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ANALYSIS OF SOME SEED OILS FROM ARID ZONE OF RAJASTHAN FOR THE CHARACTERISATION OF UNUSUAL FATTY ACIDS

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ABSTRACT

Seed oil from indigenous species of lesser known botanical family is reported. This species was collected from Western Rajasthan State and analysed for its physicochemical characteristics and fatty acid compositions using chromatographic and spectroscopic techniques viz. I.R., U.V., TLC, GLC, GC-MS. Seed oil of this species have been found to contain a new class of lipids along with normal triglycerides, identified as cyanolipid. These cyanolipids are of four types all having long chain fatty acids esterified with an unsaturated isopropenoid hydroxy or dihydroxy-nitrile. The presence of large amounts of C_{20} acids usually found in these oils indicate high content of cyanolipid because these acids are preferentially incorporated in nitrogen containing lipid fractions, this preference is probably related to the observation that a seed oil with insignificant C_{20} acids from the same family also contain no cyanolipid. Out of these one seed oil has been reinvestigated and the data were compared with previous results.

KEYWORDS: Fatty acid composition, Chromatographic and Spectroscopic characterization, Incorporation of C_{20} acids, Sapindaceae, seed oils.

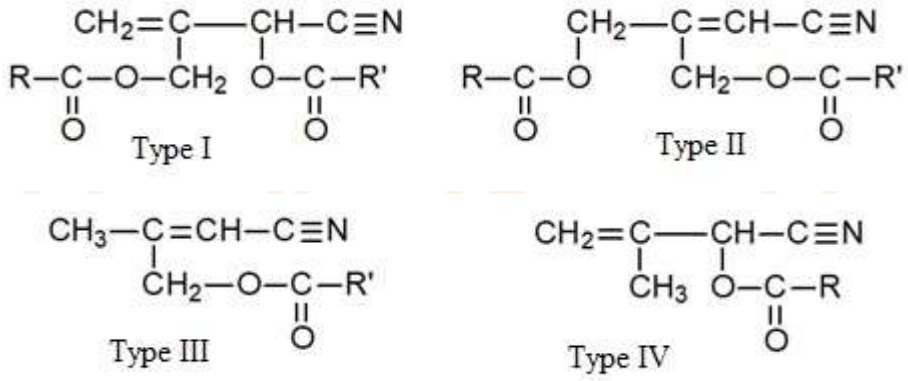
1. INTRODUCTION

The arid region of Western Rajasthan is home to a diverse range of wild flora, which presents significant potential for the discovery of novel sources of oils and fats. In our laboratory, comprehensive physico-chemical analyses have been conducted on these oils, utilizing AOCS-recommended methodologies for chemical and spectroscopic

evaluation [1-3]. The primary objective of this study is to investigate non-traditional oil sources, with a focus on establishing their potential applications in both medicinal and industrial sectors.

While non-traditional oils are generally unsuitable for direct human consumption unless appropriately processed, they can serve as valuable sources of fatty acids. These fatty acids are increasingly utilized in the oleo-chemical industry for various applications. Moreover, a substantial portion of the global population continues to rely on traditional medicine, with a predominant focus on herbal remedies [4]. Over the past few decades, there has been a significant surge in the demand and supply of seed oils at both domestic and industrial levels, leading to a corresponding rise in the consumption of natural products.

Cyanolipids were probably first discovered in ***Schleichera trijuga*** seed oil [5-10] but the nature of the nitrogen containing lipid fraction (NCLF) was not established, later on seed oils from many other plants especially from sapindaceae and some from Boraginaceae were found to contain cynolipids in appreciable amounts between 20 - 40 % of the total triglycerides along with C_{18} and C_{20} carbon fatty acids esterified with mono or dihydroxy-nitrile moiety. A number of sapindaceae seed oils have been studied for their cyanolipid fractions. These cyanolipids have been identified as four different types but all consist of long chain fatty acids esterified with an unsaturated isopropenoid hydroxy or dihydroxy nitrile component. It has been noticed that the large amount of C_{20} acids usually found in the oils remain incorporated in the NCLF part of the oil. The various types of NCLF are:



Type I – (Diester) : 1-cyano-2-hydroxymethylprop-2-ene-1-ol

Type II – (Diester) : 1-cyano-2-hydroxymethylprop-1-ene-3-ol

Type III – (Monoester) : 1-cyano-2-methylprop-1-ene-3-ol

Type IV – (Monoester) : 1-cyano-2-methylprop-2-ene-1-ol

RCO / R'CO = octadecenoyl or eicosenoyl

Structure of NCLF

Most of the seed oils [11] studied were from sapindaceae family and the major amount of C_{20} acids mainly found in the seed oils remain incorporated in the NCLF part.

