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Research Article
Phytochemical and Antimicrobial Properties of Oil Extracts from the Seeds of *Ricinodendron heudelotii*

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Abstract

Objective: The aim of this project is to extract the chemical components of various parts of this tree and to characterize the constituents of these extracts and to test for their biological activity. **Methodology:** Agar-well diffusion assay was used to determine the antimicrobial activity of the oil extract on the test isolates: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus* and the yeast *Candida albicans.* The Minimum Inhibitory Concentration (MIC) for each test organism was determined by the broth dilution method using 0.5 McFarland's standard. **Results:** Preliminary proximate and phytochemical analysis of the oil extracts from the seed showed the presence of the following minerals, Na, K, Ca, Mg, Mn, Fe, Cu and Zn as well as the following family of compounds: Steroids, saponin and terpenoids. Antimicrobial and antibacterial studies also revealed that the oil extract was active against Gram's negative and positive bacteria and fungi. The test organisms were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus* and the yeast *Candida albicans. Pseudomonas aeruginosa* was resistant to the oil extract at all the concentrations used. **Conclusion:** Amongst the bacteria used, highest MIC of 150 mg mL^{-1} was recorded for *Staphylococcus aureus*, while lowest MIC of 50 mg mL^{-1} was observed in *E. coli*. Highest activity was observed against the fungus, *Candida albicans* with MIC of 25 mg mL^{-1}.

Key words: *Ricinodendron heudelotii*, extraction, antimicrobial, phytochemical

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ricinodendron (African nut tree or African wood oil nut tree) is a dioecic plant genus in the family Euphorbiaceae. There are two varieties, the *R. heudelotii* and *R. africanum* found along the west coast to the central regions of Africa, of which the *R. africanum* is unique to Nigeria. The tree is known locally in Nigeria as Okhuen, Okwe, ekku or erínmadó or ológbóigbó and wawankurmi[1].

The kernel is the edible part of the plant because of its high nutritive content. The kernels are dried and ground and then used as a flavoring agent in some dishes and also to thicken soups and stews[2-8]. The oil obtained from the kernel has a yellowish color and it is very stable because of its high content of γ-tocopherol[9,10].

All parts of the tree have found many uses, for example the bark and roots have therapeutic properties, used by traditional doctors as an antidote against poison. Extracts from the stem bark are also used to cure various diseases as cough, malaria, yellow fever, stomach pain, rheumatism constipation, miscarriage, painful menstruation, female infertility, diarrhoea, dysentery and asthma[11-14].

The extract is also thought to function as an aphrodisiac and to possess anti-inflammatory properties. Seed husk and latex, leaf decoction and sap are also used to treat diverse illnesses[1].

The fruits of *R. heudelotii* are not eaten by humans[11,15,16], but the seeds have many uses for example, the seeds and husks have been processed to obtain oil, which is light yellow in colour. Analysis of this oil indicated that it contained unsaponifiable matter, saturated fatty acids, 9: oleic acid, 9:12-linoleic acid, linolenic acid, elaostearic acid, glycerol residue and volatile materials[17]. The oil, it has been suggested will be suitable for manufacture of soaps and pharmaceutical preparations[17,18].

All parts of the tree from the tree trunk, stem bark, roots, leaves, seeds and kernel are used in medicines and treatment of ailments with the trunk being the most effective and frequently used part. Many of the medicinal uses range from use as a laxative, treatment of diarrhea, coughs, dysentery, anaemia and blennorrhoea and to increase blood, treatment of edema, elephantiasis, leprosy, sexual and fertility problems, pains associated with menstruation and childbirth, prevention of abortion, gonorrhea and venereal diseases[19-25].

With these claims in mind the efficacy of, the antibacterial and antifungal efficacy of seed extracts of *R. heudelotii* was investigated with an initial main objective of identifying and characterizing the chemical constituents of the oil extract from the seeds.

MATERIALS AND METHODS

The fruits of Ricinodendron were collected, dried in the shade and then deseeded. The kernels were then removed from the shells and ground. About 62.2 g of the ground kernel were put into a thimble for exhaustive Soxhlet extraction using petroleum ether (40-60) as solvent[26]. Petroleum ether was removed on a rotary evaporator to give clear golden yellow oil. The oil obtained was stored away from light prior to analysis.

Agar-well diffusion assay was used to determine the antimicrobial activity of the oil extract on the test isolates: *Staphylococcus aureus, Pseudomonas aeruginosa Escherichia coli*, *Bacillus cereus* and the yeast *Candida albicans*[27]. Positive and negative controls were set up with gentamycin and dimethyls sulfoxide (DMSO), respectively. The minimum inhibitory concentration for each test organism was determined by the broth dilution method using 0.5 Macfarlands standard. The least concentration of the extract which inhibited the growth of the inoculums was considered as the min inhibitory concentration.

