PHYTOCHEMICAL INVESTIGATION OF SEEDS OF *PONGAMIA PINNATA*

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ABSTRACT: *Pongamia pinnata* seed oil was extracted through sohxlet and its components were identified through gas chromatography. Physicochemical analysis of oil indicate the acid value (2.5mg KOH/g), iodine value (117), saponification value (183), flash point (183°C),kinematic viscosity (40.3 CST). Fatty acid composition was estimated through GC and it shows the presence of strearic acid, palmitic acid, arachidic acid, behenic acid, lignoceric acid, oleic acid, linoleic acid, and ecosonic acid, 6.5%, 1.2%, 2.51%, 6.5%, 1.6%, 46.22%, 18.5% and 12.9% respectively. Mono-glyceride (5.7%) di-glyceride (1.8%) and tri-glycerides (2.43%) were determined through thin layer chromatography. Elemental analysis revealed the presence of Ca, Mg, K, Zn, Mn, Cu, Fe and Na (5.0,0.5,2.0,1.0,2.0,0.7,0.1 and 0.2 mg/ L respectively).

Key Words: *Pongamia pinnata, physicochemical, viscosity, fatty acid, chromatography.*

INTRODUCTION

Pongamia pinnata, locally known as sukhchein, belongs to genus *Pongamia* and family *fabaceae* (Chopra *et al.,* 1958). It is small evergreen tree that is widely distributed in Pakistan, Bangladesh, India, China, Philippine and Australia (Ali, 1980, Ghani, 1998 and Kirtikar, 1994). The Leaves are soft, shiny burgundy in early summer and mature to a glossy, deep green as the season progress. Small cluster of flowers blossom on their branches throughout the year, maturing in to brown pods. The leaves are good source of green manure and being leguminous, they enrich the soil with nitrogen (Kumar, 2007). It is predominantly cultivated through seeds and genetic diversity has been conserved through storage of seeds, the most common conventional and economical method (Robert, 1972 and Hong, 1996). Seeds are elliptical, reniform, compressed, reddish-brown, fairly hard and 2-3 cm long (Kumar *et al.,* 2007). The seeds contain 30-48% oil, which has been identified as source of bio-fuel and has medicinal value. Seed oil has been used in bronchitis, chronic fever, whooping cough, chronic skin diseases, leprosy, piles, and ulcer (Ghani, 1998, Kirtikar, 1994). Pongamol isolated from seeds of this plant has been used as anticonvulsant CNS depressant (Basu, 1994 and Mahli, 1989). Oil of *pongamia pinnata* is UV radiation absorber and it is used in indian Ayurevedic herbal medicine and cosmetic preparations (Barki *et al.,* 2007, M$_c$cutchtchen *et al.,* 1994, Khan and Evan, 1996, Kapoor, 2001 and Wagh *et al.,* 2007). Its constituents have been studied by Sharmeel *et al.* (1996) and Kiran *et al.,* 1998 and its antimicrobial activities have also been investigated by Simin *et al.* (2002) and Wagh *et al.* 2007). *Pongamia pinnata* found in Pakistan in Karachi has only been studied by Shameel *et al.,* 1996 for its fatty acid composition. We have designed this study to investigate the physicochemical properties, fatty acid composition and elemental analysis of seeds oil of *pongamia pinnata* found in Lahore.

MATERIALS AND METHODS

Seeds of *Pongamia pinnata* were collected from the local market. 500 gm seeds were ground and dried in the shade for ten days. 250 grams of dried seed powder was subjected to Sohxlet apparatus using n- hexane solvent. After eight hours of reflux the oil was extracted and solvent was separated under vacuum. Separated oil was dried over anhydrous sodium sulphate and yield was calculated.

CHROMATOGRAPHIC ANALYSIS

The seed oil was saponified with alcoholic potash and extracted with diethyl ether to remove the non-saponifiable matter. Fatty acids were then generated from the soap by acidification, followed by extraction with petroleum ether. This extract was washed three times with water and dried over anhydrous sulphate (AOAC, 2003). Methyl esters were prepared by using methanol and boron tri-flouride (Kumar and Sunoda, 1978; Morison and Smith, 1964).

The gas chromatographic analysis was carried out with a GC-14A gas chromatograph fitted with

flame ionizer detector and data processor. A PEG capillary column (25m X 0.2mm id) was used for fatty acid. The column was operated with temperature programming from 150 to 200°C. The injection and detector temperature were maintained at 250-300°C respectively. Flow gas of carrier gas (nitrogen) was 20ml/min. at split ratio of 1: 50. Identification of components was based on their retention time as compared with those obtained from methyl esters of known fatty acids, analyzed under the same conditions.

