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Proximate Composition and Fatty Acid Profile of *Pongamia pinnata*, a Potential Biodiesel Crop

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Abstract The chemical characteristics of *Pongamia pinnata* seeds, focussing on proximate composition and the fatty acid profile of its oil, are presented. The proximate composition of *P. pinnata* seeds was: 3.8% ash, 9.7% sugar, 7.07% protein, 24% oil, 10.7% free amino acids, and 0.24% free fatty acids. The oil was extracted from seeds by use of different solvents and the highest yield (29%) was obtained by use of *n*-hexane. Monounsaturated and polyunsaturated fatty acids accounted for 63.3 and 22.9%, respectively, of the seed oil. Oleic acid was the major fatty acid but a substantial amount of erucic acid was also detected; this was not reported in previous studies. The level of erucic acid and the presence of toxic flavonoids, for example karanjin, pongapin, and pongaglabrin, render the oil inedible according to WHO recommendations. However, low levels of saturated and polyunsaturated fatty acids with desirable cetane number and iodine value suggest potential for application as a biodiesel fuel.

Keywords Biodiesel · Fatty acids · Karanja · *Pongamia pinnata* · Proximate composition

Abbreviations

CN	Cetane number
FAME	Fatty acid methyl esters
IV	Iodine value
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
SN	Saponification number
WHO	World Health Organization

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Introduction

Pongamia pinnata (L.) Pierre synonym *Derris indica* and *Pongamia glabra* [1], belonging to the family Fabaceae, is an Indo-Malaysian species. This medium-sized evergreen tree is commonly found in alluvial and coastal situations from India to Fiji, from sea level to a height of 1,200 m. In India, it is commonly known as Karanja [2]. After 5–7 years of growth it bears fruits containing one to two kidney-shaped brownish-red kernels. It is one of the few nitrogen-fixing trees producing seeds/kernels containing 18–27% oil [3]. It is a medicinally important plant used for its anti-inflammatory [4], anti-plasmodial [5], anti-hyperammonic and antioxidant [6], anti-diarrhoeal [7], anti-ulcer

[8, 9], anti-lipidperoxidative and anti-hyperglycaemic [10], and anti-lice [11] activity. Karanja oil also finds use as a raw material in the soap and leather-tanning industries. Freshly extracted karanja oil is yellowish orange to brown in colour having a disagreeable odour and a bitter taste. The presence of toxic flavonoids, for example karanjin, pongapin, and pongaglabrin, renders the oil inedible. However, after detoxification the meal can be used as a feed ingredient for cattle [12]. *Pongamia pinnata* oil has recently been reported in India as a substitute for diesel [15]. Despite this, karanja is an underutilized plant in the Rajasthan state of India. Although various studies report its fatty acid composition, results are highly variable [3, 13, 16]. Because information about the biochemical constituents of its seed is scarce, this study was undertaken to determine the proximate composition of *P. Pinnata* seeds. Extraction of the total oil using different solvents was also undertaken, and hexane extract was used to determine its fatty acid profile.

Materials and Methods

Pongamia pinnata mature seeds were collected from plants growing along the roadside in Jodhpur and Bharatpur cities of Rajasthan state in India. *Pongamia pinnata* seeds were analyzed for various biochemical constituents, viz: moisture and ash content [17], soluble sugar [18], protein [19], free amino acids [20], and free fatty acid content [21]. Total lipids were isolated by use of the cold extraction method [22] by macerating the crushed kernel in chloroform–methanol 2:1 overnight and then separation by use of a separatory funnel. Seed oil was extracted with benzene, ether, and hexane by Soxhlet extraction [23]. The hexane extract was analyzed by use of a GCMS-QP-2010 to determine the fatty acid profile. Methyl esters of the fatty acids were prepared by transesterification, by addition of sodium methoxide and sodium chloride, and one microlitre of the methyl ester extract was injected in splitless mode on to an Rtx®-wax (crossbonded PEG) column (30 m long × 0.25 mm i.d. × 0.25 μm d_f), at an injector temperature of 250 °C, initial oven temperature 180 °C, and final oven temperature 240 °C (programmed at 5 °C min^{-1}), oven equilibration time 1.01 min, helium carrier gas pressure 100 kPa, total flow 30 ml min^{-1}, and interface temperature of 250 °C. Fatty acids were identified by use of the standard MS library of Nist 05. Saponification number (SN) and iodine value (IV) were calculated from the FAME composition of the oil by using the method of Kalayasiri et al. [24] and cetane number (CN) was calculated by use of the equation given by Krisnangkura [25]. All data are expressed as mean ± standard error of three replicates.

