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RESEARCH ARTICLE

LIPID PROFILES OF FIVE VARIETIES *ARTOCARPUS* SEED OILS BY GC-MS- A COMPARATIVE STUDY.

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Key words:-

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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 endeavors on providing qualitatively the fatty acid composition of hexane 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. integer* with seven peaks subsequently *A. heterophyllus* through five peaks, next to that *A.integrifolia* and *A.hirsitus* by four peaks each, lastly *A.inscicus* seed oil had merely three peaks. Essential fatty acid 9, 12 – Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) and 10, 13-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) were found in all tested hexane seed oil free fatty acid methyl ester fractions except *A. inscicus* and in *A. heterophyllus* 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.

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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 C14 to C24), 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]. 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

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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 hexane 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* hexane seed oils were extracted using soxhlet extraction method with analytical grade hexane 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

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

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°C 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°C with 2 min hold up time to the final temperature of 250°C with 5°C/min ramp. The final temperature hold time was 20 min. The inlet and GC/MS interface temperatures were kept at 250°C and 280°C respectively. The temperature of EI 70 eV source was 200°C 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). Hexane was used as solvent for extracting the oil from seeds and these were passed out for 10 hrs. All the extracted oils were thick yellowish in colour having pungent odour with a yield of 40%. GC-MS analysis of the *A. heterophyllus* seed oil free fatty acid methyl ester fraction showed the presence of prominent five peaks indicating the presence of five compounds, namely methyl - 9, 9, 10, 10 - 4 - Octadecanoate (RT: 14.408 and Peak area: 2.42), no information available for its biological significance. Methyl Octadecanoate (RT:15.750 and Peak area:2.58), a saturated fatty acid, generally called as stearic acid, has an enormous variety of uses as an ingredient in food, cosmetics and in industrial products. It is also often used to change the consistency or melting temperature of a product, as a lubricant or to prevent oxidation [6]. 9-Octadecenoic acid [Z] - methyl ester (RT: 15.952 and Peak area: 2.27), an unsaturated fatty acid, commonly entitled as oleic acid, methyl ester. It is used as a defoamer and solvent for inks and coatings and as a tar remover and as a raw material of emulsifiers or oiling agents for foods, spin finishes, surfactant and base material for perfume and solvent or co solvent and oil carrier in agricultural industry. 9, 12-Octadecadienoic acid, methyl ester (RT: 16.334 and Peak area: 2.63), familiarly called as linoleic acid, an essential omega-6-fatty acid. The main physiological requirement for omega-6-fatty acid is attributed to arachidonic acid. It is the major precursor of prostaglandins, leukotrienes and anandamides that play a vital role in cell signaling [2]. Z, E-3, 13-Octadecadien-1-ol (RT: 22.524 and Peak area: 90.10), feel right to pheromones chemical class. The GC-MS chromatogram and table were displayed in Fig. 1 and Table 1. In favour of *A. integrifolia* seed oil confirmed four peaks, representing the presence four compounds, explicitly Eicosanoic acid, methyl ester (RT: 14.397 and Peak area: 8.32), wax like solid, soluble in alcohol and ether, used in medical research and as a reference standard for gas chromatography. It is also known as

