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Amino Acids and Other Biochemical Components of *Ricinus communis* (Variety Minor), an Anti-conceptive Seed

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Abstract: *Ricinus communis* (Minor) seeds used as an anti-conceptive drug in traditional medical practice in Nigeria have been analyzed for their amino acids and other constituents. Results showed high concentrations of fat (40.22%), crude fibre (22.05%) and protein (20.77%). Sixteen amino acids were obtained with concentrations of the essential amino acids comparing favorably with those of the FAO reference protein, whole hen's egg and defatted soy meal. Thin Layer Chromatography (TLC) and Proton-Nuclear Magnetic Resonance analyses revealed the presence of sterols, unsaturated fatty acids and hydroxy fatty acids which may have implications in the anticonceptive attributes of *Ricinus communis* RC (minor).

Key words: *Ricinus communis* (minor), amino acids, lipids, anti-conceptive seed .

Introduction

Ricinus communis plant commonly called "castor plant" is of the family Euphorbeacae. Based on the size of the seeds, *R. communis* (RC) has been classified into three varieties namely: major, intermedia and minor. Of the three varieties, RC (minor) is the most commonly used in traditional medicine in Plateau State of Nigeria and it grows luxuriantly in the State. Claims by traditional medical practitioners that RC (minor) is an oral contraceptive have been reported (Okwuasaba *et al.*, 1991). It is said to prevent pregnancy for one year, when one or two RC minor seeds are taken by an adult female once a year. However, no scientific proof has been documented for human subjects in this respect. Nevertheless, Okwuasba *et al.* (1991) showed that the ether-soluble portion of the methanolic extract of RC (minor) possesses anti-implantation, anti-conceptive and estrogenic activity in rats and mice when administered subcutaneously. On the other hand, the *R. communis* oil popularly known as castor oil and classified as a strategic material (Carlson *et al.* 1990) has been shown to stimulate labour at term (Remington,1975). Similarly, Oslo and Prat (1973) reported that castor oil causes side effects such as nausea, vomiting, purgation and discomfort. Consequently, the oil is commonly used as a stimulant cathartic particularly in cases of chemical poisoning. The oil also finds use in ointment formations for the treatment of seborrheic dermatitis and other skin diseases, and as a solvent for removing irritating substances from the eye (Tyler, 1976).

Generally, many workers (Remington, 1975; Tyler, 1976; Temple *et al.*, 1991; Onwuliri and Anekwe, 1993a; 1997) are in agreement over the potential significance of medicinal plants and their products in health care delivery system in developing countries especially, as many of these are commonly grown, are effective, and to a great extent easily available and affordable. Additionally, they are not prone to much adulteration as is the case with conventional and imported drugs in use in Nigeria.

Nevertheless, most of these plants including RC minor used in traditional medical practices have not been fully assessed scientifically. Although, Temple *et al.* (1991) studied the physical parameters including some mineral constituents of the seeds of RC minor, the amino acids and proton-nuclear magnetic resonance (pNMR) analyses of these seeds were not investigated. The present study was therefore undertaken to determine the amino acids and other biochemical constituents of the seeds of RC minor in Nigeria.

Materials and Methods

The seeds of *R. communis* (RC) were obtained from the Department of Pharmacology and Clinical Pharmacy, University of Jos and identified at the Department of Botany, University of Jos, Nigeria, West Africa.

Proximate Composition of RC Seeds: The seeds were analyzed for moisture, ash, crude fibre, crude oil and crude protein by standard methods recommended by AOAC (1990). Crude protein was derived using the factor Nx6.25. Carbohydrate content was calculated by difference, based on the total seed composition (Ologunde *et al.*, 1990) . Energy contents were then calculated using the Attwater conversion factors for protein, carbohydrate and fat (4,4 and 9 cal per gram, respectively).

Amino Acid Content of the RC Seeds: Protein hydrolyzates were prepared from the seeds according to earlier methods (Spackman *et al.*, 1958; Onwuliri & Obu 2001) and their amino acids were analyzed by Technicon-Sequential Amino Acid Multisample Analyzer. Chemical Score was calculated based on the amino acids of FAO reference protein. (Onwuliri & Anekwe 1993b)

Thin Layer Chromatographic Analysis of the Oil: The crude oil was extracted from the seeds of *R. communis* by Soxhelt extraction with petroleum ether; (60^{o}-80^{o}C) for 8 hours, was then purified and analyzed by Thin Layer Chromatography (Tashiro *et al.*, 1990; Onwuliri and Anekwe, 1993a).