Results and Discussion

Biochemical evaluation revealed that *Pongamia pinnata* seeds contained 3.8% ash, 9.7% total sugar, 7.07% total soluble protein, and 10.7% free amino acids. Cold extraction resulted in isolation of 24% lipid whereas Soxhlet extraction yielded 29% lipid. Free fatty acid content was found to be 0.24%. Our results showed little variation from those of previous workers, who reported 27.5% oil, 17.4% protein, 6.6% starch, 7.3% crude fibre, and 2.4% ash content [14]. The remaining material comprised 19% moisture, and other biochemical constituents, for example starch, crude fibre, etc. The defatted cake can be used as a nutritious feed after detoxification [12].

Seed oil yield obtained by Soxhlet extraction with different solvents was in the range 24–29% (*v*/*w*). The highest yield of oil (29%) was obtained by use of hexane and the lowest by use of chloroform (24%). Our results are in accordance with the findings of Kesari and co-workers who also reported hexane to be the best solvent for oil extraction from Pongamia [26].

The fatty acid profile of total lipid extracts was further analyzed by use of GC–MS (Fig. 1). These data (Table 1) revealed that mature seeds contained oleic (44%), linoleic (17.4%), erucic (15.9%), palmitic (7.2%), linolenic (5.5%), 11-eicosenoic (3.4%), stearic (3.3%), behenic (2.5%), and eicosanoic (0.78%) acids. However, this composition profile was different from those in other reports [3, 13, 26]. This different fatty acid composition might be because of variation in species or different ecological conditions, because local edaphic and environmental factors are of crucial importance to the growth and characteristics of a particular plant, including the richness and uniqueness of the germplasm; they also impart characteristic traits specific to a region.

The oil was dark in colour with a disagreeable odour, and the ratio SFA:MUFA:PUFA in pongam oil was higher (1:4.60:1.66) and the $\omega 6$:$\omega 3$ ratio was lower than the respective recommended values of 1:1–3:1 and 5–10 for edible oils [27], which make this oil inedible. *Pongamia pinnata* oil analysed in this study contained 13.76% saturated, more than 60% monounsaturated, and ~23% polyunsaturated fatty acids. These data reveal that the total saturated fat (13.76%) present in *Pongamia pinnata* oil is lower than reported elsewhere [26]. Any vegetable oil can be used as raw material for the production of biodiesel. However preference is given to oils with low saturation and low polyunsaturation [28]. SN, IV, and CN of fatty acid methyl esters of the oil were determined empirically and found to be 128.8, 96.5, and 51.4, respectively. Iodine value is the degree of unsaturation and CN is the ability of fuel to ignite quickly after being injected and a higher value indicates a better ignition quality of fuel. CN of 51

