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ARTOCARPUS METHANOL EXTRACT SEED OILS - A COMPARATIVE STUDY

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Keywords:

Artocarpus, Essential Fatty Acids, GC-MS and Seed Oil

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ABSTRACT: In recent years, the significance of polyunsaturated fatty acids analysis has gained much attention because of their various biological activities in health and disease, especially the n-3 and n-6 fatty acids. The current proportional cram endeavors on providing qualitatively the fatty acid composition of methanol extracted seed oils of five *Artocarpus* species specifically *Artocarpus heterophyllus, Artocarpus integrifolia, Artocarpus inscicus, Artocarpus hirsitus* and *Artocarpus integer*. All the extracted oils were thick yellowish in colour having pungent odour with a yield of 40%. Fallouts evidently indicate that the intricate fatty acid profile was experiential for *A. heterophyllus* through fifteen peaks, next to that *A.hirsitus* by nine peaks, *A.integrifolia* by seven peaks and, *A.inscicus* seed oil had merely five peaks lastly *A. integer* with four peaks. Essential fatty acid 9-Octadecadienoic acid ($C_{18}H_{34}O_2$) and 9, 12 – Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) were found in all tested methanol seed oil free fatty acid methyl ester fractions except *A. inscicus* in that order. These findings demonstrated that essential oils of five *Artocarpus* species seeds were a complex mixture of numerous fatty acids and have great potential to be used as a source for natural health dietary products.

INTRODUCTION: Fatty acids are compounds synthesized in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex. Fatty acids act as building blocks of lipids. In general, they contain even numbers of carbon atoms in straight chains (usually in the range C_{14} to C_{24}), although the synthases can also produce odd and branched chain fatty acids to some extent when supplied with the appropriate precursors; other substituent groups, including double bonds, are normally incorporated into the aliphatic chain later by different enzyme systems [1].

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Fatty acids can either be saturated, monounsaturated or polyunsaturated depending on the number of double bonds [2]. To analyze the fatty acid composition of food lipids, the complex lipids must be pre-treated so that the individual fatty acids are available for chromatographic analysis. Fatty acid components need to be converted into fatty acid methyl esters (FAME) in order to improve their volatility and thus ensuring better gas chromatographic peak shape [3].

FAME analysis of samples demands high chromatographic resolution because of the large number of positional and geometrical isomers of unsaturated fatty acids. Identification of the fatty acids is based on the retention times of FAME and the most common fatty acids are available in commercial reference mixtures. To confirm the identity of an analyte, gas chromatography-mass spectrometry (GC-MS) can be utilized in order to

compare the mass spectrum with a reference spectrum stored in a computer database [4]. The present comparative study aims on providing qualitatively the fatty acid composition of methanol extracted seed oils of five *Artocarpus* species specifically *Artocarpus heterophyllus, Artocarpus integrifolia, Artocarpus inscicus, Artocarpus hirsitus* and *Artocarpus integer.*

MATERIALS AND METHODS:

Extraction of oil by Soxhalation: The *Artocarpus heterophyllus, Artocarpus integrifolia, Artocarpus inscicus, Artocarpus hirsitus* and *Artocarpus integer* methanol seed oils were extracted using soxhlet extraction method with analytical grade methanol as refluxing solvent. At the completion of the extraction process, the oils were recovered from the mixture by distillation and stored in dessicator until required to exploit [5].

The percentage of oil content can be calculated as below

$$\% \text{ of oil} = \frac{\text{Wt. of oil obtained in gm.}}{\text{Wt. of seed taken in gm.}} \times 100$$

Determination of fatty acid composition by GC–MS: Fatty acid composition of hexane extracted seed oils specifically *Artocarpus heterophyllus, Artocarpus integrifolia, Artocarpus inscicus, Artocarpus hirsitus* and *Artocarpus integer* were determined using GC-MS according to the method described by Lepage and Roy [3]. Test seed oil samples (20 mg) were separately mixed with 20ml of methanol and acetyl chloride (20:1, v/v) solution and to this 20 ml hexane was added. Mixture was heated at 100^0C for 30 mins under continuous stirring. After cooling to room temperature, 20 ml of water was added and using separating funnel, the fatty acid methyl esters were extracted in hexanic layer. Three more extractions with hexane were made to ensure complete removal of methyl esters (REMI centrifuge, R-8C, India).

The clear supernatant (2 ml) was transferred to an auto sampler vial and injected with auto injector (AOC-20 i) into GC-MS for analysis. The GC-MS analysis carried out in a Shimadzu GC-MS-QP2010SE, equipped with a KRATOS mass detector model MS25RF (sector instrument) and a capillary column of DB×LB (30 m x 0.32 mm, 0.50 µm film thickness), carrier gas helium, constant pressure 90 kPa, split 1:10. The oven was programmed initially from 70^0C with 2 min hold up time to the final temperature of 250^0C with 5^0C/min ramp. The final temperature hold time was 20 min. The inlet and GC/MS interface temperatures were kept at 250^0C and 280^0C respectively. The temperature of EI 70 eV source was 200^0C with full scan (25-450m/z), scan time 0.3 s. The mass spectra of essential oil components were identified by comparing the mass spectra of the analytes with those of authentic standards from the mass spectra of Wiley 229.LIB and Mass Spectra Library NIST 05.LIB as well as on comparison of their retention indices of literature.