In studying the occurrence of eicosenoic acid in the seed oils, C_{20} unsaturated acid has been reported as a component in the seed oils [12] as 11-eicosenoic acid, the structural identification of the oil components was

carried out by chemical, chromatographic and spectroscopic means. The GC-Mass analysis showed that the fatty acids were predominantly present in the NCLF part of the oil [13] were cis-11-eicosenoic acid, cis-11-octadecenoic acid and eicosanoic acid as the only esterified fatty acyl chains.

The physiological role of NCLF in the plants has still not been completely understood [14,15]. These components may serve as a major nitrogen source for the developing seedings [16] and their co-occurrence with hydroxynitrile glycosides [17] in some species suggested that they represent a biosynthetic variation of hydroxynitrile glycosides with esterification to lipids possibly serving specific functions related to storage and transport.

Many species belonging to sapindaceae family are used commercially as food and also as medicine. Therefore identification and quantification of cyanolipids in food and forage plants containing this natural product is of importance to possibly allow their removal and avoid food poisoning. The presence of various chemical constituents, extract of the plant showed various medicinal properties such as antibacterial, antifungal, antiparasitic, antidiarrheal, anxiolytic, rubifacient, antipyretic, anti-inflammatory, anticonvulsant and anticarcinogenic.

Plant Description

Botanical Name: ***Cardiospermum halicacabum***

Common Name: Balloon plant, Baloon vine.

Classification:	Kingdom	Plantae
	Order	Sapindales
	Family	Sapindaceae
	Genus	***Cardiospermum***
	Species	***C. halicacabum***

Habitat : This is widely found in tropical and subtropical areas and often found as a weed along roads.

Description : The balloon vine is a overgrown, perennial herbaceous climbing plant growing over 10 meters height. Stems are hairy, slender and grooved. Leaves are triangular foliage about 5 to 6 cm long. Flowers are unisexual and zygomorphic. Fruits are conspicuous, membranous, fluffy hairy, light green with diameter of 3 to 5 cm. Seeds are kidney shaped with light heart-shaped white spot in diameter of about 6 mm.

Medicinal Uses: The root is diuretic, emetic, laxative, stomachic and demulcent and used to treat rheumatism, nervous diseases, piles. The leaves are used in amenorrhoea and pulmonic complaints. It is also used in homeopathy to treat eczematic skin. Leaves internally rubbed up with castor-oil to reduce swellings and tumours of various kinds.

Plant

Leaf

Flower

Unripe Fruit

Fruit with Seeds

Seeds

2. MATERIAL COLLECTION AND SAMPLE PREPARATION :

Oil Extraction

The seeds were collected, cleaned, dried and powdered, the collection of seeds was mainly on maturity and specifically from arid land of Rajasthan. The oil extraction was performed from the ground seeds using light petroleum ether (40 – 60 °C) using soxhlet extraction method. The solvent was removed under vacuum with rotary evaporator. The physico-chemical properties of seeds and seed oils were determined using standard procedures [18]. Methyl esters of the oils were prepared using trans-esterification technique. Direct analytical TLC tests were also carried out for the presence of any unusual fatty acid component.

Analyses of fatty acids methyl ester (FAMEs)

IR spectrum of FAMEs was carried out using Perkin Elmer RX-I FTIR on KBR cell. The UV-visible spectrum was recorded on Perkin Elmer Lambda-15 UV/Vis spectrophotometer. The fatty acid composition of the FAME was obtained using Perkin Elmer Autosystem XL gas chromatograph equipped with flame ionization detector. A capillary column of fused silica of high polarity (SP 2330; Length : 30 m; Internal diameter : 0.25 mm; Thickness of film : 0.2 μm) was used.

