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Anticonceptive and Pharmacognostic Studies on the Seed and Diethyl Ether Extract of RICOM-1013-J

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Authors' contributions

This work is the collaborative efforts of all authors. Authors AOO and OO designed, performed the experiment, analyzed the data and wrote the Pharmacognostic manuscript. Author CI read and improved the pharmacognosy manuscript. Authors OEE, AS and GGJ performed the anti-conceptive studies and proofread the manuscript. Authors OEE and IJU analyzed data, wrote the anti-conceptive part of the manuscript. KFO conceived the study and proofread the manuscript. All authors read and approved the final manuscript sent for publication.

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ABSTRACT

Aims: The anticonceptive effect of the diethyl ether extract of *Ricinus communis Linn* (RICOM 1013-1) was studied in rats alongside pharmacognostic properties, amino acids content and elemental analysis.
Study Design and Methodology: 20 adult female albino Wistar rats were randomized into four experimental groups of five rats each. The first, second and third groups received 3, 10 and 20 mg/kg of diethyl ether extract administered subcutaneously (SC) in divided doses over two days, respectively. The fourth group received 0.1 ml of corn oil for two days and served as control. They

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were then mated with proven fertile males in a ratio of 3:1 and followed for three gestation periods. The first day of mating was termed day 0 of pregnancy. In addition, amino acid and elemental analyses were undertaken as well as a phytochemical screening of the seed of RICOM-1013-J.

Results: At doses of 3, 10 and 20 mg/kg, RICOM-1013-J produced dose-dependent inhibition of pregnancy in female albino Wistar rats over three gestation periods. The total ash value obtained was 3.13 ± 0.26%, while the acid-insoluble and water-soluble ash values were 0.30 ± 0.003% and 0.20±0.003%, respectively. The yield to ethanol was much higher than the water-soluble extract. Essential and non-essential amino acids were not detected. K^+, Ca^{2+} and Mg^{2+} were present in high concentrations in the following order: defatted undecorticated>undecorticated>decorticated samples. Phytochemical screening indicated the presence of steroids and alkaloids whereas, triterpenes, tannins and flavonoids were absent.

Conclusion: The pharmacognostic parameters, including elemental values, provide some preliminary data for identification of this species among many varieties reported in the literature.

Keywords: Ricinus communis var. minor; antifertility; pharmacognostic parameters; amino acids; elemental analysis.

1. INTRODUCTION

The seed of *Ricinus communis* var minor, Linn (Euphorbiaceae) have been used for decades by the Rukuba speaking people, in Bassa Local Government Area of Plateau State, north-central Nigeria, for anti-conceptive purposes. Castor oil plant (*R. communis*) originally indigenous to India and comprising 17 varieties is now widely spread over all tropical and sub-tropical countries. In India, it may grow to a height of 40 cm and be a perennial tree, but in the temperate savannah climate, it is either a shrub or an annual herb [1]. The large arborescent forms yield large seeds, while the small annual varieties yield small seeds [1,2]. The seeds have been broadly classified into three groups depending on the size, and include the large-sized variety (variety major), medium-sized variety (variety intermediate) and the small-sized variety (variety minor).

Advances in the study of the seeds of *R. communis* var minor (RICOM-1013-J) have shown the diethyl ether fraction of the methanolic extract to have pronounced anti-conceptive effects in rats [3]. The authors further showed that when 3-4 seeds (800 mg) of *R. communis* var minor (RICOM-1013-J) were administered as a single oral dose, fertile female volunteers were protected against conception for 12 months, as practiced traditionally. Isichei et al. [4] have also reported similar outcomes in women volunteers, demonstrating that the high acceptance and usage of the seeds by the Rukuba women was informed by the absence of troublesome side effects often associated with conventional family planning pills: nausea, headache, weight gain and high blood pressure. Several other authors have reported related anti-conceptive activity of this seed in other cultures too [5-7]. Chemical pathological and toxicological evaluation of liver and renal functions in women volunteers, showed that RICOM-1013-J possessed a high margin of safety [8], while phytochemical screening has revealed the presence of steroidal compounds and alkaloids [9,10].