RESULTS: The *Artocarpus heterophyllus, Artocarpus integrifolia, Artocarpus hirsitus, Artocarpus inscicus* and *Artocarpus integer* seed oil extraction was carried out by soxhlet extraction method as per the direction of AOAC 1998 (Association of Official Analytical Chemists). GC-MS analysis of methanolic seed oil of *A. heterophyllus* showed the presence of prominent fifteen peaks indicating the presence of fifteen phyto constituents, namely Benzene (RT: 3.228 and Peak area: 31.82), Benzene, methyl ester (RT: 4.238 and Peak area: 0.33), Octanoic acid, methyl ester (RT: 8.272 and Peak area: 0.53), Decanoic acid, methyl ester (RT: 10.273 and Peak area: 0.39), Hexadeca methylcyclooctasiloxane (RT: 10.650 and Peak area: 0.05), Methyl dodecanoate (RT: 12.042 and Peak area: 3.44), Tetradecanoic acid (RT: 13.633and Peak area: 1.56), Palmitic acid (RT: 15.082and Peak area: 2.49), Octadecanoic acid, methyl ester (RT: 16.503 and Peak area: 1.07), Methyl, 9-Octadecenoate (RT: 16.699 and Peak area: 4.28), Methyl linoleate (RT: 17.097 and Peak area: 4.41), 9,12,15-Octadecatrienoic acid (RT: 17.677and Peak area: 0.23), 11-Eicosenoic acid (RT: 18.423 and Peak area: 0.30), 9-Octadecenoic acid (RT: 23.805and Peak area: 44.15) and 1,2-Benzenedicarboxylic acid (RT: 26.974 and Peak area: 4.94).

The GC-MS chromatogram and table were displayed in **Fig. 1** and **Table 1**. In favour of methanol seed extract of *A. integrifolia* confirmed seven peaks, representing the presence seven compounds, explicitly 2,4-Di-tert-butylphenol (RT: 12.799 and Peak area:3.69), Cyclononasiloxane,

octadecamethyl ester (RT: 14.525 and Peak area: 1.14), n-Docosane (RT: 15.110 and Peak area: 31.88), 3-Hexadecanol (RT:15.323 and Peak area:1.48), Hexatriacontane (RT: 18.860 and Peak area: 24.84), 9-Octadecenoic acid (RT:23.440 and Peak area: 22.72) and 10, 13- Octadecadienoic acid, methyl ester (RT: 29.142 and Peak area: 12.42).

