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Antifungal activities of *Citrus sinensis* seed oil against *Lentinus sajor-caju*

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ABSTRACT

The aim of the present study was to assess the antifungal activity of seed oil of Citrus sinensis, an evergreen tree belonging to the family Rutaceae family. It is the most commonly cultivated and commercialized species of citrus, and the fruits, by-products inclusive, have been reported to possess high medicinal and economic values. The oils extracted from the peels and seeds of C. sinensis have been reported to possess insecticidal and antimicrobial activity. 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 1.15, 2.3, 4.6 and 9.2µg/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 exhibited a concentration-dependent increase in antifungal activity and high relative percentage inhibition against L. sajor-caju compared to standard antifungal agent. These results indicated that C. sinensis seed oil is highly effective as an antifungal agent against L. sajor-caju, which caused white rot in wood; therefore it can be used as a preservative agent for the management of wood infected with white rot fungi.

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

INTRODUCTION

Wood is a very important material utilized by humans for diverse purposes. Yet despite its importance, the commercial utilization of wood is sometimes limited by its low resistance to fungi and other biodegradative agents, most especially in the semi-arid and sub-humid tropical environment [1]. One of the largest groups of fungi known to cause wood biodegradation is the basidiomycete, usually classified as brown rot or white rot fungi. Unlike the brown rot fungi which primarily degrade cellulose, white rot fungi on the other hand can metabolize and degrade both the cellulose and lignin components of wood which can thus result in serious damage to wood and wooden materials [2]. *Lentinus sajor-caju* (Fr.) Fr. is a white rot fungus that possesses the ability to secrete different enzymes including laccases and peroxidase thereby allowing them to colonize and metabolize lignocellulosic substances [3]. The ability of this fungus to metabolize wood and other lignocellulosic substrates make it an economically important fungus.

Medicinal plants and their products have been employed for thousands of years in the treatment of parasitic infections such as those due to bacteria, viruses, fungi and others. Recently, these plants have also served as lead in the synthesis of many synthetic and semi-synthetic drugs [4]. Various parts of these plants, ranging from the roots, bark, leaves, flowers, seeds and fruits, are utilized in the treatment of different diseases and ailments. The oils obtained from different parts of medicinal plants are not left out as they have also been investigated for their activity

against pests, insects, bacteria, fungi and other microorganisms [5]. *Citrus sinensis* L. (Osbeck) is an evergreen tree belonging to the family *Rutaceae* family. It is native to China but is widely cultivated throughout the world. It currently rank as the most commonly cultivated and commercialized specie of citrus [6]. It possessed a globose, oblate or oval fruit; the inner rind of which is white, spongy and non aromatic. This fruit possessed a yellow, orange or slightly red pulp comprising of about 10-14 compartments each of which may enclosed 2-4 irregularly shaped seeds [7]. Citrus fruits, including their by-products, have been reported to possess high medicinal value in addition to their economic values. The fresh fruits are consumed on a large scale and large quantities are processed to produce juice. The wastes produced, after consumption and juice extraction, such as the peels, pulps and seeds are a potential source of valuable byproducts [8]. The oil obtained from the seeds, flowers, fruits and rinds of different species of Citrus also find wide applications in the toiletry, confectionary, and perfumery industry. The oils extracted from the peels and seeds of *C. sinensis* have also been reported to possess different type of activities ranging from insecticidal activity [9], to antimicrobial activity against a wide range of microbial organisms [10] hence this study was designed to evaluate and validate the activity of *C. sinensis* seed oil on *L. sajor-caju through* an *in vitro* antifungal assay.

MATERIALS AND METHODS

Collection of plant materials and extraction of Oil

Ricinus communis (Citrus) fruits were obtained from local vendors in Oje market, Ibadan, Oyo state, and identified at Forestry Research Institute of Nigeria (FRIN) herbarium. The seeds were separated manually and sun-dried for seven days, with about eight hours of sunlight per day, to reduce the moisture content. They were then decoated and ground with a blender. The oil present in the ground seeds were exhaustively extracted with n-hexane in a Soxhlet apparatus, and the excess solvents were removed by simple distillation. The extracted oil was dried in an oven at 65^{o}C for one hour, transferred to a dessicator, allowed to cool and weighed. This process was repeated three times according to method of [11]. The extracted oils were then stored in a sterilized brown bottle and refrigerated at 5^{o}C until commencement of the tests.

Sourcing and culturing of test microorganisms

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

Antifungal assay (disc diffusion assay)

The assay was carried out by preparing 6mm diameter filter paper disks from Whatman (no 1) filter paper (England) and sterilizing it in an oven at 65^{o}C. Four concentrations were then prepared from the extracted oil and 50 µl of it were impregnated onto each disc such that each disc received a dosage equivalent to 9.2 µg, 4.6 µg, 23 µg and 1.15 µg respectively. Each disc was allowed to dry for about 15 minutes before being placed, using sterile forceps, on an agar plate previously inoculated with the test microorganism. Each of the discs was slightly pressed against the agar surface. Fluconazole (100 µg/disc) was used as positive control (PC) while Methanol (50 µl/disc) was used as negative control (NC). All the bioassay plates were then incubated for 24 hours at 37°C. The antifungal activity of the oil was assessed daily for three consecutive days through the measurement of diameter of inhibition zones, in millimeter, produced by different concentrations of the seed oil, using a transparent ruler. The experiment was aseptically conducted in triplicates 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 compared to the positive control was calculated according to [12] 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 = πr^2; where, r = radius of zone of inhibition.