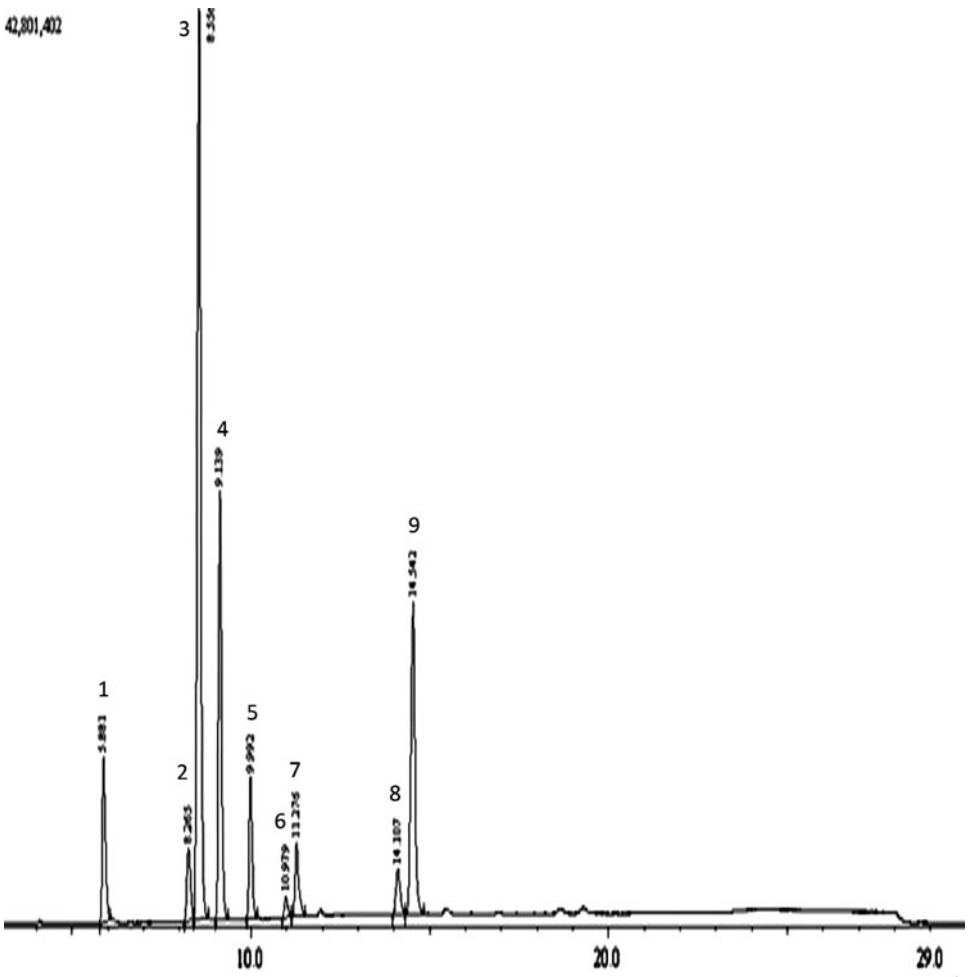

Fig. 1 Fatty acid profile of mature Pongamia seeds. *The numbers 1–9* denote the various fatty acids as listed in Table 1

Table 1 Fatty acid profile of hexane extract of *Pongamia pinnata* seeds

No.	Fatty acid	Mature seeds	Already known
1	Palmitic acid (16:0)	7.18 ± 0.13	11.65, 10.8
2	Stearic acid (18:0)	3.32 ± 0.14	7.50, 8.7
3	Oleic acid (18:1)	43.99 ± 0.92	51.59, 46.0
4	Linoleic acid (18:2)	17.38 ± 0.19	16.64, 27.1,
5	Linolenic acid (18:3)	5.51 ± 0.06	–, 6.3
6	Eicosanoic acid (20:0)	0.78 ± 0.07	1.35, 0.8
7	11-Eicosenoic acid (20:1)	3.43 ± 0.15	–, –
8	Behenic acid (22:0)	2.48 ± 0.03	4.45, –
9	Erucic acid (22:1)	15.90 ± 0.61	–, –
10	Lignoceric acid (24:0)	–	1.09, –

Each value is the mean ± SE from three replicates

and IV less than 115 is the lowest limits among biodiesel standards set by European Standard Organization [29]. Our values for CN and IV fulfil these standards so *Pongamia pinnata* could be utilized in the future as a biodiesel plant.

Conclusions

From this study it is concluded that *Pongamia pinnata* seed oil could be used for biodiesel production because it contains small amounts of saturated and polyunsaturated fatty acids and has desirable IV and CN values. After detoxification the leftover defatted cake can be used for feed purposes.