Early efforts to understand the anticonceptive action of *R. communis* var minor had come from histological and pharmacological studies in animal models. Okwuasaba et al. [11,12] demonstrated oestrogenic activity of ether extract of the seed in immature ovariectomized rats and mice, where pretreatment with the extract altered electrical activity of the uteri and fallopian tubes, precipitating a state of prolonged inertia. The authors further showed decreased uterine responsiveness to some standard smooth muscle agonists such as oxytocin, ergometrine, PGF2α and acetylcholine. In addition, they found histological changes in the ovaries that suggested anovulation (numerous vacuolation of corpora lutea and a distorted uterine histoarchitecture). Similar histological changes have also been reported in other animal models [7,13,14]. Whilst they argued that oestrogen content of the seed caused the reported disruptive tissue changes and subsequent contraceptive effect, the absence of oestrogen related side-effects often experienced with the conventional pill, but rarely reported with RICOM-1013-J [4] may imply an insignificant direct role of oestrogen. Recently, Usar et al. [15] have shown that the anticonceptive activity of RICOM-1013-J may in fact be mediated through hyperprolactinaemic induced hypogonadism.

In spite of the number of studies so far undertaken on gaining insights into *R. communis* var minor, its distinctive pharmacognostic

properties have sparsely been studied. The present study, therefore, is designed to provide further insights into the macro-morphological features, and pharmacognostic properties of the seed of the variety minor.

2. MATERIALS AND METHODS

The dried seeds of *R. communis*, RICOM 1013-J were collected from the wild shrubs in the cool savannah vegetation of Jos, North Central Nigeria, between the months of March and October, 2012. The plant was authenticated at the Department of Botany, Ahmadu Bello University, Zaria and the Forestry Research Institute, Jos both in Nigeria, and specimen vouchers were deposited at the herbarium of the Department of Pharmacognosy, University of Jos.

The weight of a seed was determined using an analytical balance (Galaxy 7m60.OHAUS) and a pair of Vernier callipers was employed to measure length, width and thickness of each seed. Seeds were also subjected to sensory characterization by sight, touch and smell. 50 seeds were crushed with the aid of a porcelain mortar and pestle and the powder used for further analytical procedures.

The extractive and ash values were determined as described in the British Pharmacopoeia [16].

2.1 Amino Acid Analysis

The seeds were defatted as described by Association of Analytical Communities [17], and the method of Spackman et al. [18], used to determine amino acid content of the seed. The seeds were hydrolyzed using 6 N HCl at 110ºC for 24 h. Separation of individual amino acids was carried out by the chromatographic procedure (reverse phase/hydrophilic interaction LC Ultra HPLC), using Technicon Sequential Multisample Amino Acid Analyser (TSM). Amino acid values were then calculated from their chromatogram peaks.

2.2 Elemental Analysis

Evaluation of the elements present in the seed of RICOM-1013-J was undertaken by use of the atomic absorption spectrophotometer (AAS model 180-811). Guided by the findings of Okwuasaba et al. [3] that the anti-conceptive activity of the seed to be confined to the cotyledons, we used a variety of samples for the elemental analysis: crushed seeds without decortication; crushed decorticated sample and crushed defatted decorticated sample. Chloride, sulphate, carbonate and bicarbonate were estimated according to Young [19], Donaldson [20], and Bossci and Mendiham [21] respectively.

2.3 Phytochemical Screening

Diethyl ether active fraction of the seed of RICOM-1013-J was obtained as described by Okwuasaba et al. [3] and screened for the presence of secondary metabolite such as flavonoids, triterpenes, alkaloids and tannins, according to the method of Yen and Yang [22].

2.4 Anticonceptive Studies

Adult female albino Wistar rats (20) weighing 115±5.3 g and having regular oestrous cycles were used in this study. They were randomized into four experimental groups of 5 rats per group. The first, second and third groups received 3, 10 and 20 mg/kg of diethyl ether extract administered subcutaneously (SC) in divided doses over 2 days, respectively. The fourth group received 0.1 ml of corn oil for two days and served as control. They were then mated with males obtained from a pool of proven fertile rats at proestrous phase of the cycle in a ratio of 3:1 (F: M ratio) and left in contact with the male rats until after three gestation periods. Mating was confirmed by the presence of spermatozoa on vaginal smears taken on the first day of mating, and termed day 0 of pregnancy.

2.5 Statistical Analysis

Data were expressed as Mean ± SEM. The test of significance was carried out using Student's t-test. P-values ≤ 0.05 were considered significant.

