oils was carried out at the laboratories of the International Centre of Insect Ecology and Physiology (ICIPE), Nairobi. One milligram (1mg) of each of the sample was separately weighed and dissolved in 1 ml dichloromethane, dried using anhydrous Na_2SO_4 to make a stock solution (1 mg/ml) from which a sample whose final concentration was

100 ng/ μ l was prepared for analysis. The samples were analyzed on an Agilent Gas Chromatograph HP-7890A (Agilent Technologies, Wilmington, USA) connected to a HP 5975 C (Agilent, Wilmington, USA) Mass Spectrometer in full scan mode. The GC equipment was fitted with a non-polar HP-5MS low bleed capillary column (30 m \times 0.25 mm internal diameter; 0.25 μ m film thickness) with 5%-phenyl methyl silicone as the stationary phase (J & W Scientific, Folsom, CA, USA). One microliter of each sample was injected in the splitless mode, and helium was used as carrier gas at 1.0 ml min⁻¹. The oven temperature was maintained at 35°C for 5 min, increased to 280°C at 10°C min⁻¹ and then held at this temperature for 5.5 min then to 285°C at 50°C min⁻¹ for 14.9 minutes. Spectra were recorded at 70 eV in the electron impact (EI) ionization mode. Compound identities were determined using NIST¹¹, 08, 05, Adams and chemocol mass spectral databases (Adams, 2007).

Results

Table 1: Retention time (min), index and mean (Mean \pm SE, n=3) percent concentration of chemical constituents of seed oil obtained from *A. indica*

No.	Retention Time(min)	Compound name	Retention Index	% Concentration (Mean \pm SE,n=3)
1	11.5227	Sulfurous acid, nonyl 2-propyl ester	998	1.41 \pm 1.00
2	16.7117	1- octadecene	1320	0.8 \pm 0.38
3	20.0696	Oxalic acid, cyclobutyl tridecyl ester	1579	5.46 \pm 0.38
4	20.3972	Oxalic acid, 6-ethyloct-3-yl ethyl ester	1606	15.55 \pm 7.26
5	20.7891	2-hexyl-1-decanol	1640	30.97 \pm 18.86
6	22.6436	Methyl hexadecanoate	1832	3.85 \pm 2.76
7	22.9946	n-Hexadecanoic acid	1865	2.38 \pm 1.52
8	24.6502	Octadec-9-enoic acid	2038	9.92 \pm 4.69
9	24.8491	Octadecanoic acid	2059	29.69 \pm 21.91

Chemical constituents of A. indica and R. communis seed oils

Seeds of *A. indica* and *R. communis* each weighing 500g were extracted with percentage oil yield of 0.59% and 9.52% respectively. Seed oil of *A. indica* was golden yellow in colour while *R. communis* seed oil was colourless. The samples of *A. indica* and *R. communis* seed oils were subjected to Gas chromatography-mass spectrometer and the results obtained are indicated in Tables 1 & 2 and Figures 3 & 5.

From the results in Table 1, the most abundant compounds in the extract of *A. indica* were 2-hexyl-1-decanol (30.97%), Octadecanoic acid (29.69%) and Oxalic acid, 6-ethyloct-3-yl ethyl ester (15.55%). Others were Octadec-9-enoic acid (9.92%), Oxalic acid, cyclobutyl tridecyl ester (5.46%), Methyl hexadecanoate (3.85%), n-Hexadecanoic acid (2.38%), Sulfurous acid, nonyl 2-propyl ester (1.41%) and 1- octadecene (0.8%).

