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## Putranjiva roxburghii Seeds: Oil Content and Fatty Acid Composition During Different Stages of Seed Maturity

Saloni Gangal<sup>[1]</sup>, Shweta Sharma<sup>a</sup> and Abdul Rauf<sup>\*\*</sup>

<sup>a</sup>Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India

<sup>[1]</sup> Present Address: Department of Chemistry, Mangalayatan University, Beswan, Aligarh 220145, India

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### ABSTRACT

New potential oilseed crops for industrial uses have been considered for agronomic traits and seed oil composition during last decades in different countries. In order to extend the knowledge of phytochemical biodiversity of higher plant lipids, seed fatty acid compositions of *Putranjiva roxburghii* (Euphorbiaceae) were investigated. This research was conducted to evaluate changes in the contents of oil and fatty acids of *P. roxburghii* seeds obtained from different maturity stages. Oleic and linoleic acids were found as abundant compounds.

**Keywords:** *Putranjiva roxburghii* Seeds, Euphorbiaceae, Oil content, fatty acid composition,

### INTRODUCTION

Seed oils of the Euphorbiaceae show a great variability in their lipid composition<sup>1</sup>. The seed oils from some members of this plant family, e.g. *Ricinus communis*, *Vernonia* species and *Aleurites* species, play an important economic role in many countries and are used widely in pharmaceutical and technical applications<sup>2</sup>. To extend the knowledge about seed oils of this plant family, the sample of *Putranjiva roxburghii* seeds at different stages during maturity was examined. *Putranjiva* is a small genus of trees from the IndoMalaysian region. The species, *P. roxburghii* is a dioecious, evergreen tree with pendent branches, attaining a height up to 18 m and a girth of 2 m, found wild or cultivated in almost all parts of India. The oil of *P. roxburghii* seed is not commercially available. The seed yield fatty oil, used for heating and cooking<sup>3</sup>. The oil could be used for the production of factice<sup>4</sup> and also be used as a herbal preservative for peanuts during storage<sup>5</sup>.

For the efficient improvement of oil quality, the accumulation pattern in developing seeds and its relation to fatty acid composition must be investigated. In general there are two substantial changes during development: one is the change in seed volume and the other is biochemical and physiological changes. The former involves division, enlargement and differentiation of seed cells and the latter involves changes in seed components such as storage lipids, fatty acids and other seed storage metabolic substances<sup>6</sup>. The oil content and fatty acid composition of the oilseeds are modified by the duration of the seed development<sup>6-12</sup> in this sense, the genetic analysis of oil and fatty acid composition needs to take into account the duration of development<sup>10</sup>. The chemical composition of the seed

oil depends on the genetic and environmental conditions as well as the stage of maturity of the seed. In our laboratory variety of seed oils have been analyzed for their fatty acids composition<sup>13,14</sup>. Information on the changes in oil and fatty acids in *P. roxburghii* seeds at different stages of seed development and in different parts of the head is still insufficient or limited. The objective of this research was to determine the oil content and fatty acid composition of *P. roxburghii* seeds during different stages of seed maturity.

### 2. Materials and methods

#### 2.1 Plant material and extraction of seed oil

The seeds were collected from the botanical garden, Aligarh Muslim University, campus in three different months. The analytical values of oil (**Table 1**) were determined according to the procedure recommended by the AOCS<sup>15</sup>. The air-dried seeds were powdered and extracted thoroughly with light petroleum ether (bp- 40-60 °C) in a Soxhlet extraction for 8 hrs to yield oil.

#### Preparation of fatty acid methyl esters (FAMES)

Every time the seeds from a given month were bulked and representative samples were taken for total fatty acid analysis. First the extracted oil was saponified. Saponification of the seed oil was carried out by refluxing it with 0.5N alcoholic KOH. The unsaponifiable material was removed by diethyl ether extraction and free fatty acids were obtained by acidification with 6N HCl of aqueous layer followed by extraction with diethyl ether.

Methyl esters were prepared by refluxing the mixed fatty acids (MFA) for 1 hr in excess of methanol containing catalytic amount of sulphuric acid. In each case, the resulting mixture was diluted to the cloud point with water chilled in ice bath, and then extracted repeatedly with diethyl-ether. Combined extracts were washed with 5 % aqueous sodium bicarbonate and dried over anhydrous sodium sulphate to yield FAMES which were subjected to gas liquid chromatog-

### \*Corresponding author.

Dr. Abdul Rauf  
Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India  
Tel.: + 91-9412545345  
Telefax: +91-  
E-mail: abdulraufchem@gmail.com

**Table 1. Variation in oil content, saponification value and iodine value during different stages of seed development**

	Area %									
	September		October		November		December		January	
	2007	2008	2007	2008	2007	2008	2007	2008	2008	2009
Oil Content %	25.8	26.1	28.2	29.1	32.5	32.8	37.4	37.9	42	42.8
Saponification value	230	230	223	223	220	220	217	217	215	215
Iodine value	66	66	82	82	92	92	97	97	101	101