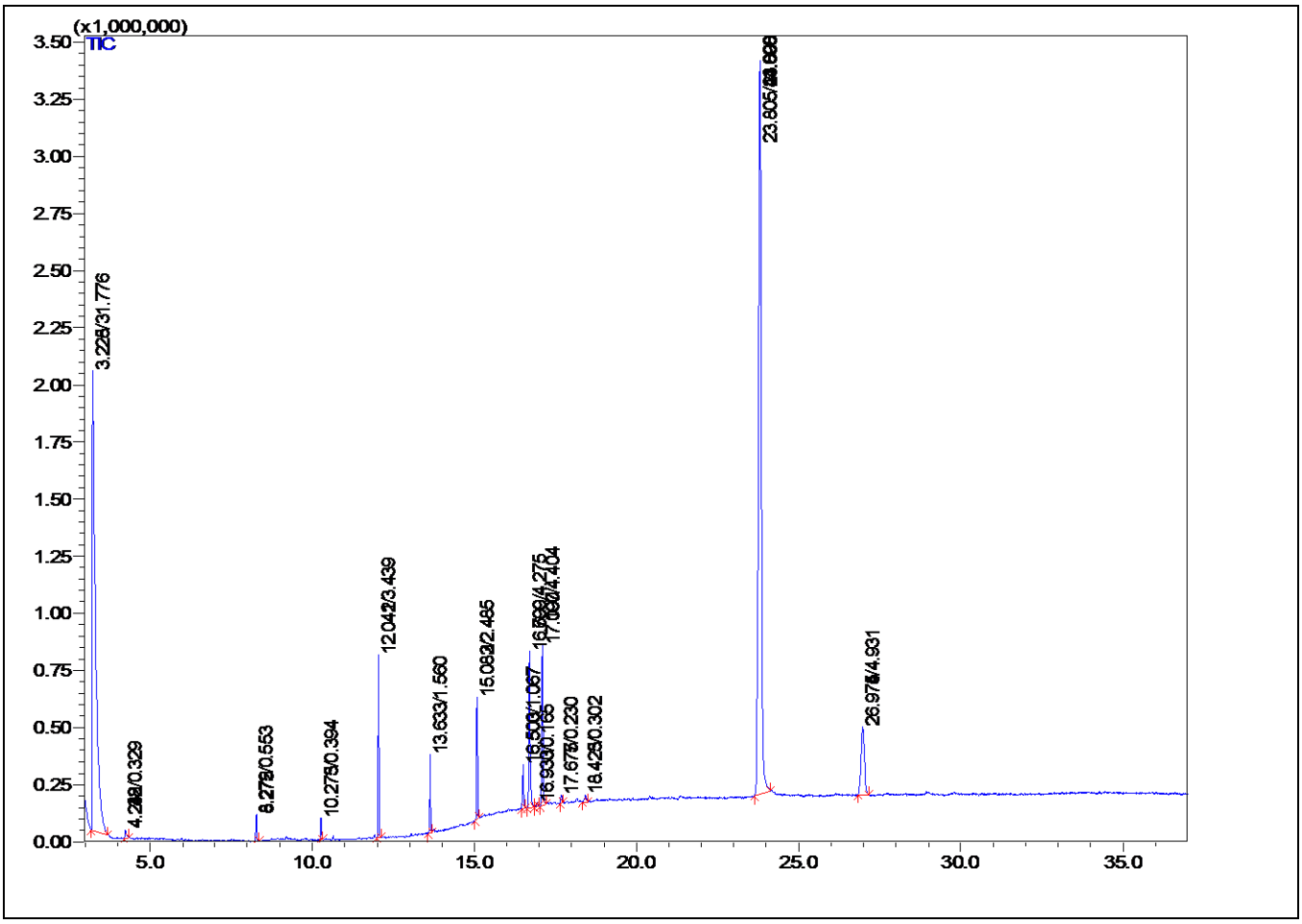

FIG. 1: GC-MS CHROMATOGRAM OF *ARTOCARPUS HETEROPHYLLUS* METHANOL EXTRACTED SEED OIL

TABLE 1: TOTAL IONIC CHROMATOGRAM (GC-MS) OF *ARTOCARPUS HETEROPHYLLUS* METHANOL EXTRACTED SEED OIL OBTAINED WITH TEMPERATURE OF EI 70 eV USING A CAPILLARY COLUMN OF DB×LB WITH He GAS AS THE CARRIER

S. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	3.228	Benzene	C_6H_6	78.11	31.82
2	4.238	Benzene, methyl ester	C_7H_8	92.14	0.33
3	8.272	Octanoic acid, methyl ester/ Caprylic acid methyl ester	$C_9H_{18}O_2$	158.24	0.53
4	10.273	Decanoic acid, methyl ester/ Capric acid methyl ester	$C_{11}H_{22}O_2$	186.29	0.39
5	10.650	Hexadeca methylcyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	593.23	0.05
6	12.042	Methyl dodecanoate [CAS] Lauric acid, methyl ester	$C_{13}H_{26}O_2$	214.34	3.44
7	13.633	Tetradecanoic acid [CAS] Myristic acid	$C_{14}H_{28}O_2$	228.37	1.56
8	15.082	Hexadecanoic acid [CAS] Palmitic acid	$C_{16}H_{32}O_2$	256.42	2.49
9	16.503	Octadecanoic acid, methyl ester [CAS] Stearic acid methyl ester	$C_{19}H_{38}O_2$	298.50	1.07
10	16.699	Methyl, 9-Octadecenoate [CAS] Oleic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	4.28
11	17.097	9, 12-Octadecadienoic acid, methyl ester [CAS] Methyl linoleate	$C_{19}H_{34}O_2$	294.47	4.41
12	17.677	9,12,15-Octadecatrienoic acid [CAS] α-Linolenic acid	$C_{18}H_{30}O_2$	278.43	0.23
13	18.423	11-Eicosenoic acid	$C_{20}H_{38}O_2$	310.51	0.30
14	23.805	9-Octadecenoic acid [CAS] Oleic acid	$C_{18}H_{34}O_2$	282.46	44.15
15	26.974	1,2-Benzenedicarboxylic acid [CAS] Phthalic acid	$C_8H_6O_4$	166.13	4.94

The GC-MS chromatogram and table were put on show in **Fig. 2** and **Table 2**. Whereas *A.hirsitus* methanol seed extract showed nine peaks, on behalf of the presence nine phytoconstituents, namely Octanoic acid, methyl ester (RT: 6.507 and Peak area: 3.71), Methyl caprate (RT: 8.437 and Peak area: 2.30), Methyl laurate (RT: 10.156 and Peak area:19.92), Methyl myristate (RT: 11.655 and Peak area: 5.90), Methyl stearate (RT: 13.023 and Peak area: 3.32), Methyl stearate (RT: 14.284 and Peak area: 2.42), Oleic acid, methyl ester (RT: 14.449 and Peak area: 7.28), Methyl linoleate (RT: 14.767and Peak area: 5.10) and Methyl ricinoleate (RT: 18.950 and Peak area: 50.05).

