



Original Research

Antibacterial activities of acetonides prepared from the seed oils of *Calophyllum inophyllum* and *Pterocarpus osun*

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Abstract

Calophyllum inophyllum and *Pterocarpus osun* are underutilized plant species in Nigeria, but these have the potential to serve as a source material for various industries, especially in the pharmaceutical industry. Over the years, the search for antimicrobial agents from cheap and renewable sources has been on the rise. In this study, oil was extracted from the seeds of *C. inophyllum* and *P. osun*, analyzed for fatty acid composition, and the oils were then converted into acetonides by simple chemical reactions. The acetonides synthesized from these oils were tested for antimicrobial activities. The oil yield from *C. inophyllum* ($41.96 \pm 2.0\%$) and *P. osun* ($10.16 \pm 0.5\%$) was fairly high. C18:1 was the most dominant fatty acid in *C. inophyllum* ($36.88 \pm 0.20\%$), whereas C18:2 was the most dominant form in *P. osun* ($38.97 \pm 0.50\%$). Acetonides were produced from the oils with a yield of 91.70% for *C. inophyllum* and 92.10% for *P. osun*. The synthesis was monitored and confirmed using the PerkinElmer Fourier transform infrared spectrophotometer. The acetonide from *C. inophyllum* inhibited the growth of organisms such as *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, whereas the oil had inhibitory activity against *Bacillus subtilis* and *Staphylococcus aureus*. Acetonides from *P. osun* did not show any inhibition activity against the studied organisms.

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1. Introduction

Seed oils are esters of glycerol and fatty acids, which are found either in the free state or in a combined state with other compounds such as proteins and carbohydrates.^{1–3} Apart from the possibility of being a food source, many of the known seed oils are used in the manufacture of cosmetics, varnishes, paint, lubricants, and plastics. In addition to having agricultural potential, seed oils are renewable resources for developing novel products with superb qualities and multifunctional

purposes. These credentials have given seed oil a face-lift in the oleochemical industries.

The modification of seed oil properties is important to extend their applications in various industries. One area of these modifications involves the introduction of branched chains and other functional groups such as the hetero atoms. Such a modification could play a vital role in the medicinal values of some of these seed oils, because some of them have been reported to serve as important purgatives and are also useful in the manufacture of ointments and lotions for treating various skin diseases.^{4–6}

Although several reactions to synthesize such materials have been reported in the past, using safe, nontoxic, and environmentally friendly chemicals has been of great interest and yet challenging. To this effect, our aim is to use simple reaction pathways to produce nontoxic products such as

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acetonides from seed oil. In this study, we have chosen acetone for the cyclic ketal reaction because of its low cost and general availability. The process used is reversible as the acetonides can be hydrolyzed to diol, which in turn can produce the acetonides once again.^{7,8} Although acetonides are widely used in medicinal and pharmaceutical industries,^{9–11} they are usually prepared using only synthetic agents, and not from biomass such as *Calophyllum inophyllum* and *Pterocarpus osun* seed oils. Acetonide synthesized from renewable resources such as biomass will be environmentally safe, as these are both biodegradable and cost effective.

Underutilized seed oil can serve as a cheap renewable source to achieve this purpose. *C. inophyllum* and *P. osun* are examples of underutilized plants that can be used in this regard. *C. inophyllum* is a large evergreen plant. It is mostly cultivated in all tropical regions of the world, including several Pacific Islands, because of its decorative leaves, fragrant flowers, and spreading crown.¹² It is a popular ornamental plant. It is a low-branching and slow-growing tree with a broad and irregular crown. It is usually 8–20 m (26–66 ft) in height. The flower of the tree is 25 mm (0.98 in.) wide. The plant flowers throughout the year. The fruit is round, and upon ripening it wrinkles and its color changes from yellow to brownish red. The chemical properties of the oil have been previously reported by Adewuyi et al,¹³ and the fatty acid composition was reported by Ajayi et al,¹⁴ with C18:1 being the most dominant fatty acid in the oil.

P. osun is a forest tree that belongs to the Papilionaceae family¹⁵ and is characterized by a spreading crown. The wood of the tree is marketed as African padauk.¹⁶ The extracts (in crude form) of *P. osun* are applied locally in the eastern part of Nigeria as a medication for the treatment of chicken pox in children.¹⁷ The antioxidant potentials and attenuation of acetaminophen-induced redox imbalance by *P. osun* has also been reported previously.¹⁸

At present, there are no specific applications of the oils of *C. inophyllum* and *P. osun* in Nigeria as they are considered underutilized. The use of biomass as feedstock for the production of bio-based products is gaining increased attention and in this regard, we focused on the antibacterial potentials of acetonides synthesized from the seed oils of *C. inophyllum* and *P. osun*.

