Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2014, 6 (5):1-6
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Physico-chemical properties and *in vitro* antifungal activities of *Ricinus communis* seed oil against *Lentinus sajor-caju*

***Maruf O. Yekeen, Olayiwola O. Ajala, Rauf A. Adegbite and Ahmed B. Alarape**

Federal College of Forestry, Ibadan, Oyo State, Nigeria

ABSTRACT

The aim of the present study was to assess the antifungal activity of seed oil of Ricinus communis, a tall, fast growing, branching perennial shrub that can grow up to 3m high. It is a medicinal plant widely used in the treatment of various diseases and ailments. The oil obtained from the seed is used in the treatment of piles, chronic dysentery, ringworm, itch and other skin diseases. The seed oil was tested for antifungal activity against Lentinus sajor-caju using agar disc diffusion method. The discs were loaded with 50µl of the oil extract at concentrations of 1.2, 2.4, 4.8 and 9.6µg/disc. Fluconazole (100µg/disc) was used as positive control while 95% methanol was used as negative control. The zone of inhibition was measured after incubation at 37°C for 24 hours. The percentage yield of the extracted oil was 32.4% and physicochemical analysis showed that it is of good quality. The seed oil exhibit a concentration-dependent increase in antifungal activity and high relative percentage inhibition against L. sajor-caju compared to standard antifungal agent. These findings indicated that R. communis seed oil can be used as a preservative agent against white rot caused by L. sajor-caju.

Keywords: *Ricinus communis*, *Lentinus sajor-caju*, physicochemical analysis, disc diffusion method, relative percentage inhibition, antifungal activity.

INTRODUCTION

Wood is the most accessible and sustainable material utilized by humans since times immemorial [1]. It is a very important material which under normal circumstances will give centuries of service [2]. However like other biological materials, wood can be degraded by a variety of organisms including, bacteria, fungi, insects and mollusks [3]. Wood degrading fungi attack and metabolize the three main components of wood and wooden materials (i.e. lignin, cellulose and hemicelluloses) thereby causing dimensional changes in them hence restricting the use to which they can be put [4]. These fungi can be divided into three different categories namely white rot, brown rot and soft rot. White rot fungi belong to the class Basidiomycota. This class of fungi produces three types of extracellular phenoloxidases, which enable them to initiate lignin depolymerization, namely manganese peroxidase (MnP), Lignin peroxidase (LiP), and Laccase (Lac) [5]. *Lentinus sajor-caju* (Fr.) Fr. is a commercially important and edible mushroom belonging to the oyster mushroom family. It is the second most cultivated edible mushroom worldwide due to its medicinal and nutritional value coupled with its ability to grow on agricultural wastes and other varieties of substrates [6]. It is a white rot fungus capable of secreting enzymes such as peroxidases and laccases hence providing it with the ability to colonize and degrade many lignocellulosic substrates [7]. The degradative ability of this fungus on wood and other lignocellulosic materials make it an economically important fungal material.

Medicinal plants have been utilized for ages in the treatment of diseases and infections caused by parasitic organisms, bacteria, viruses, and fungi. These plants are important not only as the major source of herbal drugs, but also as starting materials in the synthesis of many semi-synthetic drugs [8]. Different parts of these medicinal plants or their products, believed to contain medicinal properties, such as leaf, root, bark, seed pulp and oil have been utilized in the treatment of various diseases and ailments through ingestion or external application [9]. Oils obtained from medicinal plants have been widely investigated for their activity against pests, insects, bacteria, fungi and other microorganisms [10]. Castor plant taxonomically known as *Ricinus communis* L. is a fast growing suckering perennial monoecious shrub belonging to the Euphorbaceae family. It is native to Africa, but can be found in both tropical and temperate regions of the world [11]. The plant is rich in phytochemicals such as Ricinoleic acid, ricin, p-coumaric acid, ferulic acid, o-coumaric acids, ricinine, syringic acid, cinnamic acids, stigmasterol, fucosterol e.t.c [12]. The plant is widely utilized in the treatment of different diseases and ailments, and different parts of the plant is used to treat diseases and ailments such as wound, boils, sores, cough, jaundice, asthma, painful kidneys, colic, skin and eye diseases, abscess, e.t.c. [13]. The seeds of this plant are not left out as it is used to hasten childbirth, treat colic, ringworms, itching, warts, dandruff, hairloss and haemorrhoids. The oil obtained from the seed is used in soap making, and it is also used to treat bowels inflammation or irritation, piles, chronic dysentery and skin ailments [12]. The leaf extracts as well as the oil obtained from the seeds has also been reported to possess antimicrobial activity, necessitating their use in the treatment of different types of skin diseases such as itch, ringworm, e.t.c. [14]. The aim of this study was to evaluate and validate the reported antimicrobial activity of *R. communis* seed oil on *L. sajor-caju* through an *in vitro* antifungal assay.