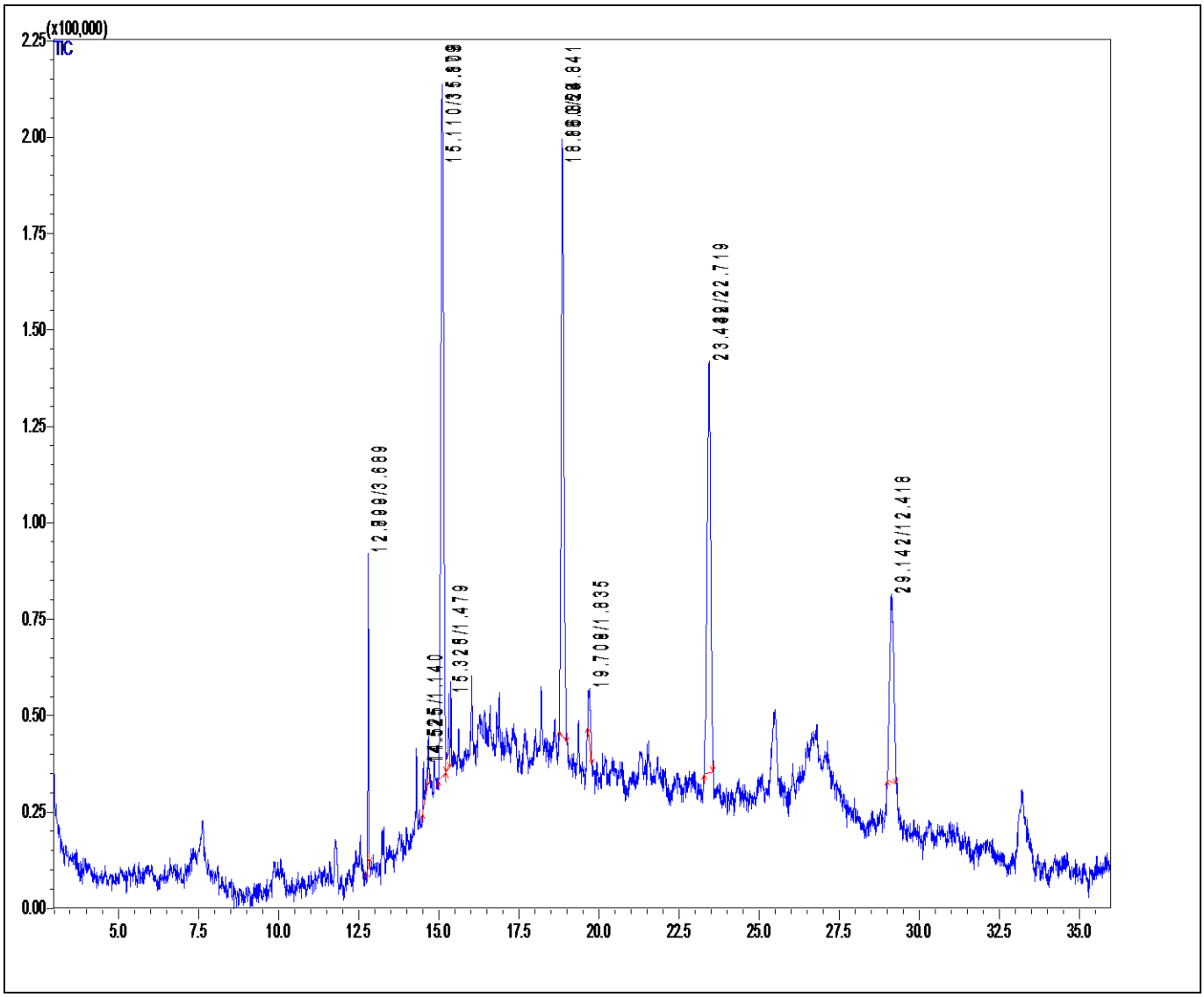

FIG. 2: GC-MS CHROMATOGRAM OF *ARTOCARPUS INTEGRIFOLIA* METHANOL EXTRACTED SEED OIL

TABLE 2: TOTAL IONIC CHROMATOGRAM (GC-MS) OF *ARTOCARPUS INTEGRIFOLIA* METHANOL EXTRACTED SEED OIL OBTAINED WITH TEMPERATURE OF EI 70 eV USING A CAPILLARY COLUMN OF DB×LB WITH He GAS AS THE CARRIER

S.No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	12.799	Phenol, 2,4-bis (1,1-dimethylethyl)- / 2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	3.69
2	14.525	Cyclononasiloxane, octadecamethyl ester	$C_{18}H_{54}O_9Si_9$	667.39	1.14
3	15.110	Docosane / n-Docosane	$C_{22}H_{46}$	310.60	31.88
4	15.323	3-Hexadecanol [CAS] Palmityl alcohol	$C_{16}H_{34}O$	242.44	1.48
5	18.860	Hexatriacontane	$C_{36}H_{74}$	506.97	24.84
6	23.440	9-Octadecenoic acid [CAS] Oleic acid	$C_{18}H_{34}O_2$	282.46	22.72
7	29.142	10, 13- Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	12.42

The GC-MS chromatogram and tabular form were put on view in **Fig. 3** and **Table 3**. Penultimate methanol *A.inscicus* seed oil demonstrated five peaks, in support of the presence five compounds, they were 2,4-Di-tert-butylphenol (RT: 12.804and Peak area: 2.73), Palmitic acid (RT: 16.724 and Peak area: 19.89), Linoleic acid (RT: 18.904 and Peak area: 46.11), Glycerol 1, 3- Dihexadecanoate (RT: 21.621and Peak area: 7.83) and (R)-(-)-14-Methyl-8-hexadecyn-1-ol (RT: 26.515and Peak area: 23.44).

