

**PHYSICO-CHEMICAL CHARACTERIZATION AND GAS CHROMATOGRAPHY-
MASS SPECTROSCOPY ANALYSIS OF *FICUS RELIGIOSA* SEED OIL FATTY ACIDS**

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Article Received on 06/01/2016

Article Revised on 26/01/2016

Article Accepted on 16/02/2016

ABSTRACT

Oil extracted from *Ficus religiosa* seed was studied to explore its suitability for ethnomedical uses with a special emphasis on its physiochemical characterization and Gas Chromatography-Mass spectroscopy analysis of seed oil fatty acids. Some of the physiochemical properties were examined and compared with those of standard oils and in particular, any common characteristics with cotton seed oil were evaluated. GC-MS analysis of free fatty acid methyl ester fraction of the *Ficus religiosa* seed oil reveals Pentadecanoic acid, methyl ester (CAS) methyl laurate, Silikonfett SE30(Grevels), Methyl ricinoleate, Thiophene 2-carboxylic acid, 4,5 dimethyl-3-(1-pyrrolyl) and 1,1,2,2-tetraethyl-Disilane. These findings demonstrated that essential oil of *Ficus religiosa* seeds was a complex mixture of numerous fatty acids and also contains some bioactive components, thus have great potential to be used as a source for natural health products. In conclusion, *Ficus religiosa* oil is a natural essential oil and could have therapeutic potential.

KEYWORDS: Physiochemical characterization; *Ficus religiosa* seed oil; Gas Chromatography-Mass spectroscopy analysis.

INTRODUCTION

Medicinal plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Numerous plants synthesize substances that are useful in the maintenance of health in humans and animals. With a view to increasing the wide range of medicinal usages, the present day entails new drugs with more potent and desired activity with less or no side effects against particular disease. The genus *Ficus* (*Moraceae*) constitutes one of the largest genera of angiosperms includes with more than 800 species and 2000 varieties of *Ficus* genus, occurring in most tropical and subtropical forests worldwide. *Ficus religiosa*, commonly known as Peepal, is the most popular member of the genus *Ficus*. *Ficus religiosa* has got mythological, religious and medicinal importance in Indian culture. Essential oils are a rich source of biologically active compounds. Therefore, it is sensible to already have identified a variety of plant compounds in these oils, for possible future therapeutic use. Therefore, the first objective of this study was to identify some physiochemical characteristics of *Ficus religiosa* seed oil using Gas Chromatography-Mass spectroscopy analysis of seed oil fatty acids. *Ficus religiosa* showed a wide spectrum of pharmacological activities like

anticonvulsant, antihelmintic, anti-amnesic, anti-anxiety, anti-asthmatic, antibacterial, antioxidant, anti-inflammatory and antiulcer.

MATERIALS AND METHODS

Seed collection

Mature dried fruits of *Ficus religiosa* were obtained from the area in and around Andhra University, Visakhapatnam, India and were explored for physiochemical characterization and Gas Chromatography-Mass spectroscopy analysis of seed oil fatty acid analysis.

Soxhlet extraction

The seed oil was extracted using a soxhlet extraction method with analytical grade hexane as a refluxing solvent. On completion of the extraction process, the oil was recovered from the mixture by distillation and stored at 40C until use. (Popoola TOS et al., 2007).

The percentage of oil content can be calculated as follows:

% of oil = Wt. oil obtained in grams / Wt. seed taken in grams × 100.

Oil characterization

The crude oil sample obtained from the hexane extraction was characterized for acid value,

saponification value, iodine value, peroxide value. Reichert-Meissl value (RMV) and Polenske value were based on official recommendations and Tentative Methods of the American Oil Chemist's Society. (AOCS., 1998).

Gas Chromatography –Mass Chromatography analysis of Seed oil

Fatty acid composition in *Ficus religiosa* seed oils was determined, using Gas chromatography –Mass Spectroscopy according to the method described by Baye and Becker., 2004.

Preparation of Fatty Acid Methyl Esters (FAMES) Method

Seed oil (100 mg) was weighed into 20 ml test tubes and dissolved in 10 ml hexane. Then, 100µl of 2N potassium hydroxide in methanol (11.2 g in 100 ml) was added into the test tube, vortexes for 30 seconds and centrifuged (REMI centrifuge, R-8C, India). The clear supernatant of test sample (2 ml) taken into individual vials and placed in an auto sampler which robotically shifted them into the auto injector (AOC -20i) 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 AND DISCUSSION

