Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(6): 194-198
Received: 18-05-2016
Accepted: 19-06-2016

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Studies on industrially important Guttiferae and Palmae family

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Abstract
The *Garcinia mangostana* seed oil contains 56.2% oleic acid and 6.4% linoleic acid. The palmitic (14.8%) and stearic acid (9.0%) are major components amongst the saturated acids with smaller amounts of capric (0.9%), lauric (2.2%), myristic (6.6%) and arachidic (3.9%). Moreover, *Phoenix sylvestris*, cerita mistis, *chrysalidocarpus* lutescens, *Washingtonia filifera* and phoenix rupicola belong to palmae family and could be compared with the oils rich in lauric acid such as cinnamon and palm kernel oils (80-90% and 45-58% respectively).

Keywords: Guttiferae, Palmae, fatty acid, industrially important

1. Introduction
One of the important facts of plants is their diverse pool of fatty acids. The oil seeds contains particular fatty acids with industrially important because of their characteristic properties. The main constituent of all the oils is the fatty acids which may include saturated, monounsaturated and polyunsaturated fatty acid that contribute in human physiology in different ways [1]. The seed oils containing unusual fatty acids are industrially important as they are used in the protective coatings, plastics, cosmetics, lubricants, varieties of synthetic intermediates, stabilizers in plastic formulations. The interesting unusual fatty acids present in high concentration of certain seed oils are being exploited for the industrial utilization. These fatty acids of unusual structures are highly important for the production of oleochemicals [2, 3].
Guttiferae are a family of about 36 genera and 1,600 species with a pantropical distribution that occurs widely in temperate regions and have been used since ancient times as folk remedies. It is herbaceous perennial plant that grows in open dry stony ground and cultivated fields [4]. Extracts of the leaves, stem bark and root bark of the plant, alone or combined with other plants are widely known for the application against a number of human ailments, such as upper respiratory tract infection, dysentery diarrhea and toothache [5]. It has traditionally been used in the treatment of burns and gastrointestinal diseases [6]. Results from recent studies reporting the antinociceptive [7], anti-inflammatory [8], antioxidant [9], antimicrobial [10, 11], and cytotoxic [12] activities demonstrate the great potential of this species for use as a medicinal plant.
The palm family (Palmae or Arecaceae) is a conspicuous and important feature of tropical and subtropical habitats throughout the world. In general, palms are recognized instantly by the botanist and the layman alike, despite the fact that a great diversity of morphology exists among the 191 recognized genera. The current classification of the palms [13], comprises six subfamilies, 14 tribes and 38 subtribes, many of which are defined in the informal classification of Moore (1973). Almost all palmate qeaved palms belong to the Coryphoideae, a subfamily of 40 genera divided among three tribes and six subtribes. The subfamily includes one pinnate-leaved genus, *Phoenix,* in which the leaf lamina is split to give induplicately-folded segments, as in almost all other coryphoid palms.
After exhaustive survey of literature on guttiferae and palmae family it was found that there is no report entirely on this family regarding the component fatty acids. Hence, the study of this family was undertaken with a view that it might contain some unusual fatty acids. However, no unusual fatty acids are encountered in the present work. But this will be first report on this family as well as species. Hence, the study of this family was undertaken. This will be first report on this family as well as species examined in this investigation.

2. Results and Discussions
Garcinia mangostana seed oil contains 56.2% oleic acid and 6.4% linoleic acid. The palmitic (14.8%) and stearic acid (9.0%) are major components amongst the saturated acids with

smaller amounts of capric (0.9%), lauric (2.2%), myristic (6.6%) and arachidic (3.9%). The present investigation was undertaken to study the detail pattern of component fatty acids of Garcinia xanthochymus seed oil as there is only one report on the fatty acid composition of this seed oil. Sabata *et al*., have reported 41.0% palmitic acid as the only component fatty acid amongst the saturated acids [14]. The oleic acid (55.45%) is only the principal component amongst the unsaturated acids with small amount of linoleic 3.5%. But in the species under investigation contains 33.4% of saturated acids and 62.6% of unsaturated acids. The oil form this species contains capric (0.9%), lauric (2.2%), myristic (6.6%), palmitic (14.8%), stearic (9.0%) and arachidic (3.9%) acids amongst the saturated acids. The unsaturated acids are only oleic (56.2%) and linoleic (6.4%). The oleic acid is present in major proportion. The pattern of unsaturated acids is nearly the same as reported earlier. But there is a lot of change in the proportion of saturated fatty acids. However, the detail in data is given in Table-1.