specialty fatty acid. 9- Hexadecenoic acid, methyl ester [Z] (RT: 15.933 and Peak area: 7.93), frequently called as Palmitoleic acid, methyl ester. 9, 12 –Octadecadienoic acid, methyl ester (RT: 16.315 and Peak area: 8.91), conferred above and 10, 13- Octadecadienoic acid, methyl ester (RT: 22.447 and Peak area: 74.84), no information accessible for its significance. The GC-MS chromatogram and table were put on show in Fig. 2 and Table 2. Whereas *A.hirsutus* extracted seed oil free fatty acid methyl ester fraction showed four peaks, on behalf of the presence four compounds, namely 2-trifluoromethyl – N- N- dimethyl 4 –nitroaniline (RT:3.758 and Peak area:2.14), no biological significance was established. 7-hexadecenoic acid, methyl ester [Z] (RT: 15.873 and Peak area: 2.23), commonly called as Palmitoleic acid methyl ester, Palmitoleic acid-based diets raise low-density lipoprotein cholesterol and diminish high-density lipoprotein cholesterol, even when dietary intake of cholesterol is maintained at a low level [7]. 9, 12 – Octadecadienoic acid, methyl ester (RT: 16.248 and Peak area: 2.98) and 10, 13 – Octadecadienoic acid, methyl ester (RT: 22.386 and Peak area: 92.65), conferred above. The GC-MS chromatogram and tabular form were put on view in Fig. 3 and Table 3. Penultimate hexane extracted *A.inscius* seed oil demonstrated three peaks, in support of the presence three compounds, they were Oxacycloheptadec -8-en-2-one (RT: 16.247 and Peak area:7.24), used as a flavour or fragrance ingredient. 2-ethoxythiazole (RT: 18.841 and Peak area: 6.71), used as a food flavor. 10, 13- Octadecadienoic acid, methyl ester (RT: 22.323 and Peak area: 86.05), discussed over. The GC-MS chromatogram and table were showed in Fig. 4 and Table 4. Finally for *A. integer* extracted seed oil displayed total seven peaks, all for the presence seven compounds, they were 2, 2, 6, 6 – tetradentorio – 3, 7, diacetyl 3, 7 –diazadodecane (RT: 14.767 and Peak area:2.66), no biological impact was established. Benzo [1, 2 –C: 3, 4- C¹- 5, 6- C¹¹] tris [1, 2, 5] oxadiazole, 1, 4, 7 trioxide (RT: 15.133 and Peak area: 3.57), moderately toxic by ingestion, a mild skin and severe eye irritant. When heated to decomposition it emits acrid smoke and fumes. It used in explosives. Silane, dimethyl (3-hexyloxy) butoxy- (RT: 15.425 and Peak area: 2.25), no biological suggestions were obtainable for the compound and 9, 12 – Octadecadienoic acid [Z], methyl ester (RT: 15.814 and Peak area: 2.73), 9, 12- Octadecadienoic acid, methyl ester (RT: 16.187 and Peak area: 3.76), conferred above. 10, 13- Octadecadienoic acid methyl ester (RT: 22.233 and Peak area: 85.52) and 1, 2, 3 – trimethoxy pentane (RT: 33.039 and Peak area: 2.51), both don't comprise biological impact. The GC-MS chromatogram and tabular form were exhibited in Fig. 5 and Table 5.

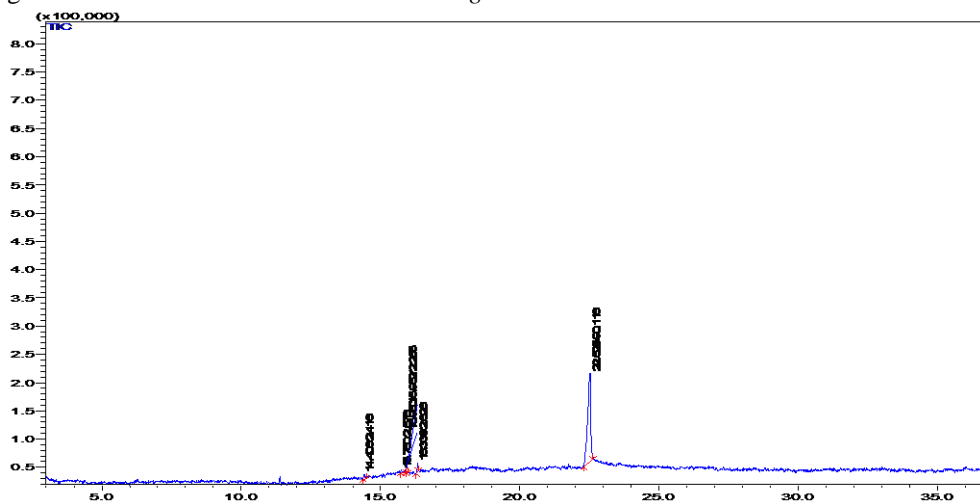


Figure 1:- GC-MS chromatogram of *Artocarpus heterophyllus* hexane extracted seed oil