Physicochemical characterization Oil extraction was carried out by a soxhlet extraction method according to Association of Official Analytical Chemists - AOAC - recommendations. Hexane was used as solvent to extract the oil from seeds and this was passed out for 10 h. The physicochemical characteristics were comparable to that of cotton seed oil (Table 1). The oil was thick and yellowish in color with a pungent odour and a 40% yield (Figure 1). The acid value of the oil was predicted to be 15, analogous to that of butter (0.46-35.0). The iodine value (degree of unsaturation) of the *Ficus religiosa* seed oil was 98, comparable to that of cotton seed oil (103-111). The saponification value of the oil was 196, identical to that of cotton seed oil (194-196). Peroxide value, which indicates the extent of oil oxidation, was 12 mEqKg⁻¹. Fresh edible oils have a peroxide value of less than 10 mEqKg⁻¹, while rancid oils have values of more than 20 mEqKg⁻¹. The RMV of the oil was 0.36, the same as volatile water-soluble fatty acids present in the oil or fat. Relatively lower RMV of the oil was an indication of low content steam volatile fatty acids. The Polenske value of the oil was 0.97, which indicates low content of the volatile alcohol-soluble fatty acids in the oil.

Table 1. Physico-chemical characterization of *Ficus religiosa* seed oil.

S.No	Parameters	Values
1	Color	Yellow
2	Odor	Pungent
3	Yield (%)	20%
4	Ph	Acidic
5	Acid value (mg KOH/g)	15
6	Saponification value (mg KOH/g)	196
7	Iodine value	98
8	Peroxide value	12
9	Reichert-Meissel value	0.36
10	Polenske value	0.97

Analysis and Identification of Fatty Acid Methyl Ester Composition in *Ficus religiosa* seed Oil Using Gas chromatography-mass spectroscopy

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. These fatty acids play an important role in the prevention and treatment of cardiovascular diseases, autoimmune diseases, eye sight and the improvement of learning ability (Nielsen *et al.*, 2005). 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. Chain length and number of double bonds determine the physical characteristics of both fatty acids and triglycerides (Mittelbach and Remschmidt., 2004). *Ficus religiosa* 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 20%. The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry

(GC-MS). For quantitative determination, gas-chromatography with flame ionization detector (GC-FID) and GC-MS are preferred (Cong *et al.*, 2007). GC-MS analysis of the *Ficus religiosa* hexane seed oil free fatty acid methyl ester fraction showed the presence of prominent five peaks indicating the presence of five compounds, namely Pentadecanoic acid, methyl ester (CAS) methyl laurate (RT:10.204 and Peak area%:1.08), Silikonfett SE30 (Grevels) (RT:16.950 and Peak area:1.62), Methyl ricinoleate (RT:19.200 and Peak area:95.15), Thiophene 2-carboxylic acid,4,5 dimethyl-3-(1-pyrrolyl) (RT:20.000 and Peak area:0.96) and 1,1,2,2-tetraethyl-Disilane (RT:26.907 and Peak area:1.19). The GC-MS chromatogram and table was displayed in Figure: 1 and Table: 2.

Table 2: Total ionic chromatogram (GC-MS) of *Ficus religiosa* hexane extracted seed oil obtained with temperature of EI 70 eV using a capillary column of DB×LB with He gas as the carrier.

Peak	Ret. Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %
1	10.204	Pentadecanoic acid, methyl ester (CAS) methyl laurate	C ₁₆ H ₃₂ O ₂	256	1.08
2	16.950	Silikonfett SE30(Grevels)	-	-	1.62
3	19.200	Methyl ricinoleate	C ₁₉ H ₃₆ O ₃	312	95.15
4	20.000	Thiophene 2-carboxylic acid,4,5 dimethyl-3-(1-pyrrolyl)	C ₁₁ H ₁₁ NO ₂ S	221	0.96
5	26.907	1,1,2,2-tetraethyl-Disilane	C ₈ H ₂₂ Si ₂	174	1.19

Pentadecanoic acid, methyl ester (CAS) methyl laurate

Molecular weight 256 (C₁₆H₃₂O₂)

Oxidative stress has been implicated in the pathogenesis of a variety of diseases ranging from cancer to neuro degeneration. The antioxidant response element (ARE) signaling pathway plays an important role in the amelioration of oxidative stress. The Cell Sensor ARE-bla HepG2 cell line (Invitrogen) can be used for analyzing the Nrf2/antioxidant response signaling pathway.

Silikonfett SE30 (Grevels)

Methyl ricinoleate

Molecular weight: 312 (C₁₉H₃₆O₃)

Methyl ricinoleate has been obtained by alcoholysis of castor oil, followed by fractional distillation of the crude esters and further purification by low temperature crystallization. According to a more recent source,

methyl ricinoleate is a product of either the esterification of ricinolic acid or the alcoholysis of castor oil; the product is purified by vacuum distillation. These are used for Lubricants and lubricant additives, paint additives and coating additives not described by other categories.

Thiophene 2-carboxylic acid,4,5 dimethyl-3-(1-pyrrolyl)

Molecular weight: 221 (C₁₁H₁₁NO₂S)

Thiophene is the simplest aromatic compound containing sulfur atom and it shares some similar chemical properties with benzene. Thiophene derivatives are also found in natural plant pigments. Biotin, a water-soluble B-complex vitamin, is a reduced thiophene derivative. Thiophene is used as a solvent and chemical intermediate. Its derivatives are used in manufacturing dyes, aroma compounds and pharmaceuticals. They are used as monomers to make condensation copolymers. Organic conductive polymers are responsible for the

important materials science for the application of polymer electro luminescence.