RESULTS AND DISCUSSION

The 27.5 g of oil was extracted from 62.2 g of kernel, an amount representing 44.2% of starting mass of the kernel. This value compares favorably with 48.8% reported in the literature[1]. The oil was then subjected to mineral analysis, phytochemical screening and antimicrobial activity.

The phytochemical screening revealed the presence of the following: Saponin, anthraquinones, terpenoids, steroids, tannins and trace amounts of phenols (Table 1). The physicochemical properties of the extracts revealed a freezing point of and density of Table 2.

Elemental analysis revealed the presence of the metals sodium, iron, zinc, manganese, copper, potassium, magnesium and calcium as indicated in Table 3. The following elements, Pb, Cd, Cr and Co, present below were the

Table 1: Phytochemical properties of crude oil of *Ricinodendron heudelotii* seeds

Cardiac glycosides	-
Saponin	+
Anthraquinones	-
Terpenoids	++
Tannins	-
Steroids	++
Phlobatinnins	-
Flavonoids	-
Alkaloids	-
Volatile	+

+, ++: Present and –: Not found

Table 2: Physicochemical properties of crude oil of *Ricinodendron heudelotii* seeds

Property	Value
Boiling point	134°C
Density	0.84 g dm^{-3}
Refractive index	1.61
Cloud point	0.5
pH	6.62

Table 3: Elemental analysis of crude oil of *Ricinodendron heudelotii* seeds

Ca (%)	Mg (%)	K (%)	Na (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
0.30	0.40	0.40	162.00	10.50	66.50	10.00	27.00

Table 4. Proximate analysis of crude oil extract of *Ricinodendron heudelotii* seeds

Parameters	Values
Acid value	3.06
Iodine	10.01
Peroxide	45.95
Saponification	184.70
FFA	1.53
Ester	176.62
Crude fibre	15.49
Ash	5.39
Moisture content	9.50
Protein	1.51
Crude fat	38.06
Carbohydrate	30.05
Total solid percentage	90.50
Organic matter percentage	94.61

Table 5: Antimicrobial activity and MIC of *Ricinodendron heudelotii* oil extract against test isolates

Organisms	Diameter of zone of inhibition (mm)						Gentamycin (10 mg mL^{-1})	MIC (mg mL^{-1})
Concentration (mg mL^{-1})	200	100	50	25	12.5	6.25		
Staphylococcus aureus	10	R	R	R	R	R	16	150
Bacillus spp.	8	R	R	R	R	R	12	100
Escherichia coli	11	10	R	R	R	R	15	50
Pseudomonas aeruginosa	R	R	R	R	R	R	6	R
Candida albicans	9	7	5	5	R	R	14	25

R: Resistant

detection limit of the instrument. Some of the elements detected here have been reported before[20].

The result of the proximate analysis carried out. Table 4 also showed the proportion of ash, protein, carbohydrate and organic content of the oil extract. The absence of alkaloids from the bark extracts had been reported in the literature[26]. The ash value of 5.39 obtained from our experiment is slightly higher than 4.63, whereas the pH value was lower in our experiment than 9.65 reported in the literature[20]. The antimicrobial tests of the oil extract of *Ricinodendron heudelotii* against Gram negative and positive bacteria and a fungus and the min inhibitory concentrations are given in Table 5. All the test organisms except *Pseudomonas aeruginosa* showed appreciable sensitivity to the extract at different concentrations. Among the bacteria, *E. coli* showed the highest sensitivity with MIC of 50 mg mL^{-1}, while *S. aureus* showed the lowest sensitivity at MIC of 150 mg mL^{-1}. The fungus, *Candida albicans* was highly sensitive to the extract at a low concentration of 25 mg mL^{-1}. Antibacterial activity of the stem bark of *Ricinodendron heudelotii* had been reported[28,29]. These results compare favorably with earlier reports from studies on the stem bark and leaves where aleuritolic acid and phenolics were the phytochemicals associated with the antimicrobial activity exhibited by the plant[25,28,29].

CONCLUSION

The results of phytochemical and antimicrobial screening of oil extract from *Ricinodendron heudelotii* observed in this study, showed that the extract contains important phytochemicals and metals that are of medicinal significance. The oil extract is also active against bacteria and fungi. These findings further support the idea that the extracts from various parts of the plant may be important sources of compounds with broad-spectrum anti-microbial properties.

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