Nitrogen was the carrier gas at a flow rate of 0.75 l/min. The injection temperature and the detector temperature was 260 °C. The oven starting temperature was 80 °C and then increased to 200 °C at a rate of 6 °C/min., held for 5 min. then increased to 250 °C at a rate of 10 °C/min. The peaks were identified using methyl ester standards (Rapeseed oil mix and PUFAs from sigma). The position of double bonds was confirmed by a Thermo Scientific TS Q 8000 Gas chromatograph – massspectrophotometer. A capillary column of polysilphenylene siloxane (BPX 70 TM; length : 25 m; Internal diameter : 0.22 mm; Thickness of film : 0.25 μm) was used. Helium was the carrier gas at a flow rate of 1 ml/min. The injector temperature was 250 °C and the detector temperature was 260 °C. The oven starting temperature was 80 °C and then increased to 200 °C at a rate of 8 °C/min., held for 10 min. then increased to 250 °C at a rate of 10 °C/min., held for 10 minutes.

3. RESULTS AND DISCUSSION

Spectroscopic and chromatographic analyses

Direct TLC tests, U.V. and I.R. spectra very clearly showed the absence of any oxygenated fatty ester, conjugation and trans-unsaturation or any other functional group. Silver nitrate impregnated TLC of FAMEs showed spots corresponding to saturated monoene, diene and triene when run along with reference standards. The physico-chemical properties of seeds and seed oils have been given in following table.

Table: Physico-Chemical Characteristics of Seed and Oil

17.50	**1.55**	**16.42**	**1.4810**	**2.21**	**110.43**	**177.51**	**15.6**

* R.I.= Refractive index

U.M. = Unsaponifiable matter

I.V. = Iodine value

S.V. = Saponification value

Fatty Acid Composition Determined by GLC

The iodine values of all the seed oils recorded by the experimental procedure were in close agreement with fatty acid composition of the seed oils. The oils as their methyl esters were subjected to GLC and GC-MS analyses for their fatty acid contents. The fatty acid composition of seed oils have been shown in table.

Table: Uncorrected Weight Percentage (Triglyceride Fraction)

16 : 0	18 : 0	18 : 1	18 : 2	18 : 3	20 : 0	20 : 1	22 : 1	Others
5.96	**9.36**	**38.72**	**15.42**	**3.52**	**8.75**	**12.63**	**3.35**	**2.28**

Table: Uncorrected Weight Percentage (Cyanolipid Fraction)

16 : 0	18 : 0	18 : 1	18 : 2	18 : 3	20 : 0	20 : 1	22 : 1	Others
7.76	5.67	41.41	18.52	4.78	11.23	14.31	-	1.09

Identification of NCLF

The chemical screening of the seed oil of ***Cardiospermum halicacabum*** revealed the presence of cyanolipid and C_{20} monoenoic acid towards chemical and spectroscopical analyses. The oil extraction from seeds gave 17.50 % yield when the finely powdered seeds were subjected with petroleum ether (40 – 60 °C) in a soxhlet apparatus. The elemental analysis of the oil gave 8.3 % nitrogen content and treatment with dilute base at 35 - 50 °C for 30 min. produced Hydrocyanic acid as confirmed by a positive prussian blue and sodium picrate tests.

The I.R. Spectrum of the oil gave bands at 2240 cm^{-1} indicated the presence of cyano group in addition to the normal I.R. bands for normal triglycerides. The NMR Spectrum of the sample in $CDCl_3$ showed signal namely singlet at δ 4.18 and δ 4.27 for the protons associated with dihydroxynitrile moiety along with the proton signals for long chain fatty acids. The analytical TLC of the oil on 0.25 mm thick layer of silica gel G using ***Heliotropium indicum*** seed oil as reference standard was found more revealing than I.R. spectrum of the oil.

The developing solvent system with petroleum ether – diethyl ether – acetic acid (85:15:1, V/V/V), gave spot of triglyceride at Rf 0.74 and that of nitrile moiety at 0.52. On changing the solvent system by ether-hexane (1:3, V/V), the nitrile proton gave spot at Rf 0.56.

Characterisation of Cyanolipid Fraction

The cyanolipid fraction from the oil was fractionated through preparative TLC using ether – hexane (1:3, V/V), into two parts. The triglycerides part 84.4 % and the cyanolipid portion as 15.6 %. The elemental analysis of this part gave 8.3 % nitrogen.