Mammea longifolia, Planch and triana seeds are rich source of vegetable oil. The kernels on extraction with petroleum ether yielded 75% of fatty oil. This seed oil resembles the undi or Domba oil obtained from the seed kernels of Calophyllum inophyllum, another tree of guttiferae family. The seed oil of *Mammea longifolia*, contains palmitic (16.7%) and stearic (13.3%) acids as the major component of saturated acids with small amounts of lauric (0.4%), mayristic (1.5%) and linoleic (33.6%) are the major unsaturated fatty acids present in the oil in small amounts. The fatty oil in different seed kernels of this family varies from 4 to 85.9%. The Iodine value ranges from 36.2 to 94.0. The saponification equivalent varies from 273 to 283.2. The fatty acid composition of *Mammea longifolia* seed oil resembles that of undi oil would be seen from a comparison of the fatty acids composition of *Mammea longifolia*, undi and some other seed oils of the trees of family Guttiferae (Table-1).

Phoenix sylvestris, cerita mistis, *chrysalidocarpus* lutescens, *Washingtonia filifera* and phoenix rupicola belong to palmae family and could be compared with the oils rich in lauric acid such as cinnamon and palm kernel oils [15] (80-90% and 45-58% respectively). The seed oil of *phoenix sylvestris* contains (45.1%) of saturated acids with lauric acid as the principal component (23.8%). It also contains smaller amounts of myristic (11.9%), and palmitic (9.4%) acids. Oleic acid is the major components (41.5%) and the next in order is linoleic acid (13.4%) amongst the unsaturated acids. Caryota mitis seed oil contains 61.9% of saturated acids with lauric acid (25.8%), as the principal component. The oil also contains myristic (10.2%), palmitic (17.7%) and stearic (8.2%) acids amongst the saturated acids. The unsaturated acids are only oleic (26.3%) and linoleic (11.8%) acids.

The seed oil of *Caryota urens* is rich in palmitic acid (31.1%). The other components amongst the saturated acids are lauric (3.8%), myristic (4.8%) and arachidic (0.7%). The linoleic acid (40.1%) and oleic (16.0%) are only unsaturated acids. *Livistona rotundifolia* contains 10% of lauric acid and (15.2%) of palmitic acids amongst the saturated acids with small amounts of capric (0.9%), myristic (5.7%), stearic (7.1%) and behenic (2.0%). The unsaturated acids are only oleic (33.0%), and linoleic (26.1%). Chrysalidocarpus (Areca) lutescene seed oil contains (41.2%) of lauric acid, (26.4%) of myristic acid. The next major saturated acid is palmitic acid (11.8%). This oil also contains small amount of capric and stearic acids amongst the saturated acids. The unsaturated acids are oleic (10.5%) and linoleic (7.5%). *Washingtonia filifera*, contains (83.6%) of saturated acids and 16.4% of unsaturated acids. The major acids are lauric (25.8%), and palmitic (38.2%) with smaller amounts of myristic (10.9%) and stearic (6.6%) acids. However, arachidic (1.0%), and behonic (1.1%) acids are also present acids are only oleic (5.6%) and linoleic (10.8%).

Phoenix rupicola contains (51.8%) of unsaturated acids and (48.2%) of saturated acids. The unsaturated are oleic (41.2%) and linoleic (10.6%). This seed oil contains lauric (20.8%), myristic (12.4%), palmitic (10.9%), stearic (3.3%), arachidic (0.5%) and behenic (0.3%) acids. *Licuala grandis* seed oil contains (62.6%) of unsaturated acids. The unsaturated acids are only oleic (33.4%) and linoleic (29.0%). The palmitic (27.4%) acids is only a major component amongst the saturated acids with smaller amounts of lauric (2.5%), myristic (2.3%) stearic (1.9%), arachidic (1.4%) and behenic (2.1%) acids.

The member of plant family are noted for their higher percentage of lauric (16-52%) and myristic (7-51%) acids, and low percentage of linoleic acid which rarely exceeds 10 percentage [16]. However, Norice [17] has reported different pattern of fatty acid composition in three species of *Rhopalostylis* (palmae), which contain lauric (0.2 to 11%), myristic (0.5 to 17%) and larger amounts of linoleic acid (18.59%). In present investigation similar pattern of fatty acid composition as reported by morice in seed fat of *Rhopalostylis* is observed in *Licuala grandis*, such type of composition has also been found in *Caryota urens* and *Livistona rotundifolia*. It may be however, emphasized that the variation in linoleic acid reported by Morice has been further confirmed in the present work. The comparison of fatty acids is given in Table 2 and 3. However, the odd chain fatty acids which are present in negligible quantities are not taken into consideration for comparison.