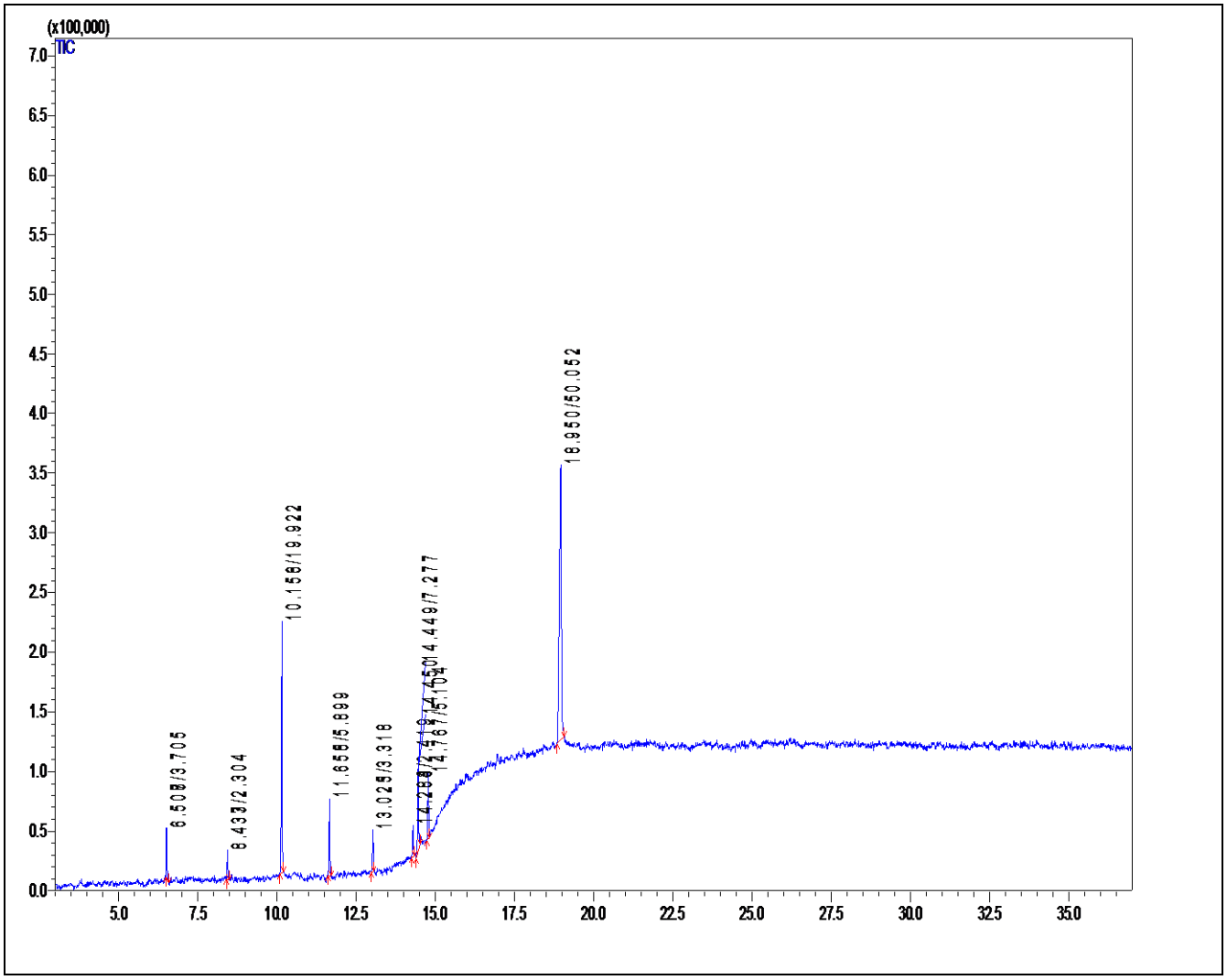

FIG. 3: GC-MS CHROMATOGRAM OF *ARTOCARPUS HIRSITUS* METHANOL EXTRACTED SEED OIL

TABLE3: TOTAL IONIC CHROMATOGRAM (GC-MS) OF *ARTOCARPUSS HIRSITUS* METHANOL EXTRACTED SEED OIL OBTAINED WITH TEMPERATURE OF EI 70 eV USING A CAPILLARY COLUMN OF DB×LB WITH He GAS AS THE CARRIER

S.No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area%
1	6.507	Octanoic acid, methyl ester/ Methyl octanoate	$C_9H_{18}O_2$	158.24	3.71
2	8.437	Decanoic acid, methyl ester [CAS] Methyl caprate	$C_{11}H_{22}O_2$	186.29	2.30
3	10.156	Dodecanoic acid, methyl ester [CAS] Methyl laurate	$C_{13}H_{26}O_2$	214.34	19.92
4	11.655	Tetradecanoic acid, methyl ester [CAS] Methyl myristate	$C_{15}H_{30}O_2$	242.40	5.90
5	13.023	Octadecanoic acid, methyl ester [CAS] Methyl stearate	$C_{19}H_{38}O_2$	298.50	3.32
6	14.284	Octadecanoic acid, methyl ester [CAS] Methyl stearate	$C_{19}H_{38}O_2$	298.50	2.42
7	14.449	9-Octadecenoic acid (Z)-, methyl ester[CAS] Oleic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	7.28
8	14.767	9, 12-Octadecadienoic acid, methyl ester [CAS]Methyl linoleate	$C_{19}H_{34}O_2$	294.47	5.10
9	18.950	Methyl ricinoleate / 9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]	$C_{19}H_{36}O_3$	312.49	50.05

The GC-MS chromatogram and table were showed in **Fig. 4** and **Table 4.** Finally for *A. integer* methanol seed extract displayed four peaks, all for the presence four compounds, they were Oleic acid methyl ester (RT:14.09 and Peak area: 0.82), 9,12-Octadecadienoic acid, methyl ester (RT:14.413 and Peak area:0.90), Cinnamic acid, m-methoxy-, trimethylsilyl ester (RT: 17.117 and Peak area:0.63) and Methyl ricinoleate (RT:18.344 and Peak area:97.66).

