Technical

Chemical and Nutritional Evaluation of *Pongamia glabra* Oil and *Acacia auriculaeformis* Oil

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ABSTRACT

Karanja seed *(Pongamia glabra)* oil contains toxic flavonoids including 1.25% karanjin and 0.85% pongamol. After refining the oil resembles peanut oil in composition and is free from toxic flavonoids, bitterness and unpleasant odors. Akashmoni seed *(Acacia auriculaeformis)* oil is rich in stearic acid (31%), and nearly two-thirds of its glyceride is GS_2U (disaturated monounsaturated), mostly SOS (saturated-stearic acid and unsaturated-oleic acid). Nutritional evaluations of these two refined seed oils were carried out in rats by feeding the respective oils and peanut oil as control at 10% level in a 20% protein diet for 12 weeks. The animals fed karanja oil showed poor growth performance, altered lipid metabolism and fatty infiltration in liver. Akashmoni oil in the diet of rats did not reveal growth retardation or any abnormalities in evaluations of lipid parameters of serum and liver or histopathological findings. The results of this study indicate that refined karanja oil is toxic to rats and may not be desirable for edible purposes, while akashmoni oil may be desirable.

INTRODUCTION

The shortages of and high prices paid for traditional edible oils (1) in livestock feeding have stimulated nutritional and economic feasibility studies of minor oils (2). India has about 86 types of minor oilseed-bearing perennial trees (3, 4), of which neem *(Azadirachta indica),* muhua *(Madhuca indica),* sal *(Shorea robusta),* karanja *(Pongamia glabra),* kokum *(Garcinica indica),* castor *(Ricinus communis),* kusum *(Scheleichera trijuga),* dhupa *(Vateria indica),* mango *(Mangiferra indica),* khakan *(Salvadora deoides)* and maroti *(Hydnocarpus laurifolia)* are the important ones. These minor seed oils are characterized by deep color, unpleasant odor and taste and high contents of free fatty acids and non-lipid components (5,6). Therefore, they need to be refined and thoroughly evaluated for their chemical, nutritional and toxicological properties before being used as supplementary oils for animal consumption. Such studies on some minor seed oils have been reported recently (7,8).

Pongamia glabra (Leguminosae), a medium sized glabrous tree, popularly known as karanja, is widely available in India. The oil content of karanja seed is about 29% and is used as a cheap, raw source of soap and leather dressing. Current annual production of this oil in India is approximately 30,000 tons and likely to increase; of this hardly one-fourth is presently used (9). Karanja oil has a yellowish orange color and contains karanjin (1.25%) and pongamol (0.85%) as major toxic flavonoids, with some other, minor flavonoids (10). Processes for the removal of these non-lipid associates have been reported (11,12), but the information on the complete chemical analysis of the oil and its nutritional status is scanty. This paper reports the data on these two aspects.

Acacia auriculaeformis (akashmoni) grows widely in the lower lands of India and is capable of growing under a wide range of agroclimatic conditions. The seed contains a high level of tannins (8-10%) and is reported to cause hypoglycemia when given in the diet of rats (13). The oil content of the seed is about 26%. Fatty acid composition data of this seed oil or its nutritional value have not, to our knowledge, been reported. In this study we present data on the chemical composition and nutritional quality of this oil, so as to promote its maximum use.

MATERIALS AND METHODS

Oilseeds were collected from the local forests of Burdwan (W.B.), India and air dried. The seeds were powdered and extracted with petroleum ether (40-60 C) in a soxhlet apparatus. Karanja oil was refined, and toxic flavonoids including karanjin and pongamol were removed by the method described earlier (12). Akashmoni oil was refined according to the method recommended by the AOAC (14).

Chemical constants were determined by conventional methods (15), and fatty acid analysis was by gas liquid chromatography (Perkin Elmer F 11 gas-liquid chromatograph) using a 15% DEGS column on chromosorb WHMDS (16).

Thirty-six male weanling rats of wistar strain, age 22 to 24 days, were divided into three groups of 12 animals each and individually caged. All the animals were maintained under controlled temperature (21 ± 1 C) and humidity (55%) conditions. Animals in each group were fed a stock standard diet (17) except for the 10% oil. Oils used in the study were peanut oil and refined karanja and akashmoni oils. All the animals in different groups received their corresponding diet ad libitum for a period of 12 weeks. Food intakes were recorded daily and the animals weighed twice weekly. Feed efficiency ratios (FER), which represent the weight gain for unit food intake, were calculated. Digestibility of the oils was determined by estimating the oil intake and oil excreted through urine and feces (18).

At the end of 12 weeks, the animals were sacrificed, blood and livers were collected and total lipids (19), phospholipids (20), free fatty acid (FFA) (21), cholesterol (22) and triglycerides (23) were determined. Organs such as liver, kidney, heart, spleen, pancreas, intestine and reproductive organs were subjected to histopathological examination under an Ortholux-Leitz microscope. Statistical analysis of the data was done by student t-test.