Statistical analysis

The bioassays were conducted in triplicate and the data obtained expressed as mean± standard deviation (SD) (where n=3) for each treatment. 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

Determination of the physicochemical properties of oil is an indispensable tool for establishing the quality and present condition of the oil. Acid and peroxide values are valuable measures of quality of oil, iodine value is a measure of degree of unsaturation of oil, saponification value is a measure of average length of the fatty acyl residues presents in the oil hence it determines what the oil can be used for, while the acid value is a measure of the degree of oxidative damage in the oil. The percentage yield of the oil extracted from the seeds of *C. sinensis* was 34.5% (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 *C. sinensis* seed oil is an indication that it contains unsaturated fatty acids, though the percentage is low, therefore this oil is a nondrying oil suitable for use as a lubricant, in soap making and in hydraulic brake fluids. The high saponification value of the oil shows that the oil probably consists, on average, high molecular weight fatty acids or high number of ester bonds. The high peroxide value of the oil is an indication that the oil is highly susceptible to lipolytic hydrolysis and oxidative deterioration by singlet or triplet oxygen. The chemical indices of the oil is comparable to that obtained by [11].

Table 1: Physicochemical properties of the extracted *R. communis* seed oil

Chemical Indices	Extracted Oil
Oil yield (%)	34.5
Specific gravity (25^{o}C)	0.92g/cm^{3}
Iodine value	106g I_2/ 100g of Oil
Saponification value	193mg KOH/ g of Oil
Peroxide value	90.3

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)	9.1±2.2	7.8±1.5	6.7±1.5	7.9±1.2^{e}	-
1.15	11.6±1.3	10.3±1.0	9.2±0.9	10.4±1.2^{e}	6.9
2.3	15.8±1.7	14.2±1.7	12.9±2.4	14.3±1.4^{d}	21.5
4.6	19.6±1.3	17.9±1.4	16.6±1.1	18.0±1.5^{c}	39.6
9.2	24.9±2.1	22.7±1.5	20.6±1.1	22.7±2.2^{b}	68.5
PC (100 µg/disc)	29.4±2.5	26.7±1.7	24.5±1.5	26.9±2.5^{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.

Results of *in vitro* antimicrobial activity of the *R. communis* seed oil against *L. sajor-caju* assessed both quantitatively and qualitatively through the presence or absence of inhibition zones and zone diameters are shown in Table 2. From the results it can be observed that the zones of inhibition increases in a concentration-dependent manner (Fig 1), as it ranges from 10.4 to 22.7 mm. Thereby showing that the oil extract exhibited *in vitro* antifungal activity against the tested *L. sajor-caju* strain. To evaluate the relative percentage inhibition of the oil extract, the result obtained in other treatment groups were compared to that of the positive control (Table 2), and it was observed that at 1.15 µg/disc concentration, the oil extract produces a 6.9 % reduction in fungal growth compared to the PC, at 2.3 µg/disc concentration it produces a 21.5% reduction, while at 4.6 µg/disc and 9.2 µg/disc concentrations it produces 39.6% and 68.5% reduction in fungal growth respectively (Fig 2). Since the widest inhibition zone and greatest increase in relative inhibition was observed at 9.2 µg/disc concentration, it is clear that this concentration is the most effective. The exhibition of antifungal activity by *C. sinensis* seed oil therefore demonstrates that the seeds indeed possessed valuable by-products which could be put to productive use and should not be discarded as wastes. This result is in accordance with previous results of [13] that discovered that neem seed oil exhibited antifungal activity against *Penicillium verrucosum* and *Penicillium brevicompactum*. It also

corroborates the findings of [7] who discovered that *Citrus sinensis* seed oil possessed antifungal activity against *Paecilomyces* sp., *Penicillium* sp. and *Rhizopus nigricans.*