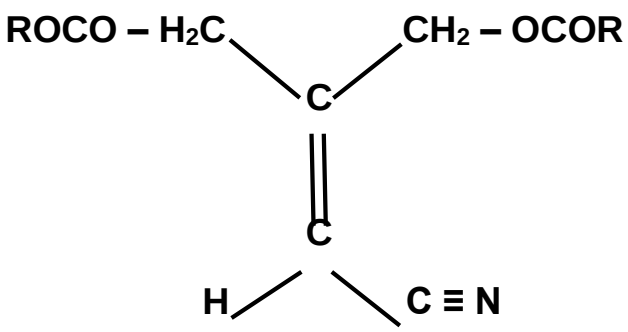
The structural identification of the nitrile moiety was very well done through comparison with TLC, I.R. and NMR results of the present study on ***C. halicacabum*** and those obtained from nitrogen containing lipid fraction reported earlier.

The pure NCLF from the oil of ***C. halicacabum*** showed IR band at 2240 cm^{-1} which was found super imposable with that obtained from ***Heliotropium*** seed oils.

The most strong and conclusive evidence further obtained from NMR Spectrum of NCLF which showed signals of normal fatty acids along with signals characteristics of NCLF as terminal methyl δ 0.88, chain methylene δ 1.26, α - methylene δ 1.65, allylic methylene δ 1.95 and other olefinic protons at δ 5.32.

As mentioned earlier [22-30] the two sets of methylene protons adjacent to the oxygen of dihydroxynitrile moiety appeared slightly up-field δ 4.19 (2H) and δ 4.27 (2H). This NMR Spectrum was found almost super imposable and identical with the spectrum of the cyanolipid reported from ***H. indicum*** from Boraginaceae seed oils.

Thus a comparison of TLC, IR and NMR data of pure NCLF obtained in the present study and those from known cyanolipid established the structure of the nitrogen fraction as a diester of 1-cyano-2-hydroxymethyl prop-1-ene-3-ol.



Identification of C_{20}–Unsaturated Fatty Acids

The nitrogen part of the oil isolated through preparative TLC was subjected to saponification using 5 % ethanolic KOH solution to regenerate the mixture of fatty acids. The mixed fatty acids (MFA) so obtained were further esterified using diazomethane. The esters were resolved on 10 % silver – nitrate – impregnated silica gel G plates using benzene as the developing solvent gave a fraction of monoenoic esters. These were subjected to oxidative degradation in tert. butyl alcohol with Von Rudloff's reagent. The GLC analysis of the cleaved products on methyl esters gave hendecandioate in addition to pelargonate and azelate, identification of hendecandioate and pelargonate placed the double bond between C_{11} and C_{12} of the fatty acid chain and there by confirmed the C_{20} – monoenoic acid as cis-11-eicosenoic acid.

The composition of the fatty acids present in the normal triglyceride part as well as in the NCLF of ***C. halicacabum*** seed oil was determined by GLC analysis of the methyl esters using both the polar and non – polar columns. The uncorrected weight percentages of the component fatty acids are given in Table VI and VII.