3. RESULTS

The seeds of *R. communis* in this study were smooth to touch without hairs or trichomes, greyish to dark grey with brown reddish patches and fine streaks and odourless. Table 1 details the physical quantitative parameters of the seed. Generally, the seed's thickness was less than half its lengths. The acid-insoluble and water soluble ash contents were very small in contrast to the high value of the total ash (Table 2). The yield to ethanol (Table 2) was about 9 times the yield to water.

Table 3 shows that RICOM-1013-J contained most essential amino acids, as well as some non-essential amino acids. Non-proteinaceous

amino acids were not detected. The elements detected in the seed were generally in high concentrations in the following order: defatted-undecorticated> undercorticated> decorticated samples, as shown in Table 4. K^+, Ca^{2+} and Mg^{2+} were particularly high in all three samples. Bicarbonate, Carbonate, Chloride and Sulphate were not detected in the ash samples (Table 4).

Table 1. Quantitative macro-morphological parameters of whole seed of RICOM-1013-J

Parameter	Size range	Mean ± SEM
Length (mm)	7.80-12.00	10.22 ± 0.26
Width (mm)	5.00-7.00	6.11 ± 0.13
Thickness (mm)	3.50-5.00	4.50 ± 0.08
Weight (g)	0.14-0.23	0.19 ± 0.17

Mean of ten separate determination ± SEM

Table 2. Ash and extractive values of the seeds of RICOM-1013-J

Types of ash extractive	Mean ± SEM
Total ash	3.13 ± 0.26%
Acid insoluble ash	0.30 ± 0.003%
Water soluble ash	0.20 ± 0.003%
Yield to water	0.97 ± 0.2%
Yield to ethanol	8.98 ± 0.06%

Each value was the mean of 5 separate determinations

Table 3. mean % amino acid levels in RICOM 1013-J

Amino Acid	% Amino acid
Lysine	1.21
Histidine	1.18
Arginine	3.56
Aspartic acid	26.4
Threonline	0.79
Serine	2.02
Glutamine	3.97
Proline	1.15
Glycine	1.42
Alanine	1.57
Cysteine	0.32
Valine	1.02
Methionine	0.52
Isoleucine	1.13
Leucine	2.02
Norleucine	1.52
Tyrosine	1.57
Phenylalanine	0.46

3.1 Phytochemical Screening

The phytochemical screening of the diethyl ether active fraction of the methanolic extract of RICOM-1013-J indicated the presence of steroids and alkaloids, while triterpenes, flavonoids and tannins were not detected, as reflected in Table 5.

3.2 Anticonceptive Studies

RICOM-1013-J (10 and 20 mg) dose-dependently protected treated rats against pregnancy throughout the three gestation period of study, while control group rats delivered pups on Day-23 of pregnancy (Table 6).

4. DISCUSSION

R. communis plant seeds have been documented to show considerable differences in size and colour: The size ranges 8. 0-10.0 mm long and from 4-12.00 mm broad, which may be coloured uniform grey, brown or black, and variously mottled with brown or black [1]. In this study, the seeds of RICOM-1013-J measured 7.80-12.00 mm in length, 5.00-7.00 mm in width and 3.50-5.00 mm in thickness and weighed 0.17 ± 0.008 g. They were glabrous, grayish with reddish-brown patches and fine streaks. The findings agree with features of castor seeds variety minor, described in the empirical literature [1,23].

Total ash estimate in this study was 3.13 ± 0.26% and does not agree with the finding of 4.22±0.11% reported by Temple et al. [23]. The relatively low acid-insoluble ash (0.30±0.26%) indicated that most of the total ash of 3.13 ±0.26% was due to its physiological contents. The absence of hairs or trichomes on the seeds was likely to have significantly contributed to the low acid-insoluble ash value as this would have reduced the seeds' ability to attract earthy matter. *R. communis* seeds are oily and contain about 46.53% fixed oil consisting of the glycerides of tricinoleic, isoricinoleic, stearic and dihydroxystearic acids [1], and this may explain the low water-soluble ash value of 0.20±0.003%.