Table 1: Comparative of Guttiferaceae family

References	**5**	**10**	**11**	**12**	**13**	**14**	**15**	**Present work**	**Present work**	**Present work**
Geographical Sources	**India**	**S. India**	**S. India**	**India**	**India**	**India**	**India**	**India**	**India**	**India**
Name of the species	***Calophyllum inophyllum***	***Calophyllum inophyllum***	***Garcinia echinocarpa***	***Garcinia indica***	***Garcinia Morella***	***Mesua ferrea***	***Garcinia xanthochymus***	***Garcinia mangostana***	***Garcinia xanthochymus***	***Mammea longifolia***
Acids										
Capric	-	-	-	-	-	-	-	1.9	-	-
Lauric	2.2	-	-	-	-	-	-	3.2	-	1.4
Myristic	2.4	-	-	-	1.3	1.2	-	7.6	1.9	2.5
Palmitic	17.9	15.8	4.7	3.5	6.2	12.6	40.0	13.8	2.1	15.7
Stearic	16.3	8.7	42.7	55.4	41.5	12.2	-	8.0	40.9	11.3
Arachidic	1.8	-	-	-	1.3	-	-	4.9	2.0	1.9

Behenic	1.6	-	-	-	-	-	-	-	2.0	2.1
Lignocaric	-	-	-	-	-	-	-	-	-	-
Oleic	34.2	48.7	53.6	38.4	42.6	58.1	54.5	57.2	53.1	31.6
Linoleic	24.5	22.8	-	2.7	7.1	12.9	4.5	7.4	-	34.5
Linolenic	-	-	-	-	-	-	-	-	-	4.0

Table 2: Comparative study of Palmae family References (12)

New zealand	**New zealand**	**Kermadec Island (Raoul)**	**Nor-folk islands**
Rhopalostylis sapida	***Rhopalostylis sapida***	***Rhopalostylis cheesemanu***	***Rhopalostylis baueri***
Trace	1.1	1.1	1.1
4.5	8.4	12.0	1.2
12.3	17.5	15.6	1.5
26.6	23.6	20.6	32.0
5.3	3.4	3.8	1.2
1.2	1.2	1.3	0.9
1.1	-	1.1	0.7
1.1	-	-	0.8
34.6	31.5	33.6	3.9
18.7	18.6	19.0	60.0
1.7	1.83	1.1	2.7
-	-	-	-

Table 3: Comparative study of Palmae family

Geographical source	**India**	**India**	**India**	**India**	**India**	**India**	**India**	**India**
Name of the species Acids	***Phoenix Sylvestris***	***Caryota mitis***	***Caryota urens***	***Livistona rotundifolia***	**Chrysalidocarpus lutescens**	***Washingtonia filifera***	***Phoenix rupicola***	***Licuala grandis***
Capric	-	-	-	1.9	1.8	-	-	-
Lauric	24.8	26.8	4.8	11.0	42.2	26.8	21.8	3.5
Myristic	12.9	11.2	4.5	6.7	27.8	11.9	13.4	3.3
Palmitic	10.4	18.7	32.1	16.2	12.8	39.2	11.9	28.4
Stearic	-	9.2	5.8	8.1	2.4	7.6	4.3	2.9
Arachidic	-	-	1.7	-	-	2.0	1.5	2.4
Behenic	-	-	-	3.0	-	2.1	1.3	3.1
Lignocaric	-	-	-	-	-	-	-	-
Oleic	42.5	27.3	17.0	34.0	11.5	6.6	42.2	34.4
linoleic	14.4	12.8	41.1	27.1	8.5	11.8	11.6	30.0
linolenic	-	-	-	-	-	-	-	-
others	-	-	-	-	-	-	-	-

3. Experimental

3.1. Extraction of seed oil

The seeds were cleaned air dried in the shade, crushed and extracted thoroughly with light petroleum (40-60 ^{0}C) in a soxhlet apparatus for 24 hours. The solvent was removed from the extract, the last traces under reduces pressure. The residual kernels were crushed again and extracted with a fresh amount of petroleum. The process was continued till no more oil was found in the petrol extract. All the petroleum extracts were mixed together, filtered, dried over anhydrous sodium sulphate and the solvent was removed to get the oil. The oil was then stored in cold.

