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Short Communication

Extraction of Alkaloids and Oil from Karanja (*Pongamia pinnata)* Seed

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Abstract

The present study deals with the extraction of alkaloids and oil from Karanja seed. It was observed that Karanja seed grown under the soil and climatic condition of Bangladesh contains alkaloids and 32% of bitter, red brown thick oil. Using hexane as the only organic solvent alkaloids and oil were recovered from the seeds and defatted kernels by two extractions.. The oil and those alkaloids which occurred as free bases were recovered first with hexane from the dried crushed seeds. Then hexane-insoluble salts of the alkaloids retained in the defatted kernels were converted into hexane-soluble free bases by the treatment with aq. sodium carbonate or ammonium hydroxide. Then after the free bases, soluble in hexane thus obtained were recovered by another extraction with hexane. Thus a proteinaceous meal was obtained containing very low alkaloid without losing any proteins. The alkaloids which were dissolved in the oil were then converted into water-soluble salts by treating with aq. hydrochloric acid. Thereafter, the water soluble salts were completely removed by repeated extraction with water.

Keywords: *Pongamia pinnata;* Oil; Alkaloid; Extraction.

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1. Introduction

Karanja (*Pongamia pinnata* Linn), a small handsome evergreen shade tree with glabrous bright green foliage, grows wildly almost in all the districts of Bangladesh [1]. Karanja is often planted in homesteads as a shade or ornamental tree and it is also planted as avenue plantings along road sides and canals.

It is one of the few nitrogen fixing trees which produced seeds containing pongam oil, bitter, red brown thick, non-drying non edible oil. The seeds contain about 27-39% oil which may be used for tanning leather, for making soap, as a liniment to treat scabies, for curing rheumatism and as illuminating oil [2]. The oil is treated as fuel in diesel engine, showing a good thermal efficiency [3]. Karanja oil is a promising source of biodiesel, an

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alternative diesel fuel which is completely biodegradable and non-toxic [4-10]. Several groups of researchers [11,12] had reported the fatty acid composition of the Karanja oil. The tree is also a host plant for Lac insect. The seeds gave positive test for alkaloids [13]. Plants containing alkaloids (cocaine, morphine, codeine) are capable to exhibit extensive and well marked pharmacological activities like analgesic antiamoebic and emetic (emetine) etc. [14].

Until recently, most of the attention on any oil seed crushing had dealt with oil recovery, with little attention for meal by-products. But with increased attention to protein for human diets, the industry is taking a careful look at the effects of pre- and post-oil removal conditions on meal characteristics [15]. So, if the *Pongamia pinnata* seed meal, which is a byproduct of oil recovery plants, containing a higher percentage of protein, can be free from alkaloids and could be a source of supplementary protein for human diet and the alkaloids might be also the effective natural drugs for suffering humanity.

To make the oil and oil cake free from alkaloids the seeds were dehusked and the kernels were crushed into small pieces by hammer mill which were then extracted with hexane to yield the oil and a minor portion of the alkaloids. The alkaloid containing oil was then refined, which leads to a complete removal of the alkaloids.

Protein concentrates, virtually free of alkaloids can be obtained from hexane defatted meal of any bitter seeds by extraction with aqueous alcohol [16]. But according to Horn *et.al*. [17] solvents like alcohol have the advantage of removing most of the alkaloids from meals, but meals are difficult to desolventize and may have undesirable odors remaining in them [18]. Thus the use of two different solvents i.e hexane for defatting and aq. alcohol for the removal of alkaloids might be difficult, which required additional sophisticated equipment for solvent recovery.

So, an attempt has been made to explore the possibility of recovering alkaloids and oil with hexane as the sole extracting organic media from Karanja seed. The main objective of our present study is to find out a easier methodology which can be easily adopted to the existing technology of the extraction of seed to produce an low-alkaloid proteinaceous meal and edible oil.

2. Materials and Methods

Locally collected Karanja seeds were dehusked manually and the kernels thus obtained were crushed with a hammer mill and dried in the oven at a temperature of 105^0C to about 4-6% moisture. The moisture content in the fresh kernel was determined by IUPAC methods [19]. The oil was then extracted with hexane in a Soxhlet apparatus for about 12 hours. The solvent was then removed using a rotary vacuum evaporator at reduced pressure and the percentage of oil was determined by AOCS methods [20]. The total nitrogen of the defatted kernel was determined using Micro Kjeldahl method. Protein contains were calculated by multiplying total nitrogen by 6.25. Total alkaloids were determined according to the procedure given by Ortiz and Mukherjee [21].

3. Extraction of Alkaloids from the Defatted Kernel and Oil

According to the usual procedure [22] the alkaloids from the defatted kernel and oil were removed. Portions of the defatted kernel, 10 g each, were taken in glass stoppered Erlenmeyer flasks and mixed uniformly with definite amounts (up to 3.0 ml/g kernel) of sodium carbonate or ammonium hydroxide at various concentrations (up to 30%). Hexane of definite volume (5-25 ml/g kernels) was then added and the mixture was shaken for different periods (5-20 minutes) in a water bath at various temperatures (30-60^{0}C). Therefore, the extract was separated from the kernels by suction on a heated Buckner funnel and the extracted kernels were dried and analyzed.

The oil was extracted 3 times in a separatory funnel with 2% of its volume of 5% hydrochloric acid in order to recover the alkaloids dissolved in the oil. The fat was then washed several times with water until neutral and its alkaloid content was determined titrimetrically.