MATERIALS AND METHODS

Collection of plant materials

Seeds of *R. communis* were obtained from Moniya, Ibadan, Oyo state in January 2013. A voucher specimen of the plant was deposited in the Forestry Research Institute of Nigeria (FRIN) herbarium.

Oil extraction

The seeds were dehulled, air-dried to reduce the moisture content and ground with a blender. The oil present in the ground seeds were then exhaustively extracted in a Soxhlet apparatus using n-hexane as solvent.

Physico-chemical properties of extracted oil.

The percentage yield of extracted oil was determined according to method of Rao et al.[15]. Physico-chemical properties of the oil such as specific gravity, acid value, saponification value, and iodine value were determined immediately after oil extraction. Specific gravity (at 32^{o}C) was determined using specific gravity bottle, Refractive Index (using water as reference) was determined using Abbe's refractometer, while acid, saponification and iodine values were determined according to procedure of Pearson [16].

Sourcing and culturing of test microorganisms

The *L. sajor-caju* strain P32-1 used was obtained from the culture collection at pathology laboratory of the FRIN. This stock culture was sub-cultured, and maintained on potato dextrose agar medium at Nigeria Institute of Science Laboratory Technology (NISLT), Ibadan.

Antifungal assay (disc diffusion assay)

Filter paper disks of 6mm diameter were prepared from Whatman (no 1) filter paper (England) and sterilized in an oven at 65^{o}C before use. Four concentrations were prepared from the extracted oil and 50 µl were impregnated onto each disc such that each of them received a dosage equivalent to 9.6 µg/disc, 4.8 µg/disc, 2.4 µg/discand 1.2 µg/disc respectively. Each disc was allowed to dry for 15 minutes before being placed on the agar plate, which had previously been inoculated with the test microorganism, by means of sterile forceps. Each of the discs was slightly pressed against agar surface. Fluconazole (100 µg/disc) served as positive control (PC) while Methanol (50 µl/disc) was used as negative control (NC). All the bioassay plates were incubated overnight at 37°C. The antifungal activity was assessed daily for three days by measuring the diameter of inhibition zones in millimeter (considered as zone of inhibition) produced by different concentrations of the seed oil, using a transparent ruler. The experiment was done in triplicates under strict aseptic conditions to minimize errors and ensure consistency of all findings.

Determination of relative percentage inhibition of oil extract
The relative percentage inhibition of the extracted oil with respect to positive control was calculated according to the Gaurav [17] method as illustrated below.

Relative percentage inhibition of the test extract = **100** X **(x-y)/ (z-y)**

Where,
x: total area of inhibition of the test extract
y: total area of inhibition of the solvent
z: total area of inhibition of the standard antifungal agent

The total area of the inhibition was calculated by using area = πr2; where, r = radius of zone of inhibition.

Statistical analysis
The bioassays were conducted in triplicate and the data obtained were expressed as mean± standard deviation (SD) (where n=3) for each treatment. The data obtained were subjected to analysis of variance (ANOVA) for a completely randomized design using SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Duncan multiple range test (DMRT) was used to separate differences among means. A *p* value <0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