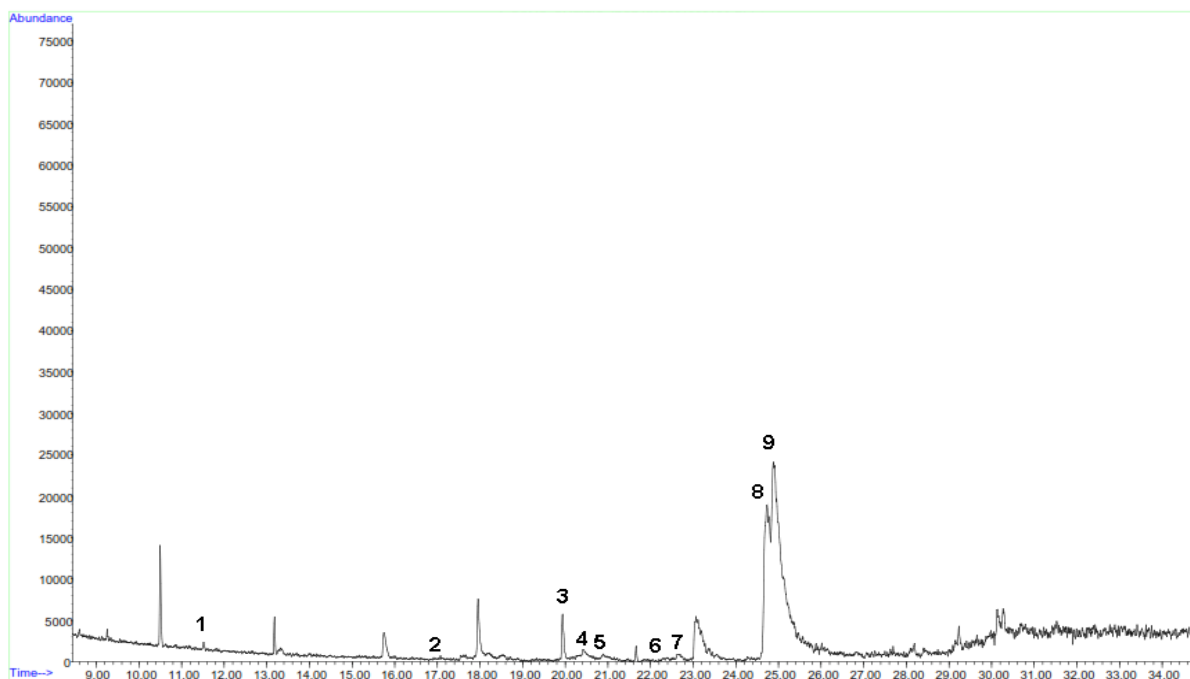


Figure 3: Chromatogram of the seed oil of *Azadirachta indica*. Peaks 1-9 show the components identified (Table 1)

The structures of the nine compounds obtained from the seed oil of *A. indica* were identified (Figure 4).