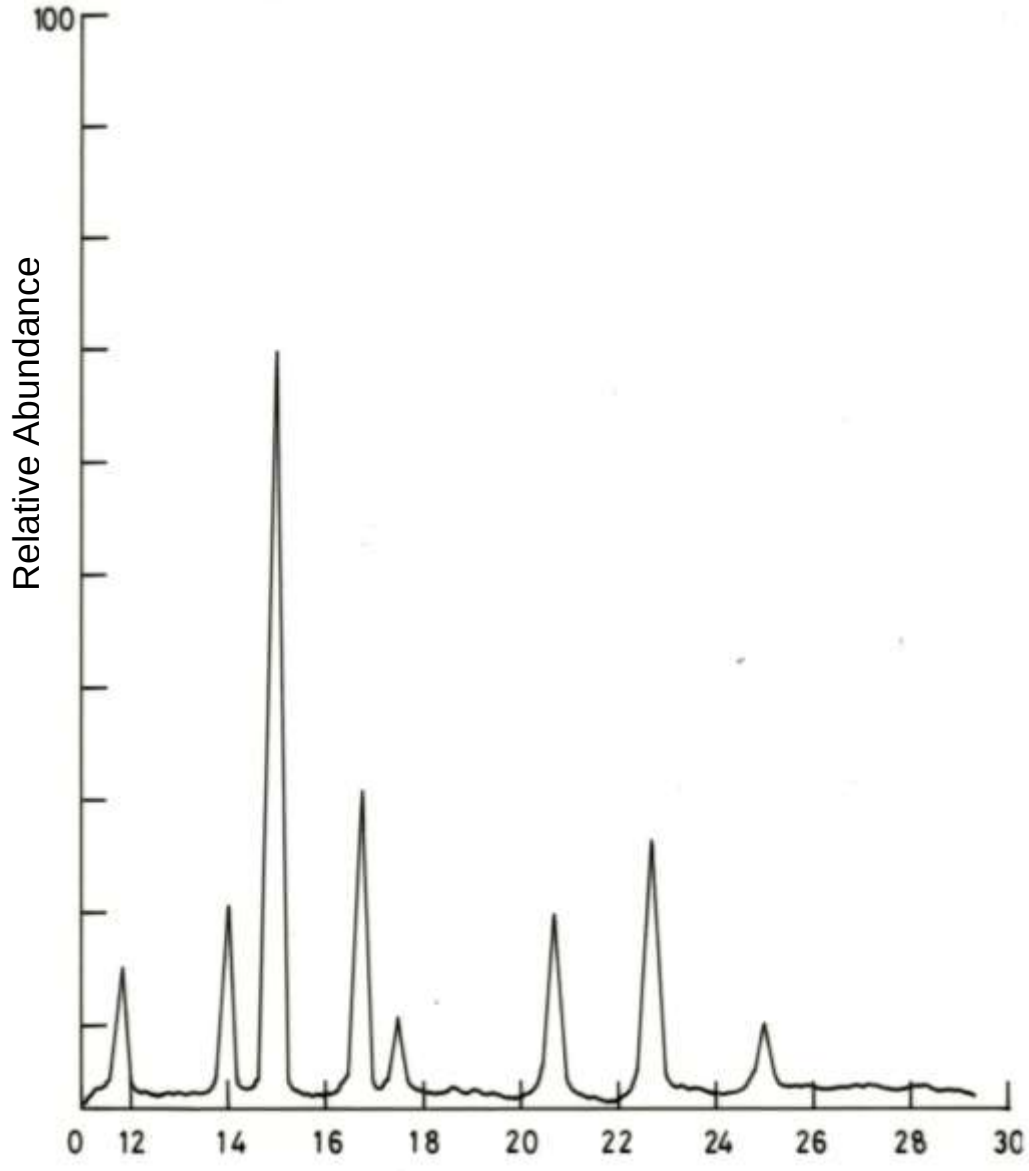
The literature survey [31-38] has revealed that the fruits and leaves of ***C. halicacabum*** were studied mainly for their anti – inflammatory, hyperalgesic migraine, and other pharmacological activities.

Another report by Mary and co-workers has also revealed that seed oil of ***C. halicacabum*** carried cyanolipid fraction as identified by spectroscopical and chromatographical techniques along with C_{20} – monoenes.