One of the most important properties establishing the quality and present condition of oil is the physicochemical properties of the oil. The acid and peroxide values of oil are valuable measures of its quality, iodine value is a measure of its degree of unsaturation, saponification value is a measure of average length of the fatty acyl residues presents in the oil and it therefore determines the use to which the oil can be put while the acid value is a measure of the degree of oxidative damage. The percentage yield of the oil extracted from the seeds of *R. communis* was 32.4% (Table 1). This percentage is fairly high compared to some oil-rich seeds; therefore this oil can be classified as high yielding. This oil is a viscous pale amber liquid with distinct odour. The low iodine value of *R. communis* seed oil shows that though it contains unsaturated fatty acids but the percentage of these unsaturated fatty acids is low, hence the oil can be classified as nondrying oil thereby justifying its use as a lubricant, in hydraulic brake fluids and in soap making. The low acid value of the oil shows that the oil is of high quality and has been minimally hydrolyzed by degradative agents and lipases. The slightly high saponification value of the oil shows that the mean molecular weight of fatty acids present in the oil or the number of ester bonds present in the oil is slightly high. The low peroxide value of the oil is an indication that the oil has been minimally oxidized by singlet or triplet oxygen. Since the result of the physicochemical analysis of the extracted *R. communis* seed oil falls within the specification range specified by ASTM for quality castor oil, the extracted seed oil is of good quality.

The *in vitro* antimicrobial activity of the *R. communis* seed oil against *L. sajor-caju* was assessed both quantitatively and qualitatively by the presence or absence of inhibition zones and zone diameters. Results of the antimicrobial screening tests carried out are shown in Table 2. It can be observed that there is a concentration-dependent increase in the zones of inhibition (Fig 1), as it ranges from 11.2 to 27.7 mm. This shows that the oil extract exhibit *in vitro* antifungal activity against the tested *L. sajor-caju* strain. A comparison of the antifungal activity of the oil extract to evaluate their relative percentage inhibition compared with Fluconazole (PC), (Table 2), showed that at 1.2 µg/disc concentration the oil extract caused an 8.0 % reduction in fungal growth compared to the PC, at 2.4 µg/disc it caused a 25.6% reduction, at 4.8 µg/disc it caused a 49.6% reduction and at 9.6 µg/disc it caused a 79.7% reduction in fungal growth (Fig 2). It is apparent from this result that 9.6 µg/disc concentration is the most effective as it records the widest inhibition zone and the greatest increase in relative inhibition. The demonstration of antifungal activity by *R. communis* seed oil therefore scientifically justifies the ethnomedicinal use of the plant, especially in the treatment of skin diseases and wounds. This result is in agreement with the findings of Akpomie [18] who discovered that *Citrus sinensis* seed oil possessed antifungal activity against *Paecilomyces* sp., *Penicillium* sp. and *Rhizopus nigricans*. In another study, Mossini et al. [19] also discovered that neem seed oil exhibited antifungal activity against *Penicillium verrucosum* and *Penicillium brevicompactum*. The result is also in accordance with previous results of Zarai et al. [20] who discovered that essential oil from the leaves of *R. communis* exhibited antifungal activities against *Penicillium digitatum, Fusarium solani, Botrytis cinerea* and *Aspergillus niger*. Jain and Nafis [21]

also investigated and discovered that aqueous leaf extracts of *R. communis* possess antifungal activity against *C. glabrata* and *C. albicans.*

Table 1: Physicochemical properties of the extracted *R. communis* seed oil and American Society for Testing and Materials (ASTM) specification for quality Castor oil

Chemical Indices	Extracted Oil	ASTM Specification (Range)
Oil yield (%)	32.4	_
Specific gravity (27°C)	0.96g/cm^3	0.957-0.968 (20-25°C)
Acid value	2.7mg KOH/ g of Oil	0.4-4.0
Iodine value	84g I_2/ 100g of Oil	82-88
Saponification value	181mg KOH/ g of Oil	175-187
Peroxide value	7.50	_

Table 2: Antifungal activity of oil extract of *R. communis* against *L. sajor-caju*

Treatments	ZI Diameter (mm) ± SD			ZI Grand Mean	
	Days after Incubation				Relative
(Conc. (µg/disc))	1st Day	2nd Day	3rd Day	(mm) ± SD	Inhibition (%)
NC (50µl/disc)	8.8±2.2	7.1±1.5	6.0±1.5	7.3±1.4^e	-
1.2	13.2±1.3	11.2±1.0	9.1±0.9	11.2±2.1^d	8.0
2.4	18.5±1.7	16.6±1.7	15.2±2.4	16.8±1.7^c	25.6
4.8	24.5±1.3	22.4±1.4	20.0±1.1	22.3±2.3^b	49.6
9.6	29.5±2.1	27.7±1.5	26.0±1.1	27.7±1.8^a	79.7
PC (100 µg/disc)	31.6±2.5	30.4±1.7	29.5±1.5	30.8±1.6^a	-

**Each value represents the mean of three determinants.*

Note: Means with different letters are significantly different from each other at p< 0.05 as determined by DMRT.