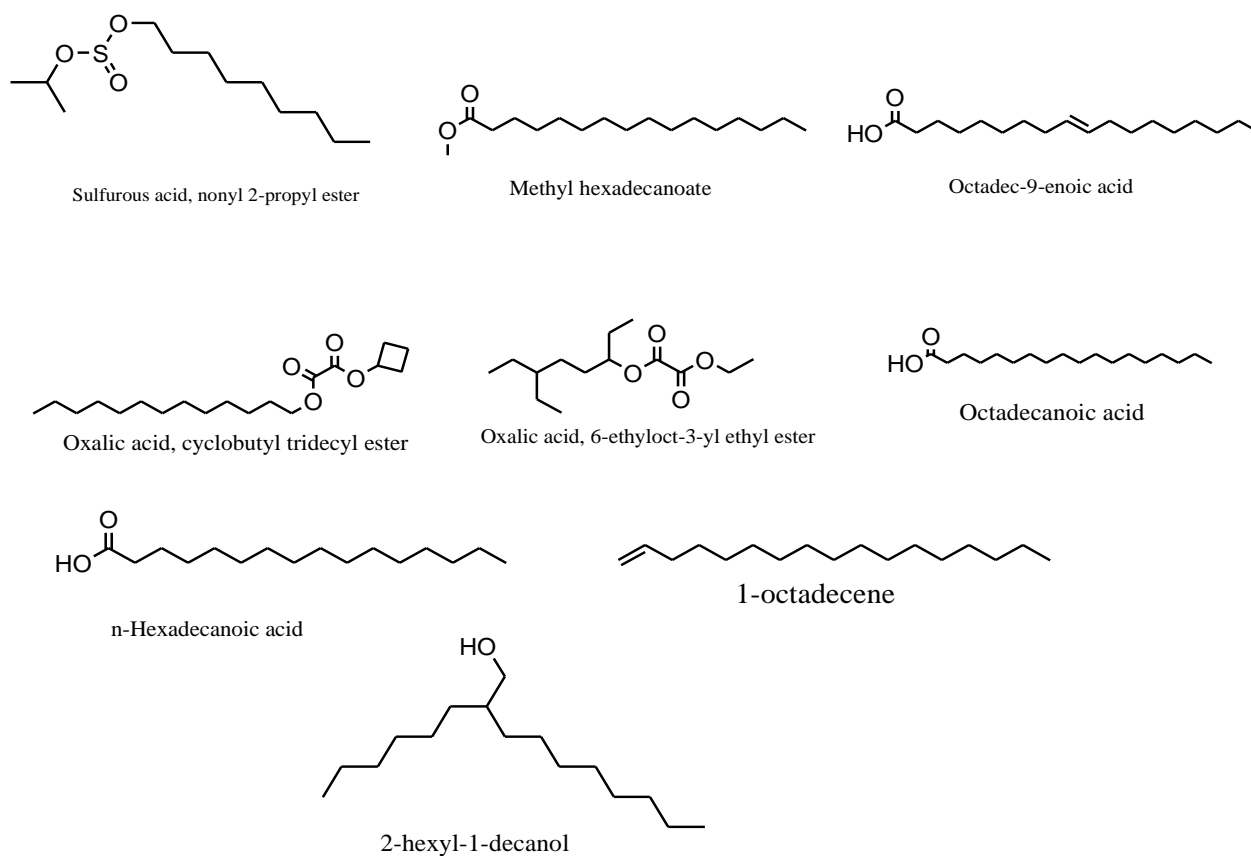


Figure 4: Structures of the nine compounds obtained in the seed oil of *A. indica*

It was concluded that *A. indica* seed oils were dominated by esters 44.4%, fatty acids (33.3%), alkanes and alkenes each at 11.1%.

In Table 2, the most abundant compounds in the extract of *R. communis* were (Z)- 6-

Octadecenoic acid (37.33%), Ricinoleic acid (30.22%) and 13-Hexyloxacyclotridec-10-en-2-one (26.67%). Others were Tetradecane (3.73%) and 2-Ethylbutyric acid, 4-methylpent-2-yl ester (2.06%).

Table 2: Retention time (min), index and mean (Mean \pm SE, n=3) percent concentration of chemical constituents of seed oil obtained from *R. communis*

No.	Retention Time	Compound name	Retention Index	% Concentration (Mean \pm SE, n=3)
1	11.5169	Tetradecane	997	3.73 \pm 0.25
2	22.3043	2-Ethylbutyric acid, 4-methylpent-2-yl ester	1800	2.06 \pm 1.42
3	24.0301	13-Hexyloxacyclotridec-10-en-2-one	1971	26.67 \pm 1.40
4	24.7087	(Z)-6-Octadecenoic acid,	2044	37.33 \pm 12.64
5	26.4169	Ricinoleic acid	2231	30.22 \pm 12.37