1,1,2,2-tetraethyl-Disilane

Molecular weight: 174 (C₈H₂₂Si₂)

1,1,2,2-tetraethyl-Disilane is a colourless, acrid gas. Disilane and ethane have similar structures, although disilane is much more reactive. Other compounds of the general formula Si₂X₆ (X = hydride, halide, alkyl, aryl and mixtures of these groups). The presence of traces of disilane is responsible for the spontaneous flammability of silane produced by hydrolysis by this method (analogously diphosphine is often the spontaneously pyrophoric contaminant in samples of phosphine). It also arises by thermal decomposition disilane via both photochemical and thermal decomposition of silane.

treat various ailments. It could be concluded that *Ficus religiosa* seed oil contains various bioactive compounds of medicinal importance. However, further studies are needed to evaluate its bioactivity and toxicity profile. The fatty acid profile of *Ficus religiosa* seed oil plays a key role to the physico-chemical properties and GC-MS analysis are useful knowledge base for further advanced research.

REFERENCES

1. Popoola TOS, Yangomodou OD, Akintokun AK. Antimicrobial activity of cassava seed oil on skin pathogenic microorganisms. *Research Journal of Medicinal Plant*, 2007; 1: 60-4.
2. AOCS. Official methods and recommended practices of the AOCS, 5th edition. Champaign, IL: American Oil Chemists' Society, 1998.
3. Baye T, Becker HC. (2004). Analyzing seed weight, fatty acid composition, oil and protein contents in *Vernoniagalamentisgermplasm* by near-infrared reflectance spectroscopy. *J. Am. Oil. Chem. Soc.*, 81: 641-645.
4. A.O.A.C. (2000). Official Methods of Analysis. Association of Official Analytical Chemist. EUA.
5. Nielsen NS et al (2005). Effect of structured lipids based on fish oil on the growth and fatty acid composition in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture.*, 250: 411-423.
6. Mittelbach Mand Remschmidt C (2004). Biodiesel: The Comprehensive Handbook. Boersdruck Ges. M.B.H., Vienna.
7. Cong Z., Meiling Q., Qinglong S., Shan Z and Ruonong F (2007). Analysis of the volatile compounds in *Ligusticum chuanxiong Hort.* using HS-SPME-GC-MS. *J Pharm Biomed Anal.*, 44: 464-470.

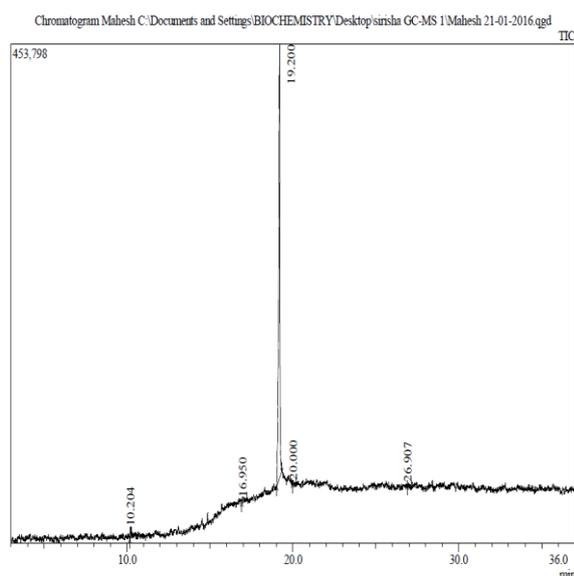
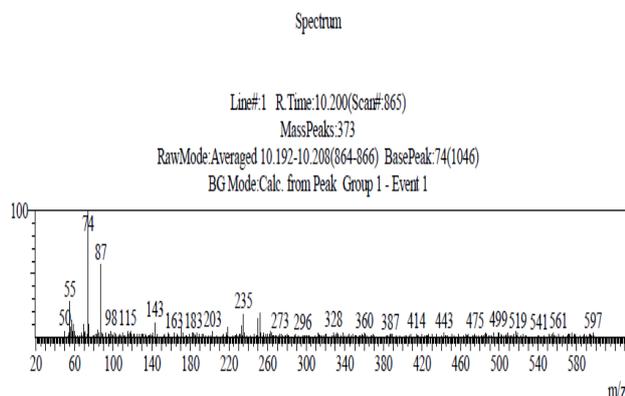


Figure 2: GC-MS Chromatogram of *Ficus religiosa* Seed Oil.



CONCLUSION

The present study found 5 chemical constituents from hexane seed extract of *Ficus religiosa* by Gas Chromatography Mass Spectrometry (GC-MS) analysis. The presence of bioactive compound justified the extensive use of the seed oil the traditional practitioner to