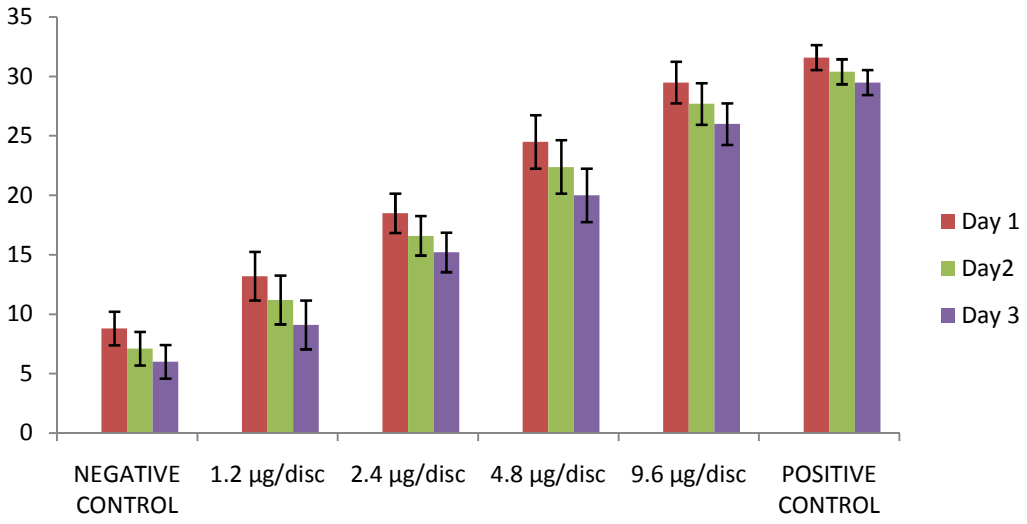

Fig 1: Summary of the zone of inhibition observed in each group after exposure to *R. communis* seed oil

CONCLUSION

This study further emphasized that *R. communis* seed oil indeed possessed antifungal activity. The high yield of the oil extracted and the antifungal activity of the oil against *L. sajor-caju*, as presented in table 2, and the ecofriendly nature of the oil shows that the extracted oil can serve and should be used as a natural drug for the treatment of white rot caused by *L. sajor-caju* or it could be used as a preservative against infections caused by *L. sajor-caju* and other white rot fungi. But further *in vitro* and *in vivo* studies are required to further investigate the bioefficacy of the oil extracts.

Also, further work is needed to determine the identity of the antifungal compound present within the oil as well as to determine its mechanism of action.