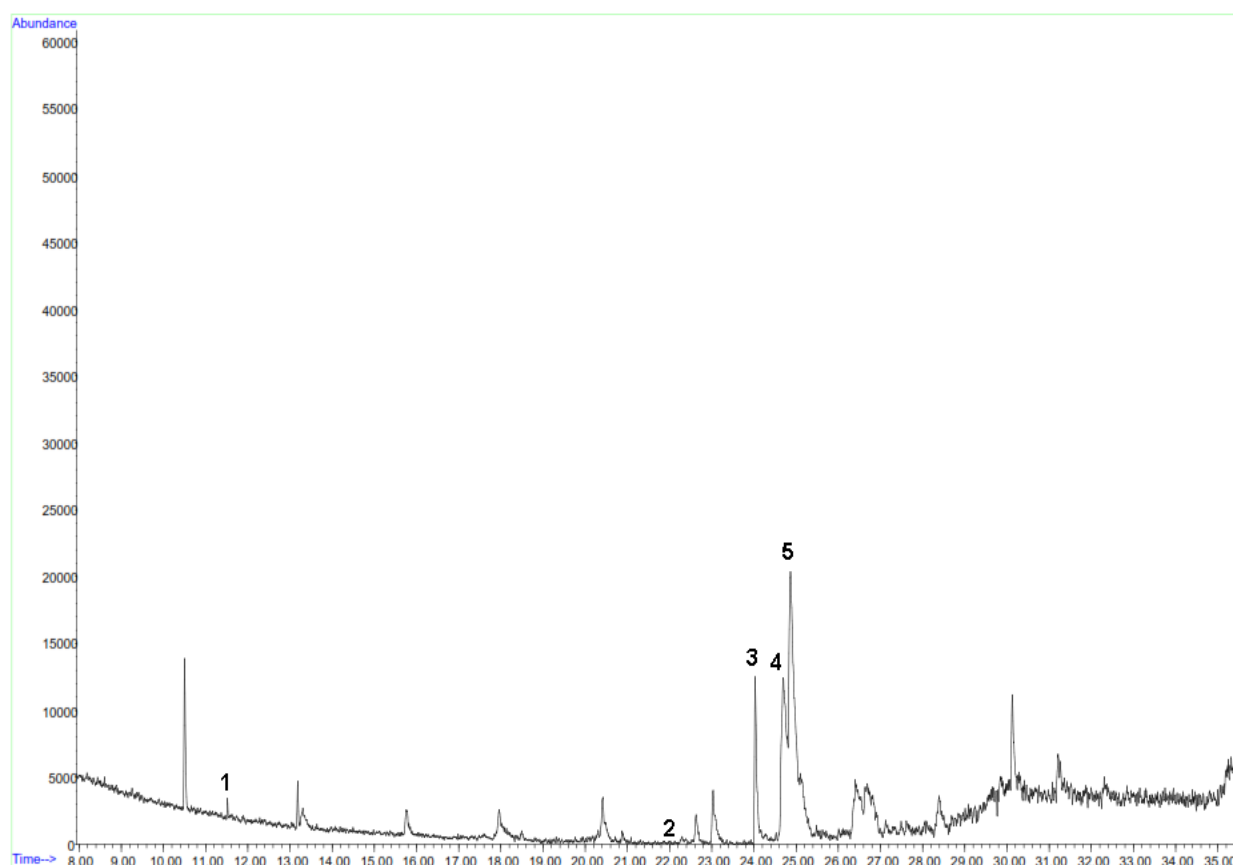


Figure 5: Chromatogram of the seed oil of *Ricinus communis*. Peaks 1-5 show the components identified (Table 2)

Structures of the five compounds found in the seed oil of *Ricinus communis* were identified (Figure 6).

