Phytochemical and Toxicity Analysis of *Ricinus communis*

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Authors’ contributions

This work was carried out in collaboration among all authors. Author JPE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CRS and AVA the analyses of the study. Author CS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants play a vital role in ensuring proper health is attained by human beings due to their antioxidants constituents. The large family *Euphorbiaceae* contain nearly about 300 genera and 7,500 species. Amongst all, *Ricinus communis* or castor bean plant has high traditional and medicinal values towards a disease-free community. The objective of this study focuses on the phytochemical constituents and phytotoxicity perspective of the *R. communis* plant. The castor bean plant is effective and is thought to have antifertility, anti-nociceptive, anticancer, antioxidant, immunomodulatory, hepato-protective, antidiabetic, antiulcer, antimicrobial bone regeneration, central analgesic, antihistamine, anti-asthmatic, cytotoxic, lipolytic, anti-inflammatory and wound healing potential. The seeds of *R. communis* were deshelled and manually separated from its shells. They were divided into three based on different methanol extraction (Fermented, Unfermented and Crystals of methanol extracts of unfermented). The quantitative phytochemical analysis showed variations in the phytochemical content of the unfermented and fermented methanol extracts respectively, alkaloids, flavonoids, tannins, soluble carbohydrates, hydrogen cyanides, steroids and phenols.

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1. INTRODUCTION

Plants serve as beneficial sources of organic compounds, many of which have been used for medicinal purposes. Medicinal plants are the plants whose parts (leaves, seeds, stems, roots, fruits and foliage), extracts, infusions, decoctions or powders are used in the treatment of different diseases of humans, plants and animals [1, 19]. In the last few decades, there has been an exponential growth in the field of herbal medicine [20]. It is getting popularized in developing and developed countries, owing to its natural origin and lesser side effects. One of such medicinal plant is Ricinus communis (Euphorbiaceae), which is commonly known as Castor. It is a small tree which is found all over India [2]. There is a wide spectrum of trees, plants and shrubs whose seeds, roots, barks and leaves are used by humans throughout the globe due to their nutritional or medicinal values [3]. In the last few years, there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in the developing and developed countries because of their natural origin and fewer side effects [4]. However, these complementary components give the plant as a whole, the safety and efficiency much superior to that of its isolated and pure active components [5]. The World Health Organisation (WHO) report in 1993 showed that nearly 80 per cent of the world population is dependent on the traditional system of medication that is the use of plants and their parts as medicine [6].

It is true that without nature, human beings can't survive. The food, clothes and shelter are the three necessities of human beings and the most important is good health, which is being provided by the plant kingdom. In traditional medicine, there are many natural crude drugs for different health purposes, one of such plants is R. communis [7].

2. MATERIALS

2.1 Plant Materials

The seeds of R. communis were used for this study. The seeds were purchased from Eke Amobi market, Otolo Nnewi in Anambra state and were identified by Mr Alfred Ozioko of the Bio-resource Development and Conservation Programme (BDCP) Research Centre, Nsukka. The fermented seeds were purchased from Ogige Market, Nsukka LGA, Enugu State, Nigeria.

2.2 Preparation of Plant Materials

The seeds of R. communis seeds were deshelled and manually separated from the shells.

2.3 Extraction of Plant Materials

The unfermented seeds (949g) of R. communis were deshelled and macerated in a mixture of methanol and chloroform (1:2) for 24 hours. The extract was filtered using Whatman No. 1 filter paper and partitioned with 0.2 volume of distilled water to obtain two layers and separated using a separating funnel. The upper aqueous methanol layer was concentrated using a rotary evaporator at a temperature of 40 to 60°C. The dry residue was used for the determination of biological activity. The unfermented concentrated extract formed crystals or precipitates after some days of concentration and their biological activities were also determined. The fermented R. communis seeds (329g) were treated similarly. The dry residue was used for the determination of biological activity.