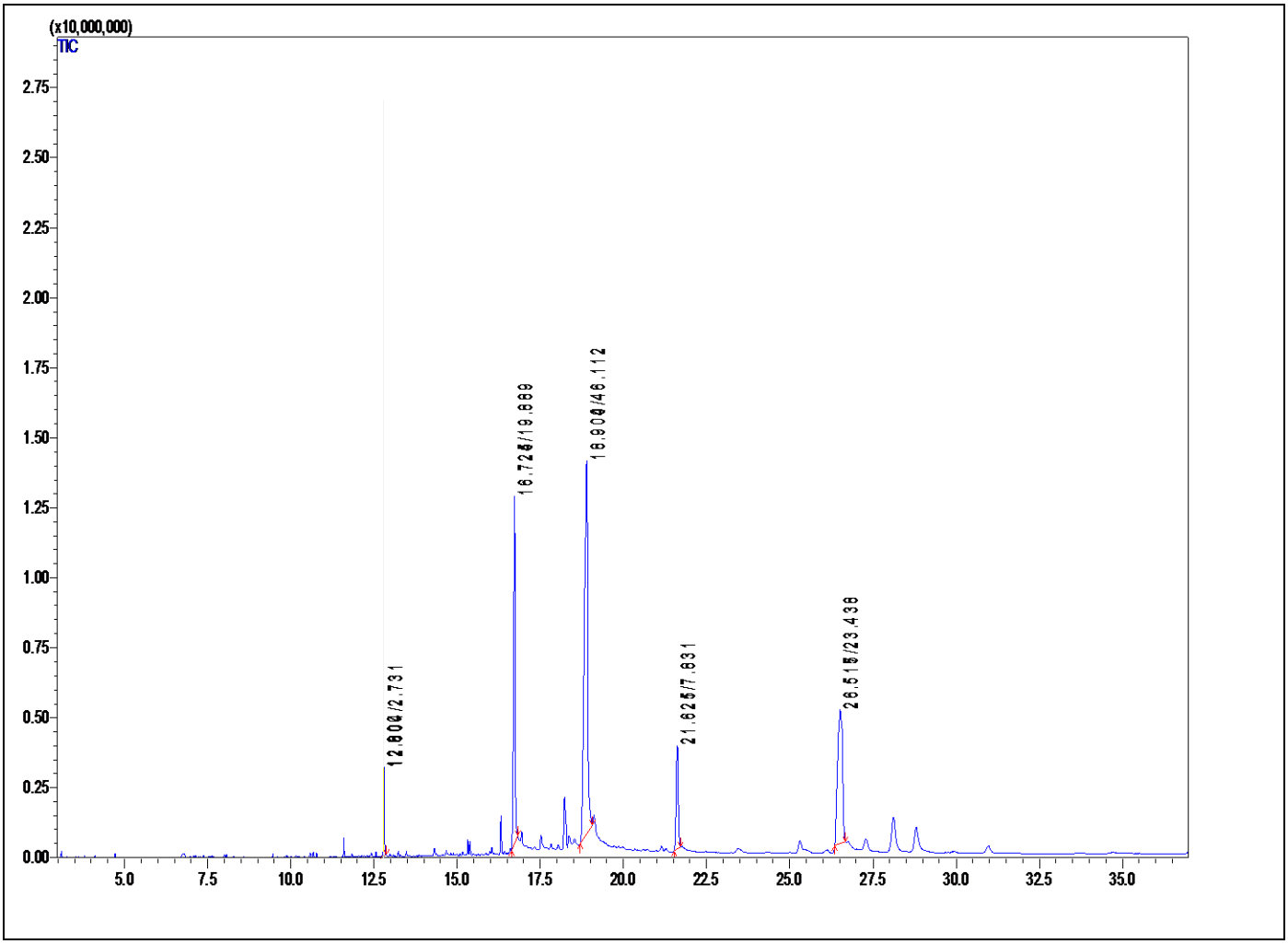

FIG. 4: GC-MS CHROMATOGRAM OF *ARTOCARPUS INSCICUS* METHANOL EXTRACTED SEED OIL

TABLE 4: TOTAL IONIC CHROMATOGRAM (GC-MS) OF ARTOCARPUS INSCICUS METHANOL EXTRACTED SEED OIL OBTAINED WITH TEMPERATURE OF EI 70 eV USING A CAPILLARY COLUMN OF DB×LB WITH He GAS AS THE CARRIER

S.No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	12.804	2, 4-Di-tert-butylphenol/ Phenol, 2, 4-bis (1, 1-dimethylethyl)	$C_{14}H_{22}O$	206.32	2.73
2	16.724	Hexadecanoic acid (CAS) Palmitic acid	$C_{16}H_{32}O_2$	256.42	19.89
3	18.904	Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid	$C_{18}H_{32}O_2$	280.45	46.11
4	21.621	Glycerol 1,3-Dihexadecanoate/Dipalmitin	$C_{35}H_{68}O_5$	568.91	7.83
5	26.515	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	$C_{17}H_{34}O$	254.45	23.44

The GC-MS chromatogram and tabular form were exhibited in **Fig. 5** and **Table 5**. From the above outcomes evidently indicate that the intricate phytoconstituents were experiential for *A. heterophyllus* with fifteen peaks subsequently *A.hirsitus* through nine peaks, next to that *A.integrifolia* and *A.inscicus* by seven and five peaks each, finally *A. integer* seed methanolic extract had four peaks. Caprylic acid methyl ester ($C_9H_{18}O_2$), Capric acid methyl ester ($C_{11}H_{22}O_2$),