RESULTS AND DISCUSSION

Karanja Oil

Data on chemical composition of the karanja oil and its fatty acid composition are given in Table I. Refining of the oil reduced the visible color and acid value from 6.3 to 0.8%. The final product was obtained in a yield of 91%. The refined oil was found to be devoid of bitterness, unpleasant odor and toxic flavonoids including karanjin and pongamol. The oil resembles peanut oil in composition and contains no oxygenated fatty acids such as epoxy, keto or hydroxy acids, as revealed by GLC.

Growth performance and the value for FER are presented in Table II, which revealed the poor growth performance and low value for FER in the group of rats fed 10%

TABLE I

Physicochemical Constants of Refined Karanja Oil and Akashmoni Oil[a]

	Karanja	Akashmoni
Oil constants		
Unsaponifiable fraction	0.6%	1.0%
Saponification value	180	105
Acid value	0.8%	0.4%
Iodine value	85	62
Refractive index	1.47	1.47
Fatty acids		
Myristic (14:0)	1.6	0.9
Palmitic (16:0)	7.9	10.1
Stearic (18:0)	3.7	31.1
Arachidic (20:0)	2.5	2.0
Behenic (22:0)	4.2	4.5
Lignoceric (24:0)	1.1	–
Oleic (18:1)	55.1	40.5
Linoleic (18:2)	18.9	8.4
Linolenic (18:3)	5.0	2.5

[a]Average of 4 samples.

karanja oil as compared to those fed refined peanut oil. Digestibility of the karanja oil was 91%, compared to 94% for peanut oil. Lipid profile of serum and liver are presented in Table III. Total lipids of serum and liver and liver triglycerides of rats fed karanja oil were significantly higher compared to those of the control group. Cholesterol, FFA and phospholipids of serum and liver and serum triglycerides were not significantly different between the two groups. Liver weight also was increased in the rats fed karanja oil over the controls. The results obtained indicated altered lipid metabolism in the rats fed karanja oil. No histopathological abnormalities were found in any organ except the liver. The liver sections from all the rats for the diet containing 10% karanja oil showed a mild to moderate degree of fatty infiltration.

Thus the rats fed 10% karanja oil showed poor growth performance, altered lipid metabolism and histopathological abnormalities in the liver. This may be due to non-lipid component(s) other than flavonoids being present in the refined oil and needs to be investigated. The present study therefore indicates that the refined karanja oil is not suitable as a dietary source of fat, unless a suitable method is found for its complete detoxification.

TABLE II

Growth Rate of Rats Fed Peanut, Karanja and Akashmoni Oils for 12 Weeks[a]

Source of oil	Av gain in body wt in g (at 84 days)	FER[b]	Digestibility of fat (%)
Peanut oil	126.4 ± 10.2	22.5 ± 2.1	94
Karanja oil	84.2 ± 11.4[c]	18.1 ± 1.8	91
Akashmoni oil	124.5 ± 12.3	21.1 ± 2.3	94

[a]Values are mean ± SEM for 12 animals.

[b]FER = feed efficiency ratio = body weight gain/food intake weight × 100.

[c]Levels of significance with respect to peanut group, $P < 0.01$.

Akashmoni Oil

Physicochemical characteristics and fatty acid composition of the seed oil are presented in Table I. Refining of the oil following the AOAC method diminished the visible color and reduced the acid value to 0.4% from 13.6% for crude oil. Toxic non-glyceride components were not found in the oil by chemical analysis, and the oil was devoid of oxygenated fatty acids. The oil has a high stearic acid content, and nearly two-thirds of its glycerides were GS_2U, i.e., of the disaturated monounsaturated type, mostly SOS (saturated-stearic acid and unsaturated-oleic acid).

Growth performance of rats fed akashmoni oil was similar to that of the control group. The value for FER and digestibility of akashmoni oil was similar to that seen with peanut oil. The groups of rats fed either akashmoni oil or peanut oil did not differ with regard to a variety of lipid parameters including liver weight (Table III). Histopathological examination of the various organs did not show any differences in either group, and all the organs were normal.

Thus, the chemical and nutritional qualities of the seed oil appear to be satisfactory and may be desirable for edible purposes. Since it is high in saturated fatty acids, akashmoni oil has a potential for use as a cocoa butter substitute/equivalent in chocolates or confectionery.