Fig. 1. The fruit and plant of R. communis [7]
2.4 Qualitative Phytochemical Analysis of *R. communis* Seeds

The phytochemical analysis of the plant was carried out on both fermented and unfermented methanol extracts according to the method of Harborne (1998) and Trease and Evans (1983) to identify the active constituents of *R. communis* [8,9].

2.4.1 Test for alkaloids

A quantity, 0.2g of the sample was boiled with 5ml of 1% aqueous HCl in a water bath for 45 mins. The mixture was filtered and 1ml portion of the filtrate was distributed evenly in two test tubes, with two drops of the following reagents [9].

- Drangendorff’s reagent: An orange-red precipitate indicates the presence of alkaloids.
- Meyer’s reagent: A creamy-white precipitate indicates the presence of alkaloids.

2.4.2 Test for glycosides

A quantity, 0.5g of the sample was mixed with 30ml of distilled water and heated in a water bath for 5 minutes. The mixture was filtered and the filtrate used for the following test;

(i) A quantity, 0.2ml of Fehling’s solution A&B was added to 5ml of the filtrate until it turned alkaline (tested with litmus paper) and heated on a water bath for 2 minutes. A brick-red precipitate indicates the presence of glycosides.

2.4.3 Test for steroids

0.5g of the sample was mixed with 5ml of 1% lead acetate solution and 10ml of aqueous ethanol. The mixture was placed on a boiling water bath for 2 minutes, it was allowed to cool and filtered. The filtrate was extracted twice with 15ml of chloroform. 5ml of the chloroform layer was evaporated. After the evaporation, 2, 3-dinitrobenzoic acid and 1ml of 1N NaOH were added. Red colouration indicates the presence of steroids. [10]

2.4.4 Test for flavonoids

A quantity, 0.5g of the sample was dissolved in ethanol, warmed and then filtered. 3 pieces of magnesium chips were added to the filtrate, followed by few drops of concentrated HCl. A pink, orange or red to purple colouration indicates the presence of flavonoids [10].

2.4.5 Test for saponin

One gram (1g) of the sample was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while still hot and the filtrate used for the following tests [10]

2.4.5.1 Frothing test

A quantity, 1ml of the filtrate was diluted with 3ml of distilled water. The mixture was shaken vigorously for 5 minutes, frothing which persisted on warming was taken as evidence for the presence of saponin.

2.4.6 Test for tannins

A known quantity, 0.5g of the sample was boiled with 5ml of 45% ethanol for 5 minutes. The mixture was cooled and then filtered and the filtrate was treated with the following solutions

- Lead subacetate solution: To 1ml of the filtrate was added 3 drops of lead subacetate solution. A gelatinous precipitate indicates the presence of tannins.
- Bromine water: To 1ml of the filtrate was added 0.5ml of bromine water and then observed for a pale brown precipitate.
- Ferric chloride solution: A quantity, 2ml of the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution were added. A transient greenish to black colour or blue-black or blue-green precipitate indicates the presence of tannins.

2.4.7 Test for reducing sugars

A quantity, 0.5g of the sample was dissolved in 5ml of distilled water and filtered, the filtrate was heated for 10 minutes with 5ml of equal volumes of Fehling’s solutions A&B and shaken vigorously. A brick-red precipitate indicates the presence of reducing sugars.

2.4.8 Test for carbohydrates

A known weight, 0.5g of the sample was shaken vigorously with distilled water and filtered. To the aqueous filtrate, few drops of Molisch reagent were added and vigorously shaken. Then, 1ml of
concentrated sulphuric acid was carefully added down the side of the test tube to form a layer below the aqueous solution. A brown ring at the interface indicates the presence of carbohydrates.

2.5 Quantitative Phytochemical Analysis of *R. communis* Seeds

2.5.1 Alkaloid determination

A measured weight, 1g of the sample was macerated in 20ml of ethanol and 20% sulphuric acid (1:1). After the maceration, the solution was filtered and 1ml of the filtrate was collected using a pipette, 5ml of 60% H₂SO₄ was added into the 1ml of the filtrate. After 5 minutes, 5ml of 0.5% formaldehyde in 60% H₂SO₄ was added into the previous solution and mixed. The solution was allowed to stand for 3 hours and the absorbance measured at 565nm.