4. Results and Discussion

The alkaloids which are contained in plant materials naturally exhibit a variety of physical, chemical, biological as well as medicinal properties [23-28]. In plant materials alkaloids generally occur partially as free bases and partially as salts which are insoluble in most of the organic solvents. Like basic compounds they form their crystalline salts with acids like hydrochloric acid, sulphuric acid, citric acid and tartaric acid. The free alkaloids are insoluble or slightly soluble in water, but their salts are freely soluble. However, they are soluble in less polar solvents such as hexane, ether or chloroform. Therefore, alkaloids are separated from non-polar solvents by salt formation, extracting the lipid soluble protein and basifying the solution with an alkali carbonate or ammonia.

A possible technological process for the recovery of alkaloids and oil from the Karanja seed had been the interest of our present study. Accordingly the alkaloids that occurred as free bases and the oil were extracted with hexane from the Karanja seed kernel. In order to liberate the alkaloids, the alkaloid containing defatted kernels which were bound as salts, were treated with an aqueous base like carbonate or ammonia. The alkaloids thus liberated were then recovered by another extraction with hexane. The results revealed that the Karanja seeds used in this study contained 32% of acrid bitter oil with a disagreeable ordour. The remaining defatted kernels (expeller cake) after the extraction of Karanja seed kernels with hexane contained 31.9% protein, 7.8% oil and 4.2% alkaloids. These alkaloids virtually occurred in the kernels as free bases which are soluble in hexane.

The defatted Karanja seed kernels (oil cake) were treated with varying proportions of an aqueous base, such as sodium carbonate or ammonium hydroxide and by the extraction with hexane, the alkaloids were subsequently recovered. In Tables 1 and 2 the effects of various parameters on the recovery of alkaloids are summarized.

Table 1. Recovery of alkaloids from defatted Karanja seed kernels by treatment with sodium carbonate and a single extraction with hexane.

Treatment with sodium carbonate solution		Extraction with hexane			Alkaloids recovered %
Amount added (ml/g kernel)	% of Na_2CO_3	Amount of hexane (ml/g kernel)	Temp (^{0}C)	Time (min)	
			30		40
			40		50
1.0	15.0	15	50	10	59
			60		68
			60	5	65
				10	68
1.0	15.0	15		15	73
				20	76
		5			52
		10			65
1.0	15.0	15	60	10	68
		20			69
		25			79
	0.0				10
	7.5				57
1.0	15.0	15	60	10	64
	22.5				67
	30.0				57
0.0					15
0.5					36
1.0	15.0	15	60	10	67
2.0					53
3.0					45

From the results in Table 1, it was quite evident that it was possible to recover as much as 79% of the remaining alkaloids from the defatted Karanja seed kernel by the treatment with sodium carbonate and by a single extraction with hexane. It was interesting to note that the recovery of alkaloids increased with the increase of temperature, time and amount of hexane. Again, the recovery of alkaloids was appreciably affected by the concentration and the amount of aq. sodium carbonate. So, from the result it was assumed that the use of 1ml 15% sodium carbonate /g defatted kernels permitted the best recovery of alkaloids.

The results in Table 2 showed that comparable amounts of alkaloids were also recovered from defatted kernel with that of sodium carbonate by aq. ammonium hydroxide prior to extraction of the alkaloids with hexane. It was observed in all the

experiments previously mentioned that the hexane extracts were free of proteins after the recovery of oil and alkaloids. Practically, no protein was lost by this process which is contained in the Karanja seed.

Table 2. Recovery of alkaloids from defatted Karanja seed kernels by treatment with ammonium hydroxide and a single extraction with hexane.

Treatment with ammonium hydroxide solution		Extraction with hexane			Alkaloids recovered %
Amount added (ml/g kernel)	% of NH_4OH	Amount of hexane (ml/g kernel)	Temp (^{0}C)	Time (min)	
				5	62
				10	69
1.0	7.5	15	60	15	70
				20	72
	0.0				12
	7.0				68
1.0	15.0	15	60	10	72
	22.5				73
	30.0				71
0.0					10
0.25					45
0.5	7.5	15	60	10	67
1.0					67
1.5					59

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The recovered oil which was extracted by hexane contained the free alkaloids. In order to remove these alkaloids, the oil was then treated with aq. hydrochloric acid which converted these alkaloids into water-soluble salts. These salts were then easily removed by repeated extractions with water. The oil obtained by this treatment was virtually free of alkaloids. The present studies showed that, most of the undesirable alkaloids can be removed effectively from Karanja seed by a single process that can be conducted in a conventional oil extraction plant. The above results for the extraction of alkaloids from Karanja seed more or less agree with the reported results [18, 21].

5. Conclusion

Karanja plant contains a large number of seeds which contain about 32% inedible oil. Like our developing countries Bangladesh is facing acute shortage of edible and industrial inedible oils. So, she has to import oils from abroad to meet up her demand in lieu of many foreign countries. To bridge the oil gap in the country extraction of oils from non-conventional oil-seeds has been taken into account. Under the above circumstances the Karanja oil can play a vital role in minimizing the shortage of inedible oil in the country to some extent. Again from these studies it is observed that most of the alkaloids contained in the seed meals and oil could have been possible to remove efficiently. So, these findings will open up a possibility to obtain an edible oil and meal from Karanja seed by refining, which will lead to a complete removal of the alkaloids.

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