Table 1:-Total ionic chromatogram (GC-MS) of *Artocarpus heterophyllus* hexane 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	14.408	Methyl - 9,9,10,10 – 4 – Octadecanoate	C ₁₈ H ₃₂ D ₄ O ₂	288.50	2.42
2	15.750	Methyl Octadecanoate/ Stearic acid methyl ester/ Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	298.50	2.58
3	15.952	9- Octadecenoic acid [Z]- methyl ester/ Oleic acid, methyl ester/ Elaidic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.49	2.27
4	16.334	9, 12-Octadecadienoic acid, methyl ester/ Methyl linoleate/Linoleic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.47	2.63
5	22.524	Z, E-3,13-Octadecadien-1-ol	C ₁₈ H ₃₄ O	266.46	90.10

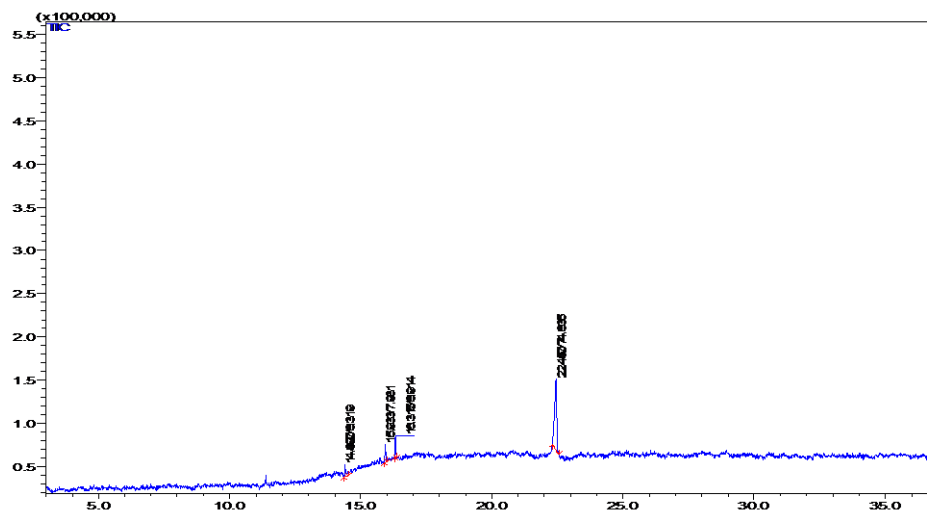


Figure 2:- GC-MS chromatogram of *Artocarpus integrifolia* hexane extracted seed oil

Table 2:- Total ionic chromatogram (GC-MS) of *Artocarpus integrifolia* hexane 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	14.397	Ecosanoic acid, methyl ester [CAS] arachidic acid methyl ester/ Methyl arachisate	$C_{21}H_{42}O_2$	326.56	8.32
2	15.933	9- Hexadecenoic acid, methyl ester [Z]/ Methyl palmitoleate/ Palmitoleic acid, methyl ester/ Methyl palmitoleinate	$C_{17}H_{32}O_2$	268.43	7.93
3	16.315	9, 12 –Octadecadienoic acid, methyl ester/ Methyl linoleate/Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	8.91
4	22.447	10, 13- Octadecadienoic acid , methyl ester	$C_{19}H_{34}O_2$	294.47	74.84