Proton-Nuclear Magnetic Resonance Analysis of the Oil: The oil was subjected to proton-NMR using a GE-300 MHZ nmr conducting system. The samples were prepared and run according to the method of Onwuliri *et al.* (1994), and the spectra obtained were described as before (Morrison and Boyd, 1987; Onwuliri *et al.*, 1994).

Results

The results of the proximate analysis of the seeds of RC (minor) are shown in Table 1.

The 16 amino acids detected in the protein hydrolyzate of RC (minor) are presented in Table 2 and compared with the amino acids of the FAO (1957) reference protein, those of whole hen's egg (Ifon and Umoh, 1987), and Defatted soy meal (Carlson *et al.*, 1990).

Thin layer chromatographic analysis of the oil (Table 3) showed that the major constituents of the oil were triacylglycerols (Rf = 0.74); free fatty acids (Rf = 0.37) and sterols (Rf = 0.16), in addition, some other minor constituents were also obtained.

The data from the proton-NMR studies are summarized in Table 4. These results revealed the presence of methylene groups, hydroxyl, and double bonds. The AB splitting at 4.15ppm also indicates the presence of triacylglycerols.

Table 1: Proximate Composition of the Seeds of *Ricinus communis* (Var. minor).

Parameters	% Concentration *
Moisture	3.34± 0.14
Ash	4.03± 0.10
Crude fat	40.22± 1.86
Crude protein	20.77±1.50
Crude fibre	22.05± 1.78
Carbohydrate	9.65±0.37
Energy value	43.66 Kcal per gram

(Mean ± SD; n = 4).

Discussion

The high concentration of crude fat, 40.22 ± 1.86%; crude fibre, 22.05 ± 1.78%; and crude protein 20.77 ± 1.5% (Table 1) are in line with the findings of Temple *et al.* (1991) and are of interest with regard to the potential feed value of the seeds.

The data (Table 2) showed that in RC (minor), glutamic acid was the most abundant amino acid (13.06g/16gN) followed by aspartic acid (12.4g/16gN). This appears to correspond with the earlier observations (Onwuliri and Anekwe, 1993b). Glutamic acid and aspartic acid represent a storage form of nitrogen in addition to being the starting compounds from which the backbones of amino acids are made. Also, it has been noted that during the acid hydrolysis step, glutamine and asparagine were converted to glutamic and aspartic acids with the liberation of ammonium (NH_4^+) ions.

This seems to explain the high concentrations of these two amino acids and the complete absence of glutamine and asparagine in the profile obtained (Fowden, 1973). Valine, tyrosine and methionine in RC (minor) with Chemical Scores of 73.81%, 82.14% and 86.36% respectively, were the first, second and third limiting essential amino acids when compared with the essential amino acids of the FAO reference protein. This agrees with earlier observations (Fowden,1973). Four amino acids, lysine, leucine, phenylalanine and threonine were present in considerably higher amounts in RC (minor) (7.3 g/16gN, 5.1 g/16gN, 4.6g/16gN, and 4.8g/16gN respectively) than in FAO reference protein (4.2, 4.8, 2.8, and 2.8 g/16gN respectively for the four amino acids). RC minor seed was found to contain more phenylalanine (Phe) (4.6g/16gN) and threonine (Thr) (4.8g/16gN) than the FAO reference protein (Phe. 2.8; Thr 2.8 g/16gN), whole hen's egg (Phe 3.78; Thr 4.0) and defatted soy meal (Phe 4.5; Thr3.7) g/16/gN. On the other hand, all the essential amino acids in RC (minor) except phenylalanine and threonine were present in lower quantities than in the whole hen's egg. When RC (minor) amino acid levels were compared with those of defatted soy meal, it was observed that lysine, methionine, phenylalanine and threonine were present in higher amounts in RC (minor). In all, the concentrations of the essential amino acids in RC (minor) compare favourably with those of the FAO reference protein, whole hen's egg and defatted soy meal with sum total of 32.9 (RC), 28.0 (FAO), 37.5 (whole hen's egg) and 35.78 (soy meal). Accordingly, RC (minor) protein can be classified to be of good quality especially, as the nutritional quality of a protein is often based on its amino acid composition (Davidson *et al.*, 1972; Tashiro *et al.*, 1990).

The presence of sterol in RC (minor) oil as indicated in Table 3, is important since sterols as steroid alcohols are key intermediates in the synthesis of related steroids as well as being major constituents of membrane. Interestingly, it has been noted that some plant steroids can be converted into animal hormones in the presence of relevant enzymes (Green *et al.*, 1995). This may help in understanding the medicinal attributes of the RC (minor) seeds.