TABLE: 1 Fatty acid composition of *pongamia pinnata* seed oil

Sr. #	Fatty acid		% Age
01	Palmitic acid	C16:0	6.5
02	Stearic acid	C18:0	1.2
03	Arachidic acid	C20:0	2.51
04	Behenic acid	C22:0	6.5
05	Lignoceric acid	C24:0	1.6
06	Oleic acid	C18:1	46.22
07	Linoleic acid	C18:2	18.5
08	Ecosonic acid	C20:1	12.09

THIN LAYER CHROMATOGRAPHY:
Mono, Di and Tri glycerides in the oil have been determined by thin layer chromatography. TLC plates (20X 20 cm) plates of 0.25mm thickness were prepared by use of quickfit TLC applicator. Silica gel (Merck TLC grade) was used as an adsorbent. Glycerides were fractionated by using the solvent system hexane: ether: acetic acid (80:20:2, v/v). The different components of the lipids were identified by comparing their Rf values with the standards and then verified by applying specific spray reagents (Javed *et al.*, 2000) to the TLC plates.

For the quantitative determination of the lipid classes, lipids were streaked on five TLC (20X20 cm) plates coated with 0.5mm silica gel. After development the bands were located and scraped after spraying the plates with 0.2% 2,7-dichlorofluorescein solution in methanol and viewing under UV light. The scraped bands were extracted with chloroform methanol mixture (2:1,v/v) separately. The solvent was removed under reduced pressure and the respective lipids were weighed from quantification in duplicate.

TABLE: 2 Percentage of Glycerides in the seed oil of *pongamia pinnata*

Sr.No.	Glycerides	Percentage
01	Monoglycerides	5.7
02	Diglycerides	1.88
03	Triglycerides	2.43

Physicochemical analysis of the seed oil for iodine value, acid value, saponification value kinematic viscosity, flash point and ash contents has been performed according to the standard methods of AOAC, 1998.

TABLE: 3 Physicochemical analysis of *pongamia pinnata* seed oil

Sr.#.	Parameter	Results
01	Iodine value	117
02	Saponification value	183
03	Acid value (mg KOH/g)	2.5
04	Kinematic viscosity (40°C) CST	40.31
05	Flash point (°C)	183
06	Ash contents %	2.6

ELMENTAL ANALYSIS: Elemental analysis of the *pongamia pinnata* seed oil was performed by following ASTM 2007 method. Perkin Elmer Atomic absorption spectrophotometer model Analyst 800 was used for this purpose. Flow rate of air and acetylene 17.0 l/min and 2.0 l/min were adjusted and analysis was performed accordingly.

TABLE: 4 Elemental analysis of *pongamia pinnata* seed oil.

Sr.#	Element	Concentration (ppm)
01	Calcium (Ca)	5.0
02	Magnesium (Mg)	0.6
03	Potassim (K)	2.0
04	Zinc (Zn)	1.0
05	Manganese (Mn)	2.0
06	Copper (Cu)	0.7
07	Iron (Fe)	0.1
08	Sodium	1.2

RESULTS AND DISCUSSION

Oil extraction was carried out by soxhlet extraction method as per direction of standard procedure of AOAC 1995. Hexane was used as extracting solvent. Seeds (250 gram) were soaked into hexane and soxhlet apparatus was operated for eight hours. Solvent was evaporated under vacuum and pure oil was dried over sodium sulphate (anhydrous). It was thick brown color oil with acrid /disagreeable smell and having yield 32.2%. This oil yield was greater than the Czech Republic,29% (Roman and Gerhard, 2007) while it was less than the yield found in India, 33-48% (Naresh *et al.*, 2006; Kesari *et al.*, 2008).

Methyl esters of this oil were studied by Gas Chromatography and compared with the standard available in the laboaratory. Unsaturated fatty acids like,oleic acid 47.12%, Linoleic acid 18.2% and Ecosonic acid 15.5% were present while saturated fatty acids stearic acid 1.2%, Palmitic acid 6.5%, Arachidic acid 2.5%, Behenic acid 6.5% and Lignoceric acid 1.6% were found. oleic acid was in maximum concentration and it was more as compared to found in Karachi (44.24%) but less than found in Egypt 84.8% (sharawy *et al.*, 2007). while stearic acid was quite less as studied (Shameel *et al.*, 1996).oleic acid was 30.4% in pongamia pinnata found in china (Yujie *et al.*, 2005) while 69.14% was in India (Wagh *et al.*, 2006). Other compositions were also comparable to the Indian varieties.

Elemental analysis of this oil was not performed before this study in Pakistan. It was found that it contain maximum quantity of calcium (5.0 ppm) followed by potassium (2.0 ppm), manganese (2.0 ppm), sodium (1.2 ppm), zinc (1.0 ppm), copper (0.7 ppm), Magnesium (0.6 ppm) and Iron (0.1 ppm). These findings are in accordance to the finding Vajpai *et al.*, (1998). *Pongmia pinnata* seed oil was bitter in taste and also have disagreeable odour. Mandal *et al.*, 2007 and Roman and Gerhard, 2007 studied that *Pongamia pinnata* seed oil contain karanjin and pongamol which are not desirable for edible purpose. So it is concluded that *Pongamia pinnata* seed oil can be used after fractionation and blending with other oils for industrial uses.

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