The total ash value for the variety minor as reported by Temple and colleagues was 4.22±0.11% while the bigger seed variety (variety major) was 3.40±0.06%. In our study, the values for the seed variety evaluated seemed to correspond with those of the variety minor of temple et al (23) in size and weight. The total ash estimate of 3.13 ± 0.26% in this study though low, is comparable to the finding of 4.22±0.11% reported by Temple et al. [23].

Table 4. Elemental analysis of digested samples of RICOM-1013-J in parts per million

Sample	K^+	Na^+	Ca^{2+}	Zn^{2+}	Mn^{2+}	Ca^{2+}	Mg^{2+}	Fe^{2+}	Cr^-
A	4600	270	28	92	13	2550	3100	230	30
B	4400	70	39	100	12	400	3100	150	15
C	9300	105	30	118	33	2800	5200	235	15

A=Undercorticated ground seeds
B=Decorticated ground seeds
C=Defatted undercorticated seeds

Table 5. Phytochemical screening of the diethyl ether fraction of RICOM-1013-J

Steroids	Present
Triterpenes	Not detected
Alkaloids	Present
Flavonoids	Not detected
Tannin	Not detected

However, it is now well documented that the amount and sometimes nature of chemical constituent(s) in plants and/or plant parts is not constant throughout the year, making the time of collection a critical factor in harvesting crude drugs [1]. It is possible that the time of seed harvesting might explain this difference in the total ash value.

R. communis seed is composed mainly of fatty constituents [1] and these are expected to be more soluble in ethanol than water which may explain the much higher yield to ethanol obtained in this study (Table 2).

The amino acid profile of RICOM-1013-J revealed the presence of essential amino acids. This may provide a logical basis for its medicinal use and wide usage as condiments/spices among south-eastern tribes in Nigeria when boiled and fermented. Moreover, the amino acid content detected in this study could be used as a marker to distinguish RICOM-1013-J (variety minor of *R. communis*).

The result of elemental analysis of RICOM-1013-J showed very high concentrations of K^+, Ca^{2+} and Mg^{2+},while K^+, Ca^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+} and Cr^- occurred in low to moderate concentrations, in all three samples analyzed (Table 4).

The present study has further provided support for the anticonceptive efficacy of RICOM-1013-J in rodents, as reported in previous investigations [3,16].

The diethyl ether fraction of the methanolic extract of RICOM 1013-J has been shown in earlier studies to possess estrogenic and progestogenic effects on the uterus of experimental animals [3,11,12]. The anti-conceptive fraction was found in this study to contain steroids (Table 5). This finding is significant since the conventional oral contraceptives contain steroidal estrogen and progestins. The detection of steroids in the active fraction may thus be a pointer to the group of secondary metabolites that may in part be responsible for the anti-conceptive effect RICOM-1013-J (*R. communis* var. minor) reported in this study, and consistent with earlier conclusions of Okwuasaba *et al.*, that the anticonceptive activity was due to steroidal induced anovulation and distortion of uterine histoarchitecture [3].

Table 6. Anticonceptive activity of RICOM-1013-J in female rats

Treatment	No of animals	No incidence of pregnancy	No of pups delivered (Mean ±SEM)	Anti-conceptive activity (%)	Fertility rate (%)
Control	10	10	6.4±0.4	0	100
Extract 3 mg/kg	10	3	5.8±0.6	30	30
Extract 10 mg/kg	10	0	0	100	0
Extract 20 mg/kg	10	0	0	100	0