The unique features of this oil as against other common vegetable oils including sunflower oil (Onwuliri, 1997), groundnut and palm oils (Onwuliri *et al.*, 1994) have the peaks, at 1.45 ppm, indicating methylene protons alpha to a hydroxy carbon, and the peak at 3.62 ppm suggests a proton on a carbon carrying the hydroxy group.

This may suggest why this oil has the anticonceptive properties which others do not have. Other peaks present (Table 4) include those at 2.05ppm, 5.4 and 5.55 ppm which confirm the presence of protons around a double bond. The peak at 2.5ppm shows protons adjacent to carbon, whereas the 1.62ppm peaks signify the protons on the carbon

Table 2: Amino Acid Content of the Seed of RC (minor): (CONCENTRATION g/16gN)

Amino acid	[a]*Ricinus communis*	[b]FAO reference protein	[c]Chemical Score	[d]Whole hen's egg	[e]Defatted soy Meal
Essential					
Lysine	7.3±0.12	4.2	173.81	8.61	6.0
Leucine	5.1 ±0.16	4.8	106.25	9.60	6.7
Isoleucine	3.8 ±0.05	4.2	90.48	4.08	4.4
Methionine	1.9 ±0.11	2.2	86.36	2.13	1.4
Phenylalanine	4.6 ±0.20	2.8	164.29	3.78	4.5
Tyrosine	2.3 ±0.08	2.8	82.14	2.66	4.58
Threonine	4.8±0.15	2.8	171.43	4.0	3.7
Valine	3.1 ±0.10	4.2	73.81	5.34	4.5
Histidine	2.2 ±0.06	-	-	3.02	2.2
Non essential					
Arginine	7.4 ±1.14	-	-	4.42	7.6
Aspartic acid	12.4 ±2.0	-	-	8.1	11.78
Serine	4.1 ±0.2	-	-	6.72	5.45
Glutamic acid	13.6±2.6	-	-	10.1	18.63
Proline	9.7 ±0.50	-	-	4.4	-
Glycine	5.7 ±0.46	-	-	2.22	4.3
Alanine	3.8 ±1.2	-	-	4.00	4.29

a. Mean ± SD, (n = 4) b. FAO pattern of amino acid (FAO, 1957) c. Chemical score based on FAO ref. Protein
d. Amino acid content of whole hen's egg (Ifon & Umoh, 1984) e. Amino acid composition of defatted soymeal (Carlson *et al.*, 1990).

Table 3: TLC analysis of the *Ricinus communis* oil

Lipids	Rf values
Monoacylglycerol	0.02
1,2-Diacylglycerol	0.12
Sterols	0.16
Free fatty acids	0.37
Triacylglycerols	0.74
Sterol esters	0.94

Thin layer chromatography in petroleum ether (60-80°C) diethyl ether-acetic acid (80:18:2 v/v/v)

Table 4: Proton-Nuclear Magnetic Resonance Spectral data of *Ricinus communis* Oil

Ricinus communis peaks (ppm)	^{X}Chemical species
0.9	CH_3
1.35	$-(CH_2)_n$
1.45	$-OHCH-CH_2-(CH_2)n$
1.62	$-CH_2CH_2COO$
2.05	$-CH_2=CHCH_2-$
2.2	$-CH_2-CH=CH-$
2.3	$-CH_2CO_2$
2.79	$-CH_2=CH-CH_2-CH-CH-$
3.62	-CHOH
4.15 AB splitting	$^{-Y}H_2C-$
4.35	-HC-
4.36	$-H_2C-$
5.25	$-^{Y}CH-O^{-}$
5.40-5.55	-CH=CH-

X – reference from (Morrison & Boyd, 1987).
Y – from glycerol moiety

atom, beta to the carboxyl carbon. The AB splitting pattern at 4.15 and 4.35ppm supports the fact that castor oil is a triglyceride oil. The peak at 2.3ppm suggests a major fatty acid with alpha methylene group adjacent to two olefinic group like in linoleic acid. However, since the peak at 2.79ppm is of very low intensity it means that linoleic acid or similar fatty acids are in minute amounts or as minor components. This seems to agree with earlier report, that castor oil contains ricinoleic acid used in manufacturing lubricants and pharmaceuticals amongst other things (Davidson *et al.*, 1972; Remington 1975; Onwuliri *et al.*, 1994). Generally, triglyceride oil confers palatability to foods. They are necessary for specific metabolic functions as energy supply, and protection of some organs. They are also of high nutritional value because of their fat-soluble vitamin contents. Finally, the presence of sterols, essential fatty acids, and hydroxyl fatty acids in the seed oil of *R. communis* (minor) may have implications in the anticonceptive role attributed to the seeds.

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