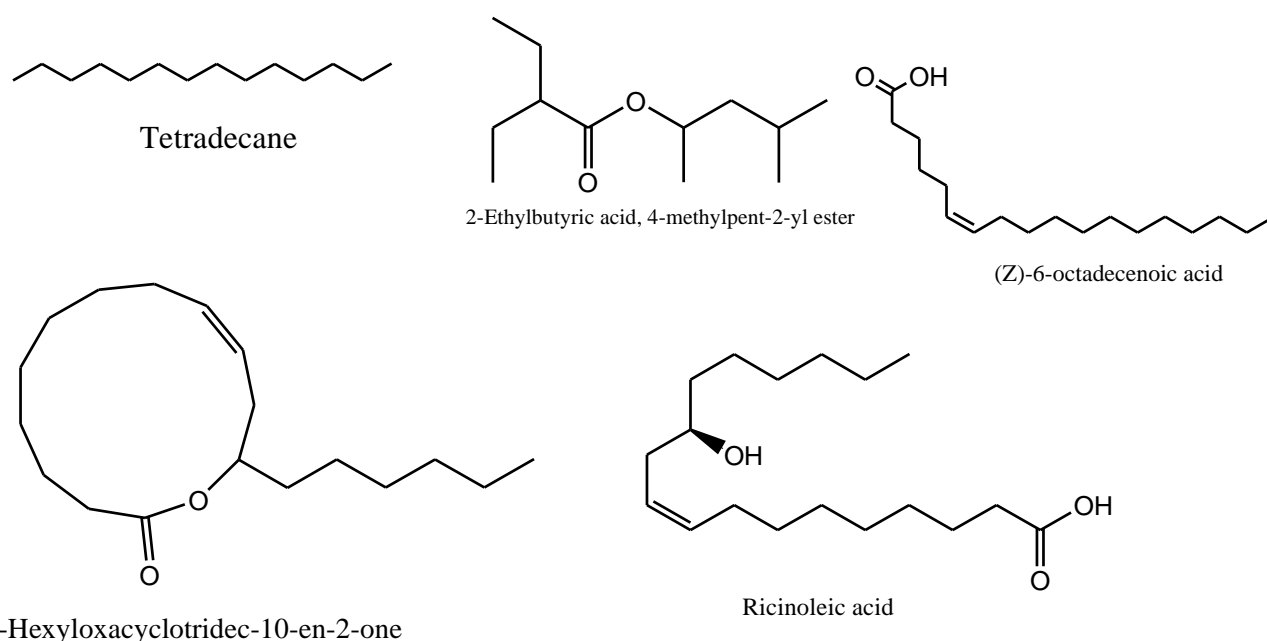


Figure 6: Structures of the five compounds found in the seed oil of *Ricinus communis*

It was concluded that *R. communis* seed oils were dominated by esters and fatty acids each at 40 % and alkanes 20% similar to *A. indica* seed oils.

Discussion

The GC-MS analysis of *A. indica* and *R. communis* seed oils showed the presence of methyl and ethyl esters, cyclic esters, alkanes and alkenes, unsaturated and saturated fatty acids. Jena and Gupta, (2012) reported the phytochemical constituents of *R. communis* seed oil of Indian origin to contain fatty acids and esters of n-hexadecanoic acid (1.2%), Octadecanoic acids (0.7%), Octadec-9-enoic acid (3.2%), 9,12-octadecadienoic acid (3.4%), Eicosanoic acid (0.3%), Ricinoleic acid (89.4%), 12-octadecadienoic acid (0.2%) and Methyl ester. Likewise, Martín *et al.*, (2010) reported predominant fatty acids of *R. communis* and *A. indica* seed oils of Cuban origin to be Ricinoleic acid (86%) and Octadec-9-enoic acid (44.5%), respectively, which were higher than those reported in the present study. Martín *et al.*, (2010) further reported that other minor fatty acids in *R. communis* were n-hexadecanoic acid (1.3%), Octadecanoic acids (1.2%), Octadec-9-enoic acid (3.6%), 9,12-octadecadienoic acid (5.5%) and 12-octadecadienoic acid (0.5%) while *A. indica* seed oils were n-hexadecanoic acid (18.1%), Octadecanoic acids (18.1%), 9,12-

octadecadienoic acid (18.3%), Eicosanoic acid (0.8%) and 12-octadecadienoic acid (3.4%).

Ricinus communis and *A. indica* seed oils of Nigerian origin had varying percentage concentration of chemical constituents. Omowanle *et al.*, (2018) reported the constituent of *R. communis* oil to be Ricinoleic acid (74.42%), Hexadecanoic acid (9.25%), 9, 12-Octadecadienoic acid (6.55%), Octadecanoic acid (7.60%) and 9-Octadecenoic acid (2.18%) while *A. indica* oil was dominated by 9,12-Octadecadienoic Acid (45.56%), Hexadecanoic Acid (27.81%) and Octadecanoic Acid (19.69%). Similarly, Yusuf *et al.*, (2018) findings on *R. communis* oil had presence of Ricinoleic acid (74.10%), 9,12-Octadecadienoic Acid (10.32%), 9-Octadecenoic acid (7.55%), Octadecanoic acid (2.81%) and Hexadecanoic acid (2.59%) by cold pressing method of extraction. Djenontin *et al.*, (2012) also reported that *A. indica* oil obtained from Benin had similar fatty acids including Octadec-9-enoic acid (43.5%), Hexadecanoic acid (17.8%) and Octadecanoic acid (17.4%) apart from 9, 12-Octadecadienoic Acid (18.7%) which was not found in the current study. From the above findings the presence of (Z)-6-Octadecenoic acid and Ricinoleic acid in *R. communis* oil and Octadecanoic acid, Hexadecanoic acid and Octadec-9-enoic acid in *A. indica* oil are consistent with the results of the