2.5.2 Flavonoid determination

A quantity, 1g of the sample was macerated in 20ml of ethyl acetate for 5 minutes. After the maceration, the solution was filtered, 5ml of the filtrate was collected using a pipette and added to 5ml of dilute ammonia. The solution was shaken for 5 minutes, after which the upper layer was collected and the absorbance measured at 490nm.

2.5.3 Glycoside determination

One gram (1g) of the sample was macerated in 20ml of distilled water for 5 minutes, followed by the addition of 2.5 ml of 15% lead acetate, the solution was filtered and 2.5 ml of chloroform added to the solution and was shaken vigorously. The lower layer of the solution was collected and evaporated to dryness. The residue was dissolved with 3ml of glacial acetic acid and 0.1 ml of ferric chloride and 0.25 ml of concentrated H₂SO₄ were added and shaken vigorously. The solution was put in the dark for 2 hours and the absorbance measured at 530nm.

2.5.4 Hydrogen cyanide determination

A sample (1g), was macerated in 50 ml of distilled water and filtered. 1ml of the filtrate was collected using a pipette and added into 4mls of alkaline picrate solution. The solution was boiled for 5 minutes and cooled at room temperature. The absorbance was measured using a spectrophotometer at the absorbance of 490nm.

2.5.5 Phenol determination

A quantity, 1g of the sample was macerated in 20ml of 80% ethanol and filtered. 5ml of the filtrate was collected using a pipette and added to 0.5ml of Folinicocaltue’s reagent, the solution was allowed to stand for 3 mins and 2ml of 20% Na₂CO₃ was added. The absorbance was measured using a spectrophotometer at 650 nm.

2.5.6 Saponin determination

A known weight, 1g of the sample was macerated in 10ml of petroleum ether and decanted into a beaker and washed twice using 10ml of petroleum ether. The filtrate of the solution was combined together and evaporated to dryness, the residue was dissolved in 6ml of ethanol and 2ml was collected using a pipette into a test tube while 2ml of chlorogen solution was added and allowed to stand for 30 minutes. The absorbance was measured using a spectrophotometer at 550nm.

2.5.7 Soluble carbohydrates determination

The sample (1g) was macerated in 50ml of distilled water and filtered. 1ml of the filtrate was collected using a pipette and added into 2ml of saturated picric acid. The absorbance was measured using a spectrophotometer at 530nm [11].

2.5.8 Steroid determination

The sample (1g) was macerated in 20ml of ethanol and filtered. 2ml of the filtrate was collected using a pipette and added into 2ml of colour reagent and allowed to stand for 30 minutes. The absorbance was measured using a spectrophotometer at 550nm.

2.6 Preparation of the Methanol Extract of the Unfermented *R. communis* Seeds for the Acute Toxicity Test

A quantity, 949 g of the cracked seeds of *R. communis* was macerated in 1265 ml and 633 ml of chloroform and methanol respectively for 24hrs. The solution was filtered with Whatman No.1 filter paper and separated, the supernatant (the methanol extract) was concentrated to a semi-solid state using rotary evaporator at a temperature range of 40 to 600C. The methanol extract was used for the study.
2.7 Acute Toxicity Test of the Methanol Extract of Unfermented *R. communis* Seeds

The method of Lorke (1983) was used for the acute toxicity test of the methanol unfermented extract of *R. communis*. Eighteen (18) albino mice were utilized in this study. The test involved two phases. In phase one, the animals were grouped into three (3) groups of three mice each. They were administered 10, 100 and 1000 mg/kg body weight of the extract respectively and in the second phase, the animals were grouped into three (3) groups of three mice each and 1600, 2900 and 5000 mg/kg body weight of the extract were administered to the animals. The administration of the extract was done orally.