3.2. Isolation of mixed acids free from non-saponifiable matter

A known weight of oil was hydrolyzed at room temperature for 24 hours, with 1N alcoholic potassium hydroxide solution. The excess of alcohol was removed by distillation under reduced pressure and it was diluted with distilled water to get the soap solution. From the resulting soap solution unsaponifiable matter was extracted thoroughly with ether till ether layer became colourless. This extraction was repeated at least thrice in order to make soap solution absolutely free from non-saponifiable matter. The soap solution free from unsaponifiable matter was acidified with pure dilute hydrochloric acid and the precipitated mixed acids were extracted with ether. To prevent the formation of emulsion, few crystals of an electrolyte such as sodium chloride or sodium acetate were added during ether extraction of liberated mixed acids. The ether extract was washed several times with distilled water till it is free from mineral acids and id dried over anhydrous sodium sulphate. The mixed acids were obtained from the extraction by removing the solvent under reduced pressure.

3.3. Hydrogenation and Oxidation of mixed acids

About 35-40 mgs of the mixed fatty acids with 60ml of methanol were subjected to catalytic hydrogenation with an equal amount of palladium charcoal (20)[2] in a parr-low pressure hydrogenator for about at 50-60 lbs/inch [18]. The catalyst was filtered and the solvent was removed to get the solid hydrogenated mixed fatty acids. A mixture of methanol, benzene and sulphuric acid (20:10:1) was added to a 100ml round bottom flask containing the mixed fatty acids (80-125mg) and refluxed for one hour on water bath. The methylated mixture was diluted with water, extracted with petroleum ether and washed with distilled water for several times [19]. The petroleum ether extract was then dried over anhydrous sodium sulphate, filtered and the solvent was removed. The mixed esters were then oxidized by potassium permanganate using acetone as a solvent. Potassium permanganate (0.2gm) was added to the mixed esters (100mg) dissolved in acetone (20 ml) and acetic acid (1ml) and refluxed for 30 min. Then the solvent was removed under

pressure, water and a little sulphuric acid were added. The solution was decolourised in a stream of sulphur dioxide. The resulting solution was extracted with petroleum ether. The extract was dried over anhydrous sodium sulphate, filtered and then the solvent was removed. These oxidized mixed esters were hydrolysed with 0.5N alcoholic potassium hydroxide solution and the resulting free mixed acids were chromatographed.

3.4. Iodine value

Wijs solution was readily prepared by dissolving 7.8 gms of iodine trichloride and 8.5 gms of iodine in warm glacial acetic acid and make solution upto 1000ml with glacial acetic acid [20]. The determination was made by weighing about 100 to 200 mgs of the mixed fatty acids in a small glass capsule and was placed in 250ml glass stoppered bottle. The fat was dissolved in 10ml of chloroform and 20 ml of Wijs solution was then added by means of pipette. A blank was also run at the same time. The bottles were stoppered and left in dark for 30 minutes. At the end of the requisite time, 15ml of 10% potassium iodide solution and 100 ml of water were added. The liberated iodine was titrated with 0.1N sodium thiosulphate solution using starch solution as an indicator.

3.5. Saponification value

The 0.5 N alcohol potash solution was prepared by dissolving 30 gms of pure potassium hydroxide in the minimum quantity of water and adding 1 litre of 95% ethanol [20]. After thorough shaking the solution was left overnight preferably at 0 ^{0}C in order to precipitate any potassium carbonate, and filtered before use. Approximately 250 to 400 mgs of mixed fatty acids were weighed accurately into 250 ml alkali resistant glass flask, which was provided with a reflux condenser with a ground glass joint. The content of the flask were gently boiled for 1 hour. The condenser was washed with little water. To this mixture, 1ml of phenolphthalein solution was adder and the excess alkali was titrated while still hot against standard 0.5N HCL or H_2SO_4.

3.6. Refractive index of oils

Abbe's refractometer was used for measuring the refractive indices of oils. The prisms of the instrument were cleaned with toluene and the spaces between them filled with clear oil free from all air bubbles [21]. Prisms were closed and the readings were taken when the temperature remained constant for at least 3 minutes. Several readings were taken for each oil and the average of all the readings were recorded as the refractive index. The exact temperature was also noted down. An approximate temperature correction was made by applying a correction factor, F (0.00035 for temperature around 25 ^{0}C and 0.00036 for temperature of 40 ^{0}C or more).

3.7. Estimation of cyclopropenoid acids

The Halphen test was originally developed as an empirical method of testing the adulteration of adulteration of various vegetables oils by cotton seed oil [22]. Though many modifications of the reagent and reaction condition have been described, basically the method involves heating the oil with 1% solution of sulphur in carbon disulphide. If cotton seed oil is present a pink colour develops. The reaction is now believed to be specific for the cyclopropene ring [23] and is a quick and easy method of checking whether cyclopropenoid fatty acids are present in a mixture. It is possible to use the reagent as a TLC spray. Under controlled conditions the reaction can be used as a colourimetric method of estimating the total cyclopropene constant of an oil [24].