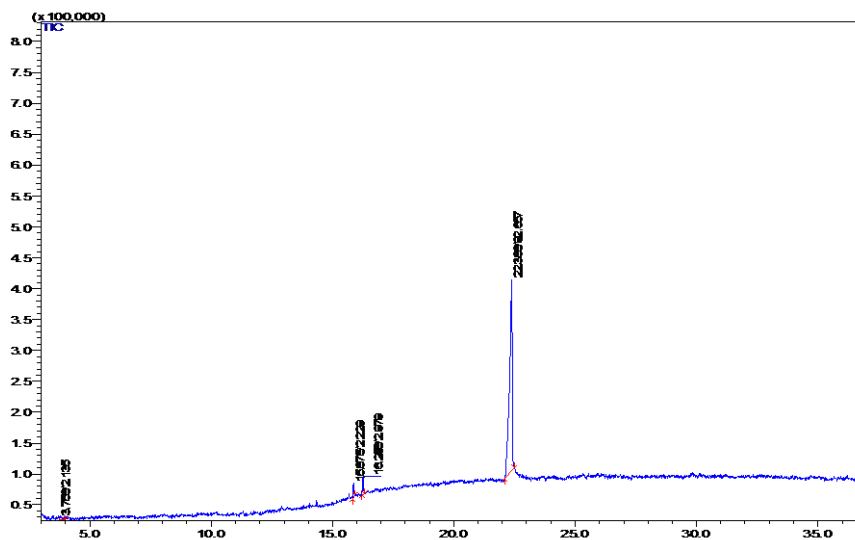
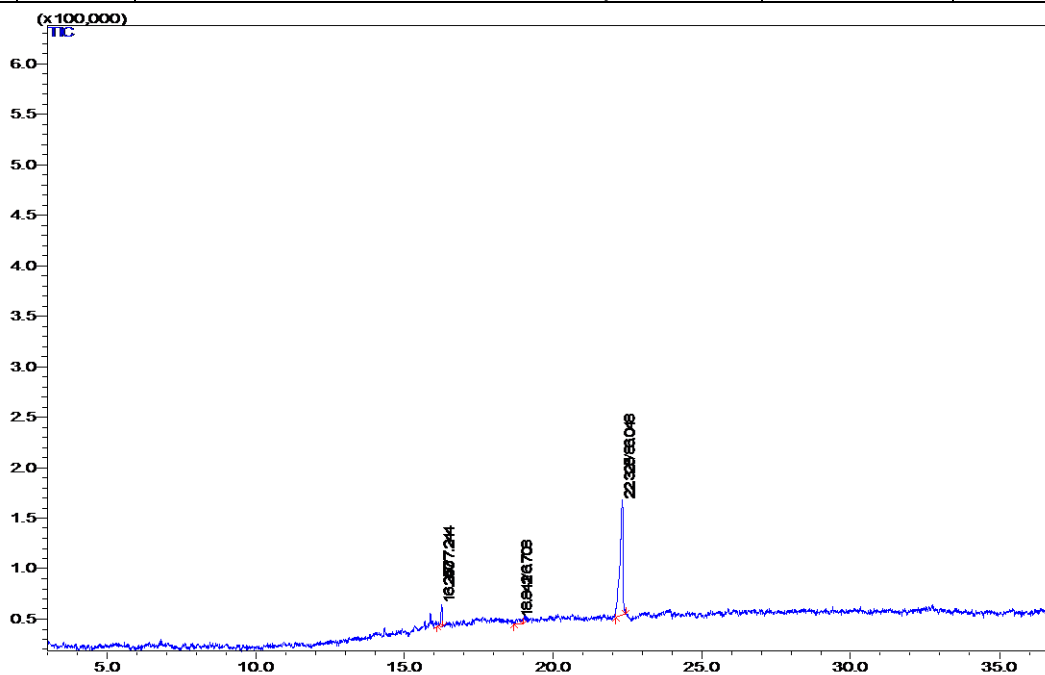


Figure 3:- GC-MS chromatogram of *Artocarpus hirsitus* hexane extracted seed oil

Table 3:- Total ionic chromatogram (GC-MS) of *Artocarpus hirsitus* hexane 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	3.758	2-trifluoromethyl – N- N- dimethyl 4 –nitroaniline / N, N-Dimethyl-4-nitro-2-trifluoromethylaniline / Benzene, 1-trifluoromethyl-2-dimethylamino-5-nitro-	C ₉ H ₉ F ₃ N ₂ O ₂	234.18	2.14
2	15.873	7-hexadecenoic acid, methyl ester [Z]/ Palmitoleic acid methyl ester	C ₁₇ H ₃₂ O ₂	268.44	2.23
3	16.248	9,12 – Octadecadienoic acid, methyl ester/ Methyl linoleate/Linoleic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.47	2.98
4	22.386	10, 13 – Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.47	92.65