2.8 Preparation of the Methanol Extracts of Fermented *Ricinus communis* Seeds

Fermented castor bean plant (392 g) was purchased from Ogige market in Nsukka, Enugu State. They were macerated in chloroform and methanol for 24 hours. The solution was filtered with Whatman no.1 filter paper and separated, the supernatant (the methanol extract) was concentrated to a solid state using rotary evaporator at a temperature of 400°C to 600°C. The methanol extract was used for biological activity determination.

2.9 Acute Toxicity Test of the Methanol Extract of Fermented *R. communis* Seeds

The method of Lorke (1983) was used for the acute toxicity test of the methanol fermented extract of *R. communis*. Eighteen (18) albino mice were utilized in this study. The test involved two phases. In phase one, the animals were grouped into three (3) groups of three mice each. They were administered 10, 100 and 1000 mg/kg body weight of the extract respectively and in the second phase, the animals were grouped into three (3) groups of three mice each and 1600, 2900 and 5000 mg/kg body weight of the extract were administered to the animals. The administration of the extract was done orally.

2.10 Percentage Yield of the Methanol Extracts of Fermented and unfermented *R. communis* Seeds

Table 1 shows that the unfermented *R. communis* seeds, 949 g and the fermented *R. communis* seeds, 392 g, gave a percentage yield of 2.93 and 7.83 respectively. The high percentage yield of the fermented extract after extraction might be as a result of high surface area of the fermented seeds which allowed the passage of solvents into the pulp for proper extraction.

2.11 Qualitative Phytochemical Screening of the Methanol Extracts of Fermented and Unfermented *R. communis* Seeds

Table 2 shows that both fermented and unfermented seeds of *R. communis* contain alkaloids, flavonoids, steroids, hydrogen cyanide, soluble carbohydrates, phenol and tannin. Reducing sugars, glycosides and saponins were highly, moderately and slightly detected respectively in the unfermented seeds but they were not detected in the unfermented seeds of *R. communis*. Resins and terpenoids were not detected in both extracts.

### Table 1. Percentage yield of the methanol extracts of fermented and unfermented *R. communis* seeds

| Unfermented extract (g) | Fermented extract (g) | Unfermented (%) | Fermented (%) |
|-------------------------|-----------------------|-----------------|---------------|
| 949                     | 392                   | 2.93            | 7.83          |

3. DISCUSSION

The preliminary phytochemical screening showed that the unfermented methanol extract of *Ricinus communis* seeds contained alkaloids, flavonoids, tannins, glycosides, steroids, soluble carbohydrates and phenols, [12, 13] while the fermented methanol extract contained alkaloids, flavonoids, hydrogen cyanides, steroids, soluble carbohydrates, tannin and phenol. From the results, some of the phytochemicals such as glycosides, saponins and reducing sugars were present in the unfermented methanol extract but were not detected in the fermented extract. The reducing sugar which was detected in the unfermented methanol extract of *Ricinus communis* seeds was not detected in the fermented extract. This could be as a result of the fermentation process which resulted to the...
Table 2. Preliminary phytochemical screening of methanol extracts of fermented and unfermented *R. communis* seeds

| Phytochemicals          | Fermented extract | Unfermented extract |
|-------------------------|-------------------|---------------------|
| Flavonoids              | ++                | ++                  |
| Glycosides              | -                 | ++                  |
| Hydrogen cyanides       | +                 | +                   |
| Resin                   | -                 | -                   |
| Saponin                 | -                 | +                   |
| Steroid                 | +                 | ++                  |
| Soluble carbohydrates   | ++                | ++                  |
| Tannin                  | + +               | +                   |
| Reducing sugar          | -                 | +++                 |
| Terpenoids              | -                 | -                   |
| Phenol                  | ++                | +                   |

Key: + slightly present  ++ moderately present  +++ highly present  - Not detected