Lauric acid, methyl ester ($C_{13}H_{26}O_2$), Myristic acid ($C_{14}H_{28}O_2$) and its ester ($C_{15}H_{30}O_2$), Stearic acid methyl ester ($C_{19}H_{38}O_2$) monitored both in *A. heterophyllus* and in *A.hirsitus*. Palmitic acid ($C_{16}H_{32}O_2$), checked in *A. heterophyllus* and in *A.inscicus*. Oleic acid ($C_{18}H_{34}O_2$) and its ester ($C_{19}H_{36}O_2$) present in all tested fractions except *A.inscicus*. Methyl linoleate ($C_{19}H_{34}O_2$) present in *A. heterophyllus*, *A.hirsitus* and in *A. integer*. Methyl ricinoleate ($C_{19}H_{36}O_3$) ensured in *A.hirsitus* and in *A. integer*. Whereas 2, 4-Di-tert-butylphenol present in *A.integrifolia* and in *A.inscicus.*

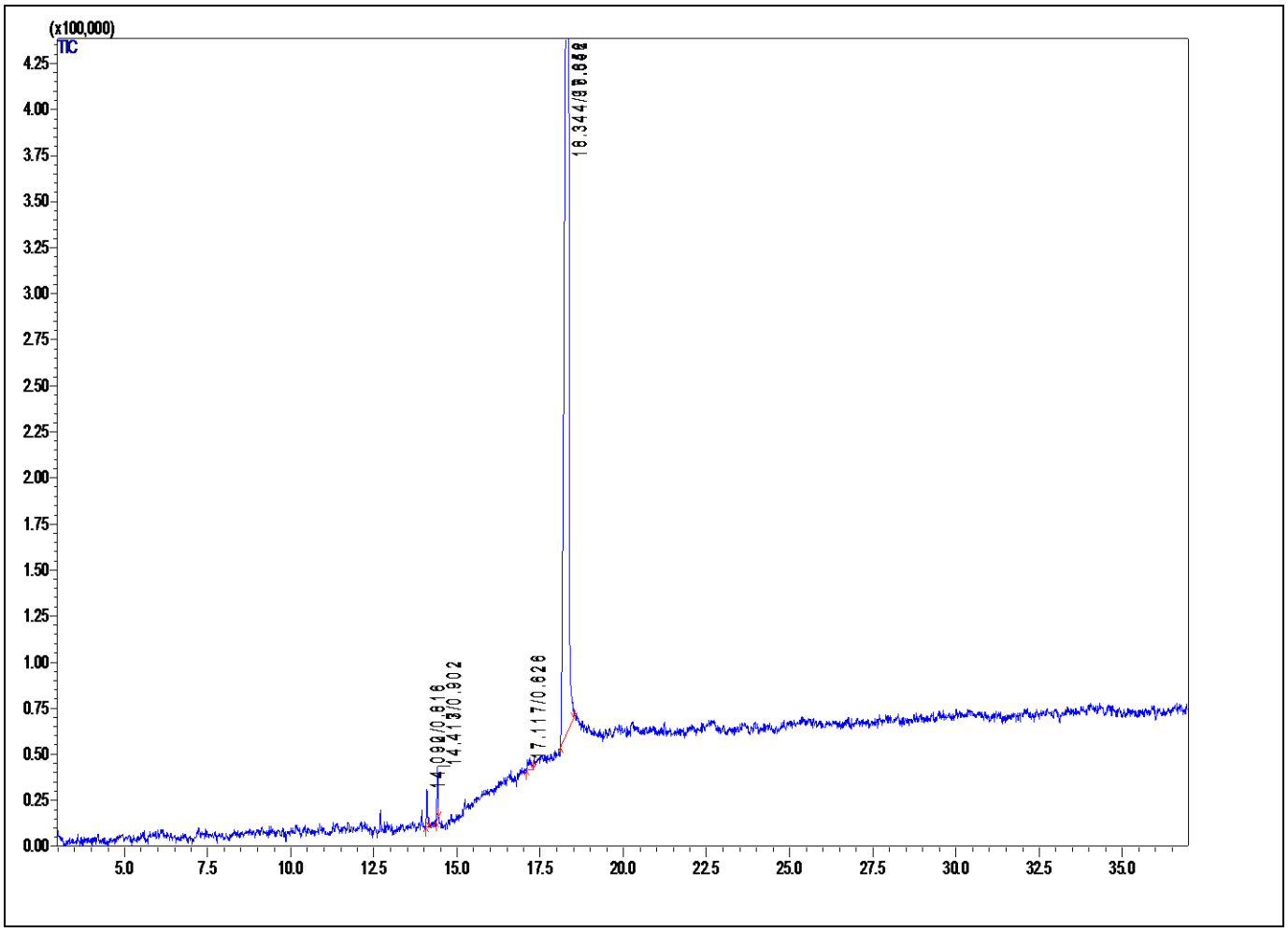

FIG. 5: GC-MS CHROMATOGRAM OF *ARTOCARPUS INTEGER* METHANOL EXTRACTED SEED OIL

TABLE 5: TOTAL IONIC CHROMATOGRAM (GC-MS) OF *ARTOCARPUS INTEGER* METHANOL EXTRACTED SEED OIL OBTAINED WITH TEMPERATURE OF EI 70 eV USING A CAPILLARY COLUMN OF DB×LB WITH He GAS AS THE CARRIER.

S. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	14.090	9-Octadecenoic acid methyl ester[CAS] Oleic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	0.82
2	14.413	9,12-Octadecadienoic acid, methyl ester[CAS] Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	0.90
3	17.117	Cinnamic acid, m-methoxy-, trimethylsilyl ester	$C_{13}H_{18}O_3Si$	250.37	0.63
4	18.344	Methyl ricinoleate/ 9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]	$C_{19}H_{36}O_3$	312.49	97.66

Previously various investigators worked out on diverse group of plant species for their fatty acid profiles by means of GC-MS. Ravi Kiran and Raghava Rao (2014) investigated lipid Profile of *Ceiba pentandra* seed oil by GC-MS and disclosed that *Ceiba pentandra* seed oil was complex mixture of numerous compounds, among them, palmitic acid appears to be the significant one along with

essential fatty acid linoleic acid. It is also found that the *Ceiba pentandra* seed oil was comparable to cotton seed oil especially in the composition of palmitic and linoleic acids. Marimuthu Krishnaveni et al (2014) analyzed fatty acid profile of Gossypium seeds through GC-MS in order to unearth the fatty acids profile. Amongst the fatty acids studied, 9, 12-Octadecadienoic acid (Z, Z)-, 9, 12-Octadecadienoic acid (Z, Z)-methyl ester, Hexadecanoic acid methyl ester were found to be high. Traces of margaric acid methyl ester were also observed along with additional methyl esters.

Syeda Farina Asghar et al (2011) explored petroleum ether extract (oil) of the whole plant *Iris germanica* with Gas chromatography-mass spectrometry (GC-MS) and identified eleven compounds specifically, 9-hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester, 8-octadecenoic acid methyl ester, 11-octadecenoic acid methyl, 10-octadecenoic acid methyl ester, 13-octadecenoic acid methyl ester, 16-octadecenoic acid methyl ester, 1,2-benzenedicarboxylic acid diisooctyl ester, bis (2- ethylhexyl) phthalate, methyl 6-methyl heptanoate and nonanoic acid, 9-oxo-methyl ester.

Vijisaral Elezabeth and Arumugam (2014) evaluated the chemical constituents of plant *Indigofera suffruticosa* leaves using GC-MS. The GC-MS analysis provided different peaks determining the presence of nine different phytochemical compounds namely pentadecanoic acid,14-methyl-,methyl ester (5.86%), n-hexanedecanoic acid (9.83%), z-[13, 14-epoxy]tetradec-11-en-1-ol acetate (6.37%), oleic acid (10.43%), 9-octadecenoic acid[z]-,2-hydroxy-1-[hydroxyl methyl]ethyl ester (10.21%), heptanoic acid, docosyl ester (6.28%), octadecanoic acid, 7-hydroxy-, methyl ester (4.89%), 6-octadecenoic acid[z]- (18.47%) and 8-octadecenoic acid, methyl ester (14.97%). The acknowledged compounds of test samples possess loads of biological properties, which were discussed here under.

DISCUSSION: The significance of polyunsaturated fatty acids scrutiny has put on much interest because of their diverse natural tricks in wellbeing and illness, especially the n-3 and n-6 fatty acids. These fatty acids play a vital role in the prevention and dealing of cardiovascular diseases, autoimmune diseases, eye sight and the improvement of learning ability [8].

From the above outcomes evidently indicate that the intricate fatty acid profile was experiential for *A. heterophyllus* through fifteen peaks, next to that *A.hirsitus* by nine peaks, *A.integrifolia* by seven peaks and, *A.inscicus* seed oil had merely five peaks lastly *A. integer* with four peaks. Essential fatty acid 9-Octadecadienoic acid ($C_{18}H_{34}O_2$) and 9, 12 – Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) were found in all tested methanol seed oil free fatty acid methyl ester fractions except *A. inscicus* correspondingly. Cheryan [9] cited that the crude oil that is extracted from the oil seeds is a mixture of triacylglycerols, partial acylglycerols, free fatty acids, phosphatides, pigments, sterols and tocopherols (compounds that present vitamin E activity).

Previously various investigators worked out on diverse group of plant species for their fatty acid profiles by means of GC-MS. Ravi Kiran and Raghava Rao [10] investigated lipid Profile of *Ceiba pentandra* seed oil by GC-MS and disclosed that *Ceiba pentandra* seed oil was complex mixture of numerous compounds, among them, palmitic acid appears to be the significant one along with essential fatty acid linoleic acid. It is also found that the *Ceiba pentandra* seed oil was comparable to cotton seed oil especially in the composition of palmitic and linoleic acids.

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methyl]ethyl ester (10.21%), heptanoic acid, docosyl ester (6.28%), octadecanoic acid, 7-hydroxy-, methyl ester (4.89%), 6-octadecenoic acid[z]- (18.47%) and 8-octadecenoic acid, methyl ester (14.97%).

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The fatty acid composition of the extracted lipid revealed using the gas chromatography (GC).

Both oleic acid (44.7%) and linoleic acid (32.8%) were detected as the dominant fatty acids while palmitic acid and stearic acid were the saturated fatty acids also found in the *Jatropha* oil.

CONCLUSION: Lipid profiles of five varieties *Artocarpus* seed oils by GC-MS showed that presence of biomedical components, which can be developed into top value-added materials of high-grade spice, cosmetic, food, and industrial chemical and solvent etc. our result as well benefit the development of new food sources and for the formulation of food supplements for the revival from malnutrition.

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