**Figure 4:-** GC-MS chromatogram of *Artocarpus inscivus* hexane extracted seed oil**Table 4:-**Total ionic chromatogram (GC-MS) of *Artocarpus inscivus* hexane 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	16.247	Oxacycloheptadec -8-en-Z-one	C ₁₆ H ₂₈ O ₂	252.39	7.24
2	18.841	2-ethoxythiazole	C ₅ H ₇ NOS	129.18	6.71
3	22.323	10, 13- Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.47	86.05

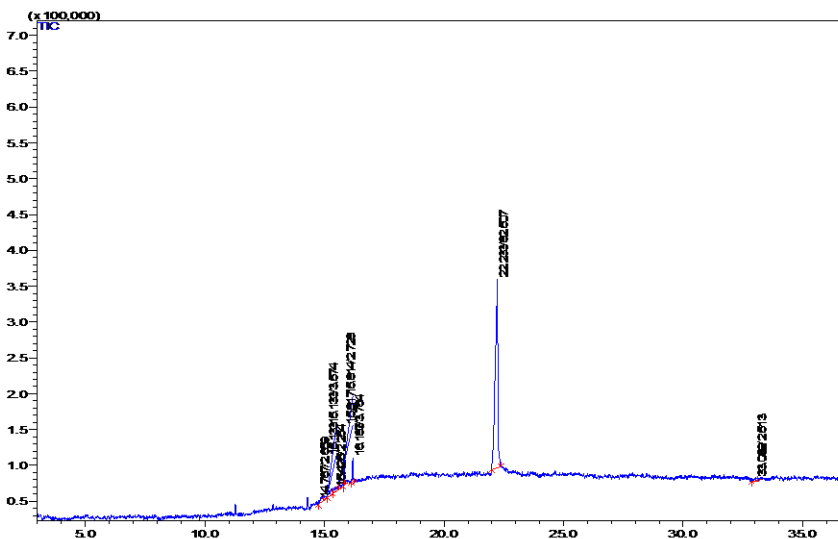


Figure 5:- GC-MS chromatogram of *Artocarpus integer* hexane extracted seed oil.

Table 5:- Total ionic chromatogram (GC-MS) of *Artocarpus integer* hexane 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	14.767	2, 2, 6, 6 – tetradenterio – 3, 7, diacetyl 3, 7 – diazadodecane	$C_{14}H_{24}D_4N_2O_2$	256.0	2.66
2	15.133	Benzo [1, 2 –C: 3, 4- C1- 5, 6- C11] tris [1, 2, 5] oxadiazole, 1, 4, 7 trioxide/ Benzenetrifuroxan/ Benzotrifuroxan; Benzotris [c] furazan-2-oxide/ Benzotris [1,2,5] oxadiazole, 1,4,7-trioxide/ BTF	$C_6N_6O_6$	252.10	3.57
3	15.425	Silane, dimethyl (3-hexyloxy) butoxy-	$C_{12}H_{28}O_2Si$	232.42	2.25
4	15.814	9,12 – Octadecadienoic acid, methyl ester/ Methyl linoleate/Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	2.73
5	16.187	9,12 – Octadecadienoic acid, methyl ester/ Methyl linoleate/Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	3.76
6	22.233	10, 13- Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294.47	85.52
7	33.039	Pentane 1, 2, 3- trimethoxy [CAS] 1, 2, 3 – trimethoxy pentane	$C_8H_{18}O_3$	162.23	2.51

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. integer* with seven peaks subsequently *A. heterophyllus* through five peaks, next to that *A.integrifolia* and *A.hirsitus* by four peaks each, lastly *A.inscicus* seed oil had merely three peaks. 9, 12 – Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) and 10, 13- Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) were found in all tested hexane seed oil free fatty acid methyl ester fractions except *A. inscicus* and in *A. heterophyllus* 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. Marimuthu Krishnaveni et al^[11] 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. Vijisara Elizabeth and Arumugam^[12] 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-hexadecanoic 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%). Syeda Farina Asghar et al^[13] 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. The lipid fraction of *Jatropha* oil seed was extracted and analyzed by Emil Akbar et al^[14]. 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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