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References

1. Allen ON, Allen EK (1981) The leguminosae. The University of Wisconsin Press, USA, p 812
2. Rangari VD (2002) Phytochemistry and pharmacognosy, 1st edn. part 2. Career Publication, Nashik, pp 259–261
3. Lakshmikanthan V (1978) Tree borne oil seeds, directorate of non edible oils and soap industry. Khadi and Village Industries Commission, Mumbai, p 10

4. Srinivasan K, Muruganandan S, Lal J (2001) Evaluation of anti-inflammatory activity of *Pongamia pinnata* leaves in rats. J Ethnopharmacol 78:151–157
5. Simonsen HT, Nordskjold JB, Smitt UW (2001) In vitro screening of Indian medicinal plants for antiplasmodial activity. J Ethnopharmacol 74:195–204
6. Essa MM, Subramanian P (2006) *Pongamia pinnata* modulates oxidant and antioxidant I imbalance in ammonium chloride induced hyper ammonem. Fundam Clin Pharmacol 20:299–303
7. Brijesh S, Daswani PG, Tetali P (2006) Studies on *Pongamia pinnata (L.) Pierre* leaves: understanding the mechanism(s) of action in infectious diarrhea. J Zhejiang Univ Sci B 7:665–674
8. Prabha T, Dora M, Priyambada S (2003) Evaluation of *Pongamia pinnata* root extract on gastric ulcers and mucosal offensive and defensive factors in rats. Indian J Exp Biol 41:304–310
9. Punitha R, Manoharan S (2006) Antihyperglycemic and antilipidperoxidative effects of *Pongamia pinnata* (Linn.) Pierre flowers in alloxan induced diabetic. J Ethnopharmacol 105:39–46
10. Vismaya, Belagihally SM, Rajashekhar S, Jayaram VB, Dharmesh SM, Thirumakudalu SKC (2010) Gastroprotective properties of Karanjin from Karanja (*Pongamia Pinnata*) seeds; role as antioxidant and H^+, K^+-ATPase inhibitor. Evid based complement. Altern Med. doi:10.1093/ecam/neq027
11. Samuel AJSJ, Radhamani S, Gopinath R, Kalusalingam A, Kumari AG, Vimala A, Husain HA (2009) In vitro screening of anti-lice activity of *Pongamia pinnata* leaves. Korean J Parasitol 47:377
12. Mandal B (1985) Protease inhibitors and in vitro protein digestibility of defatted seed cakes of akashmoni and karanja. J Am Oil Chem Soc 62(7):1124–1126
13. Shameel S, Usmanghani K, Ali MS (1996) Chemical constituents from seeds of *Pongamia pinnata* (L.) Pierre. Pak J Pharm Sci 9:11–20
14. Bringi NV, Mukerjee SK (1987) In: Bringi NV (ed) Non-traditional Oilseeds and Oils of India. Oxford IBH Publishing Co. Pvt. Ltd, New Delhi, pp 143–166
15. Shrinivasa U (2001) A viable substitute for diesel in rural India. Current Sci 80:1483–1484
16. Naik M, Meher LC, Naik SN, Das LM (2008) Production of biodiesel from high free fatty acid Karanja *(Pongamia pinnata)* oil. Biomass and Bioenerg 32:354–357
17. AOAC (1984) Official methods of the association of official analytical Chemists,14th edn, Washington, pp 330–342
18. Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
20. Lee YP, Takahashi T (1966) An improved colorimetric determination of amino acids with use of ninhydrin. Anal Biochem 14:71–77
21. Lowry RR, Tinsley JJ (1976) Rapid colorimetric determination of free fatty acids. J Am Oil Chem Soc 53:470–474
22. Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
23. AOAC (1995) Official methods of analysis of AOAC international, 16th edn. Arlington, Virginia
24. Kalayasiri P, Jeyashoke N, Krisnangkura K (1996) Survey of seed oils for use as diesel fuels. J Am Oil Chem Soc 73:471–474
25. Krisnangkura K (1986) A simple method for estimation of cetane index of vegetable oil methyl esters. J Am Oil Chem Soc 63:552–553
26. Kesari V, Das A, Rangan L (2010) Physico-chemical characterization and antimicrobial activity from seed oil o*f Pongamia pinnata*, a potential biofuel crop. Biomass Bioenerg 34:108–115
27. Kale V (2007) In souvenir National seminar on changing vegetable oil scenario: issues and challenges before India. Indian society of oilseeds research, Directorate of oilseeds Research, Hyderabad, pp 81–86
28. Korbitz W (1995) Utilization of oil as a biodiesel fuel. In: Kimber D, McGregor DI (eds) Brassica oilseeds production and utilization. University Press, Cambridge, pp 353–376
29. Biodiesel Standard (2003) EN 14214, European Standard Organization