Table 3. Quantitative phytochemical constituents of the methanol extracts of fermented and unfermented *R. communis* seeds

| Phytochemical constituents (mg/100g) | Unfermented methanol seed extract Mean ± SD | Fermented methanol seed extract Mean ± SD |
|-------------------------------------|---------------------------------------------|------------------------------------------|
| Reducing sugars                     | 39.60 ± 0.00                                | ND                                       |
| Soluble carbohydrates               | 3.25 ± 0.03                                 | 3.12 ± 0.05                              |
| Hydrogen cyanides                   | 0.02 ± 0.00                                 | 0.04 ± 0.00                              |
| Steroids                            | 4.58 ± 0.05                                 | 0.27 ± 0.04                              |
| Saponins                            | 1.36 ± 0.04                                 | ND                                       |
| Tannins                             | 5.74 ± 0.03                                 | 15.16 ± 0.04                             |
| Alkaloids                            | 3.57 ± 0.04                                 | 2.74 ± 0.04                              |
| Flavonoids                          | 3.63 ± 0.06                                 | 4.94 ± 0.03                              |
| Glycosides                          | 2.56 ± 0.04                                 | ND                                       |
| Phenols                             | 6.62 ± 0.04                                 | 12.62 ± 0.04                             |

Table 4. Phases I and II of the median lethal dose (LD$_{50}$) test of the methanol extract of unfermented *Ricinus communis* seeds

| Dosage mg/k. g. body weight | Mortality |
|-----------------------------|-----------|
| Phase I                     |           |
| Group 1                     | 10        | 0/3       |
| Group 2                     | 100       | 0/3       |
| Group 3                     | 1000      | 0/3       |
| Phase II                    |           |
| Group 1                     | 1600      | 0/3       |
| Group 2                     | 2900      | 0/3       |
| Group 3                     | 5000      | 0/3       |

breakdown of the reducing sugar to alcohols (phenols), this breakdown led to the non-detection of the reducing sugars in the fermented extract and also an increase in the quantity of the phenolic content of the fermented methanol extract of *Ricinus communis* seeds from 6.62±0.04 mg/100g to 12.62±0.04 mg/100g in the quantitative analysis. The quantitative phytochemical analysis showed the variations in the phytochemical content of the unfermented and fermented methanol extracts respectively. The flavonoids, saponins and alkaloids are said to have medicinal properties in animal [14]. The high increase in the tannin, flavonoid and phenolic content of the fermented methanol extract suggested the increase in its contractile effect on the isolated smooth muscle tissues, this is because they affect the calcium
Table 5. Phases I and II of the median lethal dose (LD$_{50}$) test of the methanol extract of fermented *Ricinus communis* seeds

| Phase | Dosage mg/kg body weight | Mortality |
|-------|--------------------------|-----------|
| I     |                          |           |
| Group 1 | 10                      | 0/3       |
| Group 2 | 100                     | 0/3       |
| Group 3 | 1000                    | 0/3       |
| II    |                          |           |
| Group 1 | 1600                    | 0/3       |
| Group 2 | 2900                    | 0/3       |
| Group 3 | 5000                    | 1/3       |

availability of cells and calcium enhances the smooth muscle contraction [16].

The acute toxicity or median lethal dose (LD$_{50}$) of the unfermented methanol extract of *Ricinus communis* indicated that the seed extract is not toxic [17]. The result showed that no casualty was recorded at a dose as high as 5000mg/kg body weight; this result also ascertains that the organic solvents used for the extraction did not extract the toxic glycoprotein, known as ricin [18, 19]. The acute toxicity or median lethal dose (LD$_{50}$) of the fermented methanol extract of *Ricinus communis* indicated that the extract is not toxic at lower concentrations but toxic at a high dose of 5000mg/kg body weight, the result showed that death was recorded at a high dose of 5000mg/kg body weight. Fermentation increases the number of organic acids in the fermented foods, organic acids such as lactic acid, citric acid, tartaric acids etc. [20] this was suspected to be the cause of the death that was recorded at high concentrations of 5000 mg/kg body weight.

4. CONCLUSION

*Ricinus communis* contains a considerable phytochemical which is suitable for all medicinal plants. The plants consider to be recommended for local treatment of some ailments due to the high phytochemical contents. The good part of the plants was found not to be toxic for consumption therefore, further studies can carry out on the plant

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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