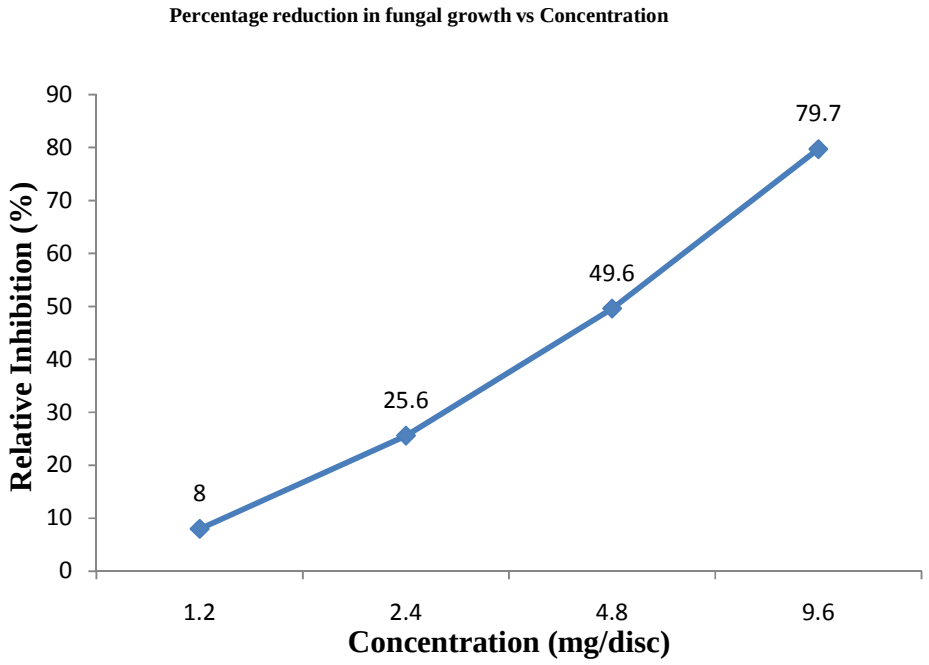

Fig 2: Relative percentage inhibition of growth of *L. sajor-caju* by *R. communis* oil extract

REFERENCES

[1] L. F. Botanini. *Wood: Types, properties, and uses*. Nova Science Publishers, Inc. *New York.* **2011,** 1-244.
[2] Forest Products Laboratory. *Wood handbook—Wood as an engineering material*. Gen. Tech. Rep. FPL–GTR–113. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. USA. **1999**, 1-463.
[3] M. Ek, G. Gellerstedt, and G. Henriksson. *Pulp and Paper Chemistry and Technology*. (Vol 1). Wood Chemistry and Wood Biotechnology. Walter de Gruyter GmbH & Co. KG, 10785 Berlin. **2009**. 1-308.
[4] Papadopoulos, A. N. *Wood Sci Technol.* **2011**, 1-8.
[5] M. Ohkuma, Y. Maeda, T. Johjima, and T. Kudo. *RIKEN Review*. **2001**. 42, 39-42.
[6] M. S. Aishah, and W. I. Wan Rosli. *International Food Research Journal*. **2013.** 20(1), 183-188.
[7] S. Patrabansh, and M. Madan. *Acta Biotechnol.* **1997**, 17(2), 107–122.
[8] S. S. Amritpal. *Herbalism, Phytochemistry and Ethnopharmacology*. Science Publishers Enfield, New Hampshire. USA. **2011**.
[9] A. Gurib-Fakim. *Molecular Aspects of Medicine*. **2006**. 27, 1-93.
[10] A. E. Sulieman, S. E. Abdelrahman, and A. M. Abdel Rahim. *Journal of Microbiology Research*. **2011**. 1(1), 1-4.
[11] M. A. Raoof, and M. Yasmeen. *J. Oilseeds Res.* **2006**. 23(2): 144-150.
[12] K. H. Ling, C. T. Kian, and T. C. Hoon. *A guide to medicinal plants. An Illustrated, Scientific andMedicinal Approach*. World Scientific Publishing Co. Pte. Ltd. 5 Toh Tuck Link, Singapore. **2009**.
[13] D. Diallo, C. Sogn, F. B. Samaké, B. S. Paulsen, T. E. Michaelsen, and A. Keita. *Journal of pharmaceutical biology*. **2002.** 40 (02), 117–128.
[14] G. T. A. Jombo, and M. N. O. Enenebeaku. *Nig. J. Physiol. Sci.*, **2008.** 23(1-2), 55-59.
[15] B. R. R. Rao, P. N. Kaul, K. V. Syamasundar, and S. Ramesh. *Industrial Crops and Products.* **2005.** 21(1): 121-127.
[16] D. Pearson. *The Chemical Analysis of Foods*. Churchill Livingstone, Edinburgh, UK. **1981**. pp. 504-530.
[17] K. Gaurav, L. Karthik, and K. V. Bhaskara Rao. *International Journal of Pharmaceutical Sciences Review and Research*. **2010.** Vol 4 (2), 141-144.
[18] O. O. Akpomie. *African Journal of Biotechnology*. **2010**. Vol. 9(5), 678-681.
[19] S. A. G. Mossini, C. C. Arrotéia, and C. Kemmelmeier. *Toxins*, **2009**. 1, 3-13.
[20] Z. Zarai, I. B. Chobba, R. B. Mansour, A. Békir, N. Gharsallah, and A. Kadri. *Lipids in Health and Disease*. **2012**. 11:102.

[21] P. Jain, and G. Nafis, *Journal of Pharmacy Research*, **2011**. 4(1),128-129.