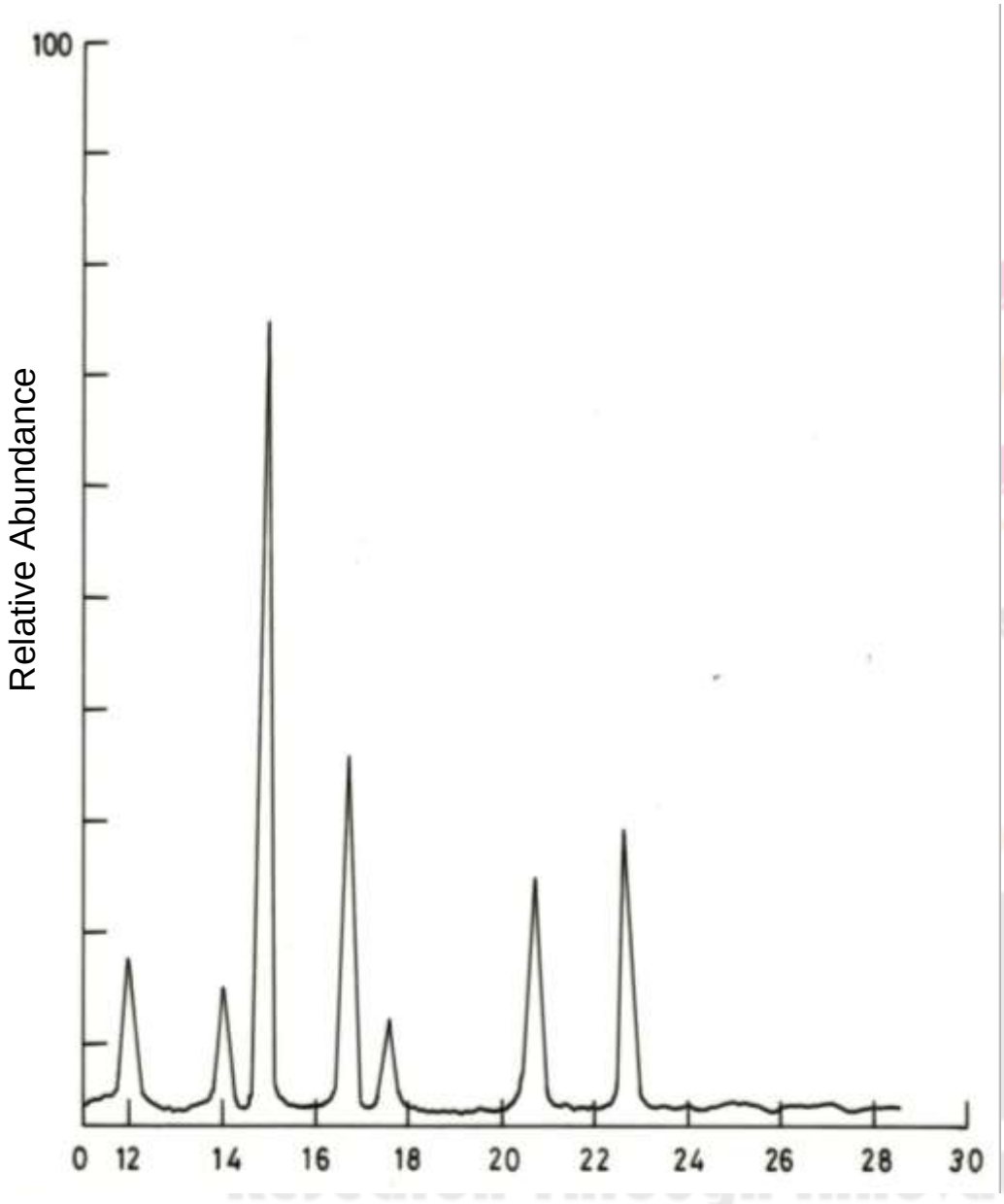
In our present study, the cyanolipid part has been obtained in an appreciable amount of the seed oil. In view of the above results it has been concluded that ***C. halicacabum*** seed oil contain a cyanolipid portion in good amount along with C_{20} – monoenoic acid and other common fatty acids.

The earlier report suggested that ***C. halicacabum*** distributed around the world may have different morphological characters. Therefore absence / or presence of any component in different populations of ***C. halicacabum*** can be attributed to the genetic / environmental difference in species.

Fatty Acid Composition (Triglyceride Fraction)



Fatty Acid Composition (Cyanolipid Fraction)



4. CONCLUSION

The seed oils of the Sapindaceae and Boraginaceae families contain an unusually high amount of cyanolipids, a class of lipids that, to date, has not been reported in any other plant family. Additionally, all four cyanolipids in these families feature a hydroxyl or dihydroxynitrile moiety with an isopropenoid skeleton, potentially resulting from various biogenetic pathways.

The results obtained from this investigation may be used as base parameters to develop these seed oils for domestic, commercial and/or medicinal purposes.

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Conflicts of Interest

There are no conflicts of interest.

References

1. Ahmad I, Sherwani MRK, Hasan SQ, Ansari AA, Osman SM. Studies on herbaceous seed oils –VIII. The Journal of the Oil Technologists Association of India, 1978; X(2):126-127.
2. Malik A, Taufeeque M, Praveen S, Sherwani MRK. Evaluation of fatty acid composition of the seed oil of some Leguminosae species from arid zone of Rajasthan. World Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(11):1344-1351.
3. Taufeeque M, Malik A, Sherwani MRK. Occurrence and characterization of Gamma-Linolenic acid in seed oil of Asteracantha longifolia (L.) ness. World Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(5):1808-1814.
4. Stanley P, Luz L. The impact of forest degradation on medicinal plant use and implications for health care in Eastern Amazonia. Bioscience, 2003; 53:573-584.
5. Rosenthaler L, Schweiz AZ. 1920; 58: 17-20.
6. Sen Gupta NN. J. Soc. Chem. Ind, 1920; 39: 88-91.
7. Kartha ARS, Narayanan R. JAOCS, 1967; 44: 350-353.
8. Kundu, MK, Bandyopadhyay C. JAOCS, 1969; 46: 23-27.
9. Kasbekar MG, Bringi NV. JAOCS, 1969; 46: 183.