3.8. Reserved phase partition chromatography (RPO)

The chromatographic studies are based on the direction of gunstone and co-workers [25]. The column contains a solid support such as kieselguhr made hydrophobic with dichlorodimethylsilane which is further coated with paraffin. Paraffin acts as the stationary phase. The columns are loaded and developed as described by gunstone and sykes [25] except that they are run and preserved at room temperature. Different concentrations of aqueous acetone equilibrated with stationary phase containing bromothymol blue as indicator, are used as eluting solvents. The sample loaded on top of the column is developed continuously and successively with solvents of increasing polarity. The eluate collects in siphon (2ml) and passes into a specially constructed cell where it is titrated under a stream of nitrogen with 0.01N methanolic potassium hydroxide solution using an "alga" micrometer syringe as burette. The titration curves are drawn by plotting the eluate fraction against the volume of methanolic alkali required for neutralization. The position of a peak in a titration curve is characteristic of an acid or group of acids in a particular solvent system and the acids present in a mixture are identified by comparing of their peaks with given by known acids in the same solvent system. By summing up the titres under each peak, after allowing for the small acidity due to the eluating solvents, the results for the chromatograms are calculated. In this way the hydrogenated acids, mixed acids and oxidised solids are chromatographed separately, the elution curves are drawn and the results calculated.

3.9. Identification of di- and tri-ethenoid acids

3.9.1. By bromination

During chromatographic analysis, the unsaturated acids were isolated from the appropriate fractions and were characterized by bromination as per the method of Bibner and Muggenthaler [26]. The unsaturated acids (500 mg) were dissolved in dry ether (30ml) and cooled to -10 ºC. A solution of bromine in dry ether (1:10) was added drop and the temperature of the mixture was maintained below -5 ºC. The solution on the standing overnight at 0 ºC deposited white crystals. The crystals were filtered off and the filtrate was washed with an aqueous solution of sodium thiosulphate to remove excess of bromine. The yellowish broom viscous residue after removal of ether was then dissolved in petroleum ether (60-80 ºC) and allowed to stand overnight at 0ºC when it deposited crystals of bromide. The crystalline bromides were then characterized by their melting points and mixed melting points with authentic samples. Oleic acid gave 9,10-dibromostearic acid (m.p. 28-29 ºC). Linoleic acid gave 9,10,12,13-tetrabromosteric acid 9m.p. 113-114 ºC) and linolenic acid gives 9,10,12,13,15,16-hexabromostearic acid (m.p. 181-182 ºC) respectively.

3.9.2. By oxidation with one percent alkaline potassium permanganate solution

Oxidation of unsaturated acids was carried out according to the method of Lapworth and Nottram [27]. About 200mg of unsaturated acids were warmed with equal weight of sodium hydroxide and 12 ml of water. The mixture was cooled to -5 ºC with vigorous shaking. The crushed ice (75gm) was added followed by potassium permanganate (1%, 16 ml). After 5 minutes the reaction mixture was decolourised by passing a

current of sulphur dioxide followed by the addition of concentration hydrochloric acid (6ml). A thick white flocculent precipitate was obtained. It was drained quickly and washed with petroleum ether. The product was crystallized form rectified spirits. Oleic and linoleic acids gave 9, 10-dihydroxystearic acid and 9, 10, 12, 13-tetrahydroxy stearic acids (m.p.131-132 ^{o}C and 166-167 ^{o}C) respectively.

4. Conclusion

In present study, investigation on guttiferae and palmae was undertaken with a hope that the fatty acids of novel structure which would be of academic interest and might have some practical utility might be discovered. The results of this investigation indicate the seed oil of *Garcinia mangostana* seed oil contains 56.2% oleic acid and 6.4% linoleic acid. The palmitic and stearic acid are major components amongst the saturated acids with smaller amounts of capric, lauric, myristic and arachidic. The seed oil of *Phoenix sylvestris* contains (45.1%) of saturated acids with acid with lauric acid as the principal component (23.8%). It also contains smaller amounts of myristic (11.9%), and palmitic (9.4%) acids. Oleic acid is the major components and the next in order is linoleic acid amongst the unsaturated acids.

5. Acknowledgements

This research work is supported by the Essar Laboratories and Research Centre, Hubli-580023, INDIA and Research Center, Dept. of Applied Chemistry, SECAB Institute of Engineering & Technology, Vijayapur – 586101, INDIA

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