5. CONCLUSION

In conclusion, this study has demonstrated the efficacy of the diethyl ether fraction of RICOM-1013-J as potential anti-conceptive phytodrug, with a novel effect, since a single oral dose protected for at least seven gestation periods. The presence of alkaloids and steroids, as well as Zn, Mn and Cr in RICOM-1013-J, may partly explain its anticonceptive efficacy. The amino acid profile and the elemental analysis could be used as a marker for classification of the many varieties of *R. communis*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the Ethical Committee of the Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The used drug for this research is a common oral contraceptive. There is absolutely no conflict of interest between the authors and producers of the drug because we do not intend to use this drug as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Evans WC. Trease and Evans Pharmacognosy. 14th ed. London: W.B. Saunders; 1996.
2. Wallis TE. Textbook of Pharmacognosy. 5th ed. London: J & A Churchill Ltd; 1967.
3. Okwuasaba FK, Osunikwo U, Ekwenchi MM, Ekpenyong KI, Onwukeme KE, Olayinka AO, et al. Anticonceptive and estrogenic effects of seed extract of Ricinus communis var minor. J. Ethnopharmacol. 1991;34:141-145.
4. Isichei CO, Das SC, Ogunkeye OO, Okwuasaba FK, Uguru VE. Onoruvwe O, et al. Preliminary Clinical Investigation of the Contraceptive Efficacy and Chemical Pathological Effects of RICOM-1013-J of *Ricinus communis* var Minor on Women Volunteers. Phytother. Res. 2000;14:40-42.
5. Scarpa A. Various uses of castor oil plant. J. Ethnopharmacol. 1982;5:177-137.
6. Daffalah AA, Al-Mutairy AR. Contraceptive effect of castor beans. Saudi Pharm J. 1994;2:152.
7. Hou Y, Ding X, Duan S, Yang Z, Gao P. Microencapsulation and pharmacological evaluations for the anti-fertility effect of castor bean extract in female mice. African Journal of Pharmacy and Pharmacology. 2010;4:700-707.
8. Das SC, Isichei CO, Okwuasaba FK, Uguru VE, Onorukwe O, Olayinka AO, Ekwere EO, et al. Chemical pathological and toxicological studies of the effect of RICOM-1013-J of *Ricinus Communis* (var. minor) in women volunteers and rodents. Phythother. Res. 2000;14: 15-19.
9. Onwuliri AV, Anekwe EG. Amino Acids and Other Biochemical Components of *Ricinus communis* (Variety Minor), an Anti-conceptive Seed. Pakistan Journal of Biological Sciences. 2001;4(7):866-868.
10. Sani UM, Sule MI. Anti-fertility activity of methanol extracts of three different seed varieties of *Ricinus communis* linn (euphorbiaceae). Nig J Pharm Scs. 2007; 6:42-50.
11. Okwuasaba FK, Dass SC, Isichei CO, Ekwenchi MM, Ononivwe O, Olayinka AO, et al. The anticonceptive and the effect on uterus of ether extract, 18312-J of *Ricinus communis*. Phyiother Res. 1997a;1:97-100.
12. Okwuasaba FK, Das SC, Isichei CO, Ekwenchi MM, Onoruvwe O, Olayinka AO, et al. Pharmacological studies on the antifertility effects of RICOM -1013-J from Ricinus communis var minor preliminary clinical studies on women volunteers. Phytother res. 1997b;11:547-551.
13. Raji Y, Oloyo AK, Morakinyo AO. Effect of methanol extract of *Ricinus communis* seed on reproduction of male rats. Asian J Androl. 2006;8:115-121.
14. McNeil RT, Noronha CC, Kusemiju TO, Okanlawon AO. The anti-ovulatory effect of a seed extract of *Ricinus communis* Linn. Nigerian Journal of Health and Biomedical Sciences. 2003;2:31-34.
15. Usar JI, Ekwere OE, McNeil RT, Okwuasaba KF. The Mechanism of action and effect on the cervix of the seed of *Ricinus communis* Var. Minor (RICOM-

1013-J) in Women Volunteers in Nigeria. Journal of Natural Science. 2016;6(24).
16. British Pharmacopoeia: Her majesty's stationary Office. Cambridge: University Press; 1980.
17. Association of Official Analytical Chemist. Official methods in analysis of the ADACW. 13th ed. Washington DC; 1980.
18. Spackman HI, Stein WH, Moore S. Automatic recording apparatus for use in the Chromatography of amino acids. Anal Chem. 1958;30:1190-1191.
19. Young RS. Chemical analysis in extractive metallurgy, 1st ed. New York: Academic Press; 1971.
20. Donaldson EM. Methods for the analysis of Related Materials, 2nd ed. New York: Longman; 1982.
21. Basset J, Mandiham JC. Vogel's Quantitative Inorganic Analysis Including Elementary Instrumental Analysis. 4th ed. New York: Longman; 1978.
22. Yen KY and Yang LL. Experimental Pharmaceutical photochemistry. National research into Chinese Medicine; 1971.
23. Temple VJ, Aliyu R, Bassa JD. The chemical composition of the seeds of two varieties of *Ricinus communis*. West Africa J. Pharm Drug Res. 1990/91;9(10):58-62.

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