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TABLE III

Serum and Liver Lipids of Rats Fed Peanut, Karanja and Akashmoni Oils for 12 Weeks[a]

Parameters studied	Oil source		
	Peanut oil	Karanja oil[b]	Akashmoni oil
Liver wt as % body wt	3.5 ± 2.1	4.1 ± 2.2	3.4 ± 2.3
Serum			
Total lipids (mg/100 mL)	278.3 ± 8.5	312.2 ± 7.4*	280.6 ± 9.3
Total phospholipids (mg/100 mL)	84.3 ± 3.9	80.1 ± 4.3	85.2 ± 4.6
Total cholesterol (mg/100 mL)	68.2 ± 4.1	70.4 ± 3.8	72.5 ± 4.2
Triglycerides (mg/100 mL)	20.3 ± 2.1	25.2 ± 2.6	21.8 ± 2.4
FFA (m mol/L)	0.32 ± 0.04	0.34 ± 0.05	0.31 ± 0.02
Liver			
Total lipids (mg/g)	132.1 ± 4.2	288.7 ± 5.8**	136.3 ± 4.9
Total phospholipids (mg/g)	78.2 ± 3.4	75.1 ± 3.8	79.1 ± 4.1
Total cholesterol (mg/g)	6.2 ± 0.4	6.8 ± 0.6	7.1 ± 0.5
Triglycerides (mg/g)	24.1 ± 1.6	48.4 ± 2.1**	26.3 ± 1.8
FFA (mg/g)	3.4 ± 0.7	3.8 ± 0.5	3.3 ± 0.4

[a]Values are mean ± SEM for 12 animals.

[b]Levels of significance with respect to peanut oil group, *$P < 0.01$; **$P < 0.001$.

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Method of Analysis for Deoxynivalenol and Zearalenone from Cereal Grains

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ABSTRACT

A method was developed to determine deoxynivalenol and zearalenone in corn, wheat, oats, rice and barley. The toxins are extracted with methanol/water (50:50, v/v) (2×) and partially purified by partitioning into ethyl acetate and then defatting with acetonitrile-petroleum ether. Toxins are isolated by silica gel column chromatography. Interfering materials are removed from the column with benzene; zearalenone is eluted with benzene/acetone (95:5, v/v), and after a column wash of chloroform/methanol (98:2, v/v), deoxynivalenol is eluted with chloroform/methanol (95:5, v/v). Zearalenone is quantitated by thin-layer chromatography and deoxynivalenol by gas-liquid chromatography of the trimethylsilyl derivative. The detection limit is about 0.02 μg/g for each toxin. Recoveries of added toxins varied with substrate and level of toxins. Recovery of deoxynivalenol ranged from 58% for 1 ppm in rice to 108% for 1 ppm in corn. Average recoveries for all levels (1, 2 and 5 ppm) ranged from 69% for barley to 89% for oats. Recovered zearalenone ranged from 40% for 5 ppm in wheat to 100% for 1 ppm in barley. Average recoveries for zearalenone at 1, 2 and 5 ppm varied from 53% for wheat to 87% for rice.

INTRODUCTION

Fusarium graminearum, a common colonist of cereal grains, produces both zearalenone and deoxynivalenol in corn and mixed feed (1,2). This coexistence of zearalenone and deoxynivalenol could pose a serious threat to animals, especially swine, due to estrogenic and feed refusal effects. Procedures have been published for the individual determination of deoxynivalenol and zearalenone which are both specific and sensitive (3-6). Also some information is available on the simultaneous determination of several *Fusarium* mycotoxins in grains and feeds (7,8). A more rapid procedure which utilizes commonly available chemicals and equipment was required to analyze inoculated cereal grains for the co-production of deoxynivalenol and zearalenone by various *Fusarium* species. Vesonder et al. (9) have determined the production of both toxins by 16 *Fusarium* isolates on corn, but recoveries of the toxins by the method used were not reported. We have developed a screening method for the determination of both toxins in the same extract and have tested the procedure on inoculated and spiked samples of corn, rice, oats, wheat and barley. This paper describes the method of analysis and reports the recoveries of both toxins from spiked substrates.

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MATERIALS AND METHODS

Spiked Substrates

Samples (50 g) of toxin-free, ground, blended corn, wheat, oats, rice and barley were individually spiked with deoxynivalenol and zearalenone to contain 1.0, 2.0 and 5.0 ppm of each toxin. Triplicate samples of the grains were prepared at these three levels. The toxins (in acetonitrile) were added to the samples with a Hamilton syringe (100 μL), and solvent was allowed to evaporate ca. 2 hr before extraction.

Extraction

A modified procedure of Scott et al. (3) was used to extract deoxynivalenol and zearalenone from the spiked substrates and inoculated substrates: blend substrate with 250 mL methanol/water (1:1, v/v) for 5 min. Transfer suspension to centrifuge bottles and centrifuge for 5 min at 5,000 rpm. Decant centrifugate and re-extract sediment with second 250 mL solvent. Repeat centrifugation and combine centrifugates. Add 100 mL saturated sodium chloride to extract and partition with 100 mL ethyl acetate (3×). Add ca. 60 g anhydrous sodium sulfate to the combined ethyl acetate fractions and swirl to remove traces of water. Filter, evaporate solvent on a rotary evaporator and dissolve residue in acetonitrile (50 mL). Transfer to separatory funnel and defat with 50 mL petroleum ether (2×). Remove acetonitrile under vacuum and dissolve residue in methylene chloride for column chromatography.