**Table 2. Variation of fatty acid composition of *P. roxburghii* seeds during different stages of seed development**

Fatty acid composition	Area %									
	September		October		November		December		January	
	2007	2008	2007	2008	2007	2008	2007	2008	2008	2009
12:0 (Lauric)	3.8	3.9	1.2	0.8	-	-	-	-	-	-
14:0 (Myristic)	1.9	2.2	1.0	0.7	-	-	-	-	-	-
16:0 (Palmitic)	29.8	29.0	18.2	17.8	13.9	13.4	9.9	8.8	6.2	5.9
16:1 (Palmitoleic)	16.4	15.9	8.2	8.4	-	-	-	-	-	-
18:0 (Stearic)	4.1	4.8	4.2	5.0	4.5	5.2	4.8	5.3	5.1	5.5
18:1 (Oleic)	28.2	28.9	46.9	46.5	55.4	55.2	56.3	56.8	57.6	57.8
18:2 (Linoleic)	15.8	15.3	20.3	20.8	25.8	25.6	28.1	28.2	29.9	29.6
20:0 (Arachidic)	-	-	-	-	0.4	0.6	0.9	0.9	1.2	1.2
Total SFA	39.6	39.9	24.6	24.3	18.8	19.2	15.6	15.0	12.5	12.6
Total USFA	60.4	60.1	75.4	75.7	81.2	80.8	84.4	85.0	87.5	87.4

raphy.

## 2.2 Instrumentation

The GLC analysis were carried out using a Varian Vista 6000 instrument equipped with FID (290 °C) detector using a stainless steel column packed with 15 % OV-275 on chromosorb-W (80-100 mesh). Separations were carried out at programmed temperature of 140-200 °C (10 °C min<sup>-1</sup>). The peaks were identified by comparing their retention times with those of standard reference samples under similar conditions.

## 3. RESULTS AND DISCUSSION

### Oil content and fatty acid composition

Analysis of variance for the oil content, saponification value (SV) and iodine value (IV) during stages of seed maturation are given in **Table 1**. Oil content and iodine value was significantly influenced by the maturity stages in two consecutive years.

The results in **Table 2** showed that the composition of fatty acids changed significantly during seed development. Changes in fatty acids are of special importance to the quality of the oil. In the present study, fatty acid accumulation patterns resulting from seed development duration were observed. The results showed that the composition of fatty acids changed significantly during seed development. Palmitic, stearic, oleic and linoleic acids comprised over 98.8% of total lipids on the average in the fully mature seeds and of these oleic and linoleic acids comprised over 86.5-87.4 % of total fatty acids **Table 2**.

Significant changes in the contents of the saturated fatty acids (palmitic and stearic) and unsaturated fatty acids (oleic and linoleic) were observed during seed development. The accumulation patterns of palmitic and stearic acids were quite different, as the seeds developed. In the month of September (2007), palmitic acid content was 29.8 % which decreased significantly with seed development, reaching minimum values of 6.2 % in the month of January (2008), while stearic acid showed a regular increase from 4.1 % to 5.1 % (i.e. from September 2007 – January 2008 as shown in **Table 2**). Similar results were observed in next consecutive year also i.e. in 2008-2009. However these types of changes were observed in other seed oils too<sup>16</sup>.

The results showed that oleic acid in the fully matured seeds

was the major component comprising 57.6 % of the total fatty acids, followed by linoleic (29.9 %), palmitic (6.2 %) and stearic (5.1%). In total these four constituted about 98.8 % of the total fatty acids in year 2007. The study displayed significant variation amongst them for individual fatty acids. Similar ranges of variation were observed for palmitic, oleic and linoleic acids in contrast with the other fatty acids over the next consecutive year.

Oil content was found to associate positively with oleic and linoleic acids but had an inverse relationship with palmitic and palmitoleic acids. Palmitic acid was inversely associated with both stearic and oleic acids, which were positively correlated. These observations agree with various reports on oil composition studies in other oil crops. Flagella et al. (2002) showed that in sunflower, an increase in palmitic acid is accompanied by a decrease in both oleic and stearic acids<sup>17</sup>. Studies on soybean<sup>18</sup>, peanut<sup>19</sup> and winter oilseed rape<sup>20</sup> also revealed strong inverse relationships between palmitic and oleic acids. The synthesis of 18-carbon fatty acids proceeds via a single step elongation of 16-carbon acyl chains, followed by desaturation<sup>21</sup>. The elongation step plays an important role in regulating the relative amounts of palmitic acid and the 18-carbon fatty acids<sup>22</sup>. A deficiency in this step leads to a reduction in the amount of the 18-carbon fatty acids and an increase in the palmitic acid content of plant tissues. This to a large extent explains the observed correlations.

## 4. CONCLUSIONS

There are many important changes in the seed and oil characteristics of *P. roxburghii* during seed maturity or development. The results could be improved by experiments including more genotypes, years and locations. However, the information obtained from this study could be helpful in agronomic, genetic and biotechnological research related to determining the ideal harvest time, applying some specific chemical agents and modifying fatty acid composition.

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