10. Kundu MK. J. Chromatography, 1969; 41: 276-278.
11. Mikolajczak KL, Smith CR Jr, Tjarks LW. Lipids, 1970; 5: 812-817.
12. Mary JC, Hopkins CY. Can. J. Chem, 1958; 36: 1537-1540.
13. Savitha G, Vishnupriya V, Suapaneni K. Asian J Pharma Clin Res, 2017; 10: 23-26.
14. Minzangi K, Kaaya AN, Kansiime F, Tabuti JRS, Samvuram B, Nielsen G. Afr. J. Food Sci, 2011; 5(4): 219-226.
15. Mikolajczak KL, Seigler DS, Smith CR Jr., Wolff IA, Bates RB. Lipids, 1969; 4: 617-619.
16. Seigler DS, Mikolajczak KL, Smith CR Jr., Wolff IA, Bates RB. Chem. Phys. Lipids, 1970; 4: 147-161.
17. Mikola jczak KL, Smith CR Jr., Tjarks LW. Biochim. Biophys. Act, 1970; 210: 306-314.
18. W.E. Link (Ed.), Official and Tentative Methods of The American Oil Chemists Society 3rd ed. AOCS USA : Champaign IL; 1973.
19. Hopkins CY, Swingle R. Lipids, 1967; 2: 258-260.
20. Kleiman R, Earl FR, Wolff IA. Lipids, 1969; 4: 317-320.
21. Bellamany LJ, "The infrared Spectra of Complex Molecules", New York : John Wiley & Sons, Inc; 1956. P. 225.
22. Rosenthaler L. and Schweiz A.Z., *Chem. Abstr*., **58** (1920) 17.
23. Sen Gupta N.N., *J. Soc. Chem. Ind*., **39** (1920) 88.
24. Kartha A.R.S. and Narayanan R., *J.A.O.C.S*., **44** (1967) 350.
25. Kundu M.K. and Bandyopadhyay C., *J.A.O.C.S*., **46** (1969) 23.
26. Kasbekar M.G. and Bringi N.V., *J.A.O.C.S*., **46** (1969) 183.
27. Kundu M.K., *J. Chromatography*, **41** (1969) 276.
28. Mikolajczak K.L., Smith CR Jr. and Tjarks L.W., *Lipids*, **5** (1970) 812.
29. Mary J.C. and Hopkins C.Y., *Can. J. Chem*., **36** (1958) 1537.
30. Savitha G., Vishnupriya V. and Suapaneni K., *Asian J. Pharma. Clin. Res*., **10** (2017) 23.
31. Minzangi K., Kaaya A.N., Kansiime F., Tabuti J.R.S., Samvuram B. and Nielsen G. Afr., *J. Food Sci*., **5(4)** (2011) 219.
32. Mikolajczak K.L., Seigler D.S., Smith CR Jr., Wolff I.A. and Bates R.B., *Lipids*, **4** (1969) 617.
33. Seigler D.S., Mikolajczak K.L, Smith CR Jr., Wolff I.A. and Bates R.B., *Chem. Phys. Lipids*, **4** (1970) 147.
34. Mikola jczak K.L., Smith CR Jr. and Tjarks L.W., *Biochim. Biophys. Act*., **210** (1970) 306.
35. Hopkins C.Y. and Swingle R., *Lipids*, **2** (1967) 258.
36. Kleiman R., Earl F.R. and Wolff I.A., *Lipids*, **4** (1969) 317.
37. Bellamany L.J., "The infrared Spectra of Complex Molecules", New York : John Wiley & Sons, Inc., (1956) 225.
38. Franceso M., Luigi L., Marco B., Alessandro P., Monica R. L. and Rosa T., *J. Enzyme Inhib. Med. Chem*., **29(5)** (2014) 677.