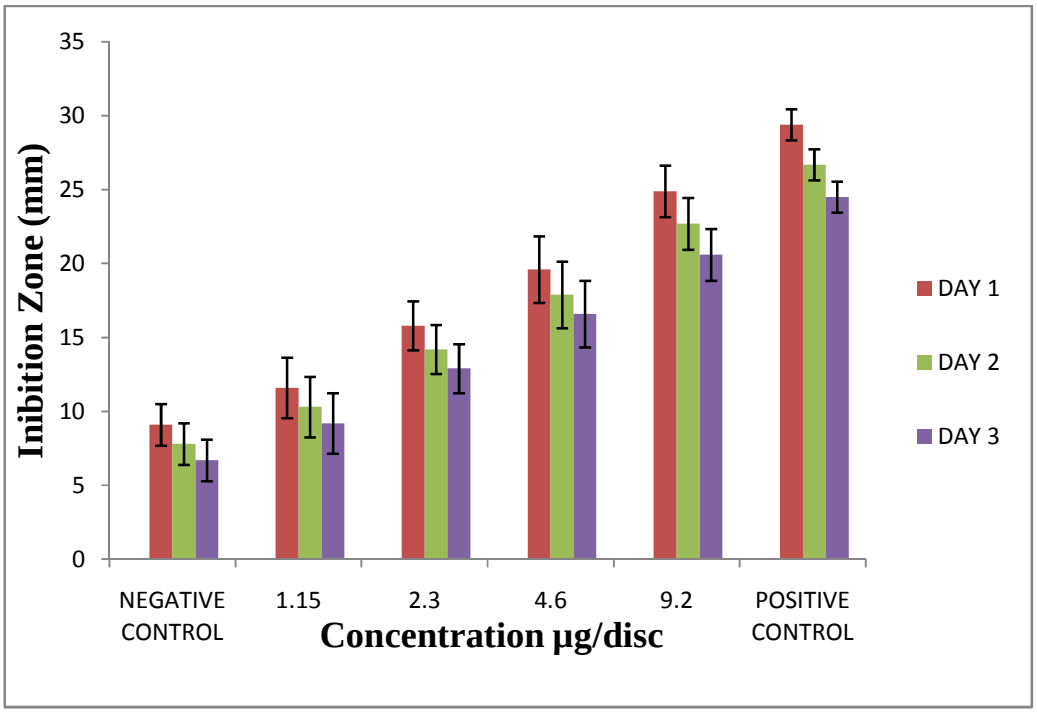

Fig 1: Summary of the zone of inhibition observed in each group after exposure to *R. communis* seed oil

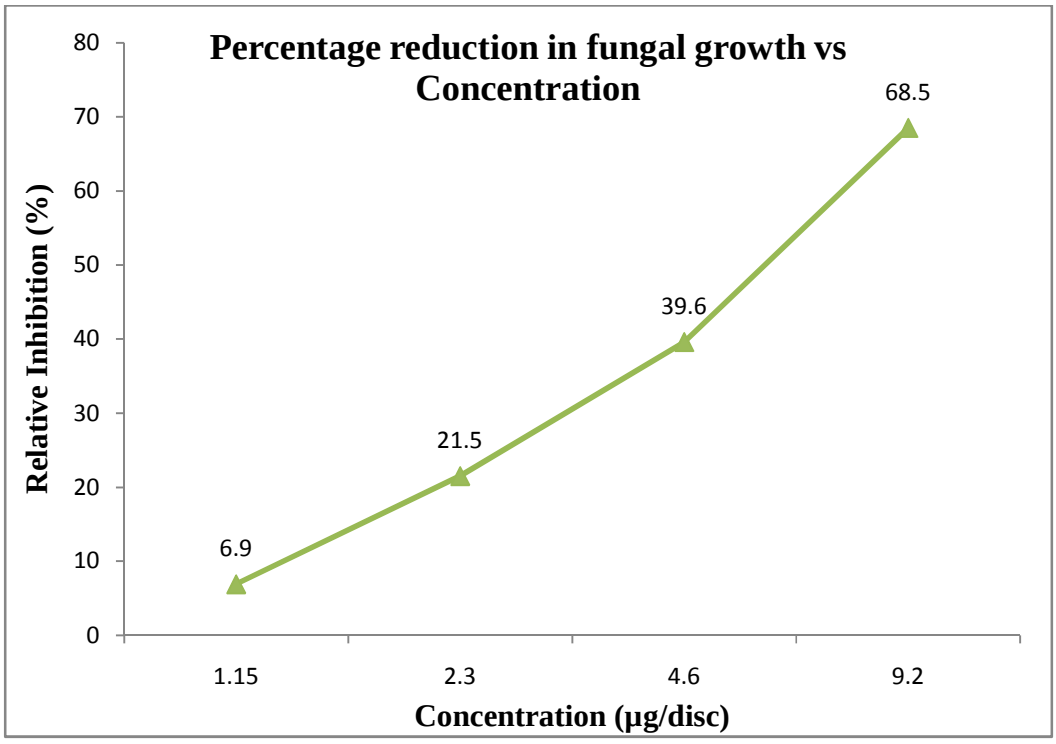

Fig 2: Relative percentage inhibition of growth of *L. sajor-caju* by *R. communis* oil extract

CONCLUSION

This study has further emphasized that *C. sinensis* seed should not be treated as a waste product as its oil content indeed possessed antifungal activity. The high yield of the extracted oil, its antifungal activity against *L. sajor-caju* (Table 2), and the fact that the extracted oil is a natural material which is ecofriendly shows that the extracted oil can be used as a preservative or in the treatment of white rot caused by *L. sajor-caju*. But further *in vitro* and *in vivo* studies still needed to be carried out in order to investigate the bioefficacy of the oil extracts.

Also, the determination of the identity of the antifungal compound present within the oil and their mechanism of action is a matter of necessity.

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