current study but differ in percent concentration. The most interesting features are the prominent concentration of 6-Octadecenoic acid (37.33%) of *R. communis* oil and Octadecanoic acid (29.69%) of *A. indica* in the current study which has not been reported elsewhere.

The active compounds available in the plant materials may vary considerably across geographical locations and depending on soil and growth limitations which directly influence the metabolism of the plant and the exposure to different biotic components (Brooker and Kleinig, 2006; Chéraif *et al.*, 2007; Tran and Hinds, 2013). Furthermore, different extracts from the same plant material using different equipment and solvents for extraction may give different responses. For instance, Suryawanshi, (2011) reported on the isolation of active compounds in *A. indica* seed oil through steam distillation method and found to be a mixture of 28 components containing sulphur compounds and esters of fatty acids and solvent extraction of *A. indica* seed oil to have triterpenoids such as azadirachtin, nimbin, nimbidin, salannin. Other studies on solvent extraction of *A. indica* seed oil showed presence of steroids, triterpinoids, reducing sugars, alkaloids, phenolic compounds, flavonoids and tannins (Sharma *et al.* 2013; Azamthulla *et al.*, 2015; Tesfaye and Tefera, 2017) while *R. communis* seed oil are alkaloids, saponins, phenols, flavonoids, ricin, ricinine, lectin and tannins (Sharma *et al.* 2013; Azamthulla *et al.*, 2015) which contrast the composition seed oils in the current study. Mechanical extraction was preferred choice in the current study because it is affordable and easily available to small scale farmers compared to use of expensive solvent extraction.

The chemical constituents found in *A. indica* and *R. communis* seed oils in the current study can be used in different sectors. For example, the presence of Ricinoleic acid in *R. communis* oil makes it unique among vegetable oils as it remains the only commercial source of a hydroxylated fatty acid (Uzoh and Nwabanne, 2016). Removal of hydroxyl group in Ricinoleic acid can be used in the production of alkyl resins for surface coating and biofuel production (Martín *et al.*, 2010; Waidee *et al.*, 2018). Other fatty acids in the present *A. indica* are Octadecanoic acid and Hexadecanoic acid which is known to be used in the production of detergents, soaps, and cosmetics such as

shampoos and shaving cream products (Gunstone, 2004; Mak-Mensah and Firempong, 2011; Warra, 2015).

In therapeutic applications, monounsaturated fatty acid (MUFA) plays an important role in human health and diseases. According to several studies, presence of Octadecenoic acid in the oil composition which was found in both *A. indica* and *R. communis* in the current study can be used in the treatment and prevention of cardiovascular and autoimmune diseases, wound injury, immunomodulation, inhibiting platelet aggregation and cancer. Octadecenoic acid further prevents endoplasmic reticulum stress, lipoapoptosis and insulin resistance in hepatocytes. It also decreases serum low density lipoproteins cholesterol, Thromboxane A₂ (TXA₂) secretion and drug absorption (Sales-Campos *et al.*, 2013; Karacor and Cam, 2015; Pardo *et al.*, 2015). Warra, (2015) reported use of Octadecenoic acid as pharmaceutical solvent and as moisturizer.

Conclusion

The GC-MS analysis of seed oils from both *A. indica* and *R. communis* showed the presence of methyl and ethyl esters, cyclic esters, alkanes and alkenes, unsaturated and saturated fatty acids. They can be used instead of synthetic chemicals as they are biodegradable, environment-friendly, affordable, less side effects and readily available to humans.

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