2. Materials and methods

2.1. Materials

The mature seeds of *C. inophyllum* and *P. osun* were collected from the trees grown in the garden of the University of Ibadan (Ibadan, Oyo State, Nigeria). They were identified at the Herbarium Unit, Department of Botany, University of Ibadan. All solvents and chemicals used in this study were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Determination of fatty acid composition

The fatty acid composition of the seed oils of *C. inophyllum* and *P. osun* was determined as described by Adewuyi et al.¹⁹ In brief, the fatty acid contents of the oils were determined as methyl esters. First, the oils were converted into methyl esters by refluxing at 70°C for 3 hours in 2% sulfuric acid in methanol. Second, the resulting methyl esters were extracted into ethyl acetate, washed free of acid, and passed over anhydrous sodium sulfate. Finally, the fatty acid composition was analyzed using an Agilent 6890 N series gas chromatography system (USA) equipped with a flame ionization detector on a split injector. A fused silica capillary column (DB-225; 30 m × 0.32 mm i.d., J&W Scientific, Folsom, CA, USA) was used with the injector and the temperature of the detector maintained between 230°C and 250°C. The oven temperature was programmed at 160°C for 2 minutes and finally increased to 230°C at 4°C/minute. The carrier gas was nitrogen at a flow rate of 1.5 mL/minute. The area percentages were recorded using a standard ChemStation Data System (Agilent, USA).

2.3. Epoxidation of oils of *C. inophyllum* and *P. osun*

The epoxidation was carried out in a 150-mL three-necked round-bottomed flask equipped with a magnetic stirrer and a thermometer to monitor the reaction temperature. The whole apparatus was kept in an oil bath to maintain the desired temperatures. Approximately 46 mL of oil from *C. inophyllum* and *P. osun* was separately added into the flask, followed by an addition of 5 mL of 100% formic acid. The mixture was continuously stirred at 15°C. To this mixture, 14 mL of hydrogen peroxide was dropwise added and the mixture was

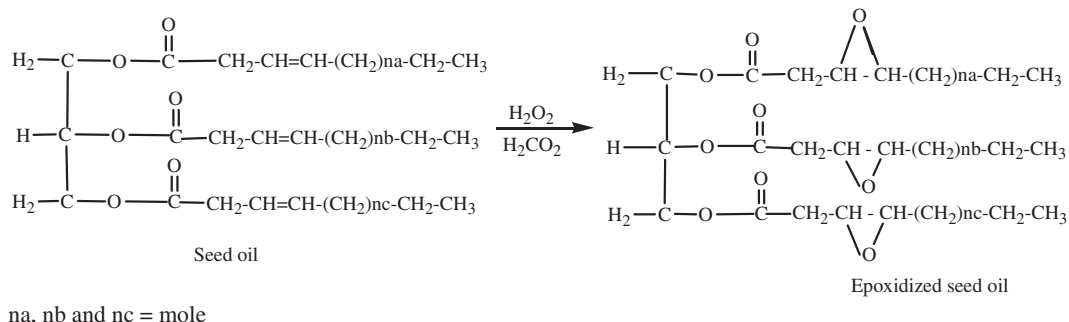


Fig. 1. Epoxidation of the seed oils of *Calophyllum inophyllum* and *Pterocarpus osun*.

respectively. By contrast, C18:2 was the most abundant fatty acid found in *P. osun* ($38.97 \pm 0.50\%$). The composition of C18:2 was found to be $23.90 \pm 0.30\%$ in *C. inophyllum*, whereas that of C18:1 was $19.30 \pm 0.10\%$ in *P. osun*. The composition of C22:0 was $10.41 \pm 0.05\%$ in *P. osun*, whereas it was $0.34 \pm 0.02\%$ in *C. inophyllum*. The unsaturation level of *P. osun* was $59.52 \pm 0.30\%$, whereas that of *C. inophyllum* was $61.41 \pm 0.20\%$. The unsaturation is an indication of the level of unsaturated bonds present in these oils. Moreover, the unsaturated bonds are the points where different functional groups can be introduced into the oils to achieve modifications or where chemical reactions can take place due to the rich electron system of the double bonds.²¹

3.2. Epoxidation and synthesis of acetonides from the oils of *C. inophyllum* and *P. osun*

The FTIR spectra showed important peaks explaining the stretching, bending, and double-bond absorptions of the oil samples. It was observed that the absorption peaks are almost the same for both oil samples. The C–H stretching of absorption for CH₃ occurred at wavelengths of 2926.12 cm^{-1} and 2925.86 cm^{-1} in *C. inophyllum* and *P. osun* oils, respectively, whereas the C–H stretching of absorption for CH₂ occurred at wavelengths of 2859.83 cm^{-1} and 2860.20 cm^{-1} , respectively. Two alkane peaks were observed showing the bending absorption for CH₂ (1451.39 cm^{-1} for *C. inophyllum* and 1455.01 cm^{-1} for *P. osun*) and CH₃ (1371.61 cm^{-1} for *C. inophyllum* and 1367.37 cm^{-1} for *P. osun*). Peaks corresponding to the vibrational frequency of the ester functional group were observed in *C. inophyllum* at 1741.82 cm^{-1} (C=O) and 1162.82 cm^{-1} (C–O), whereas in *P. osun*, they appeared at 1744.53 cm^{-1} (C=O) and 1163.48 cm^{-1} (C–O). The absorption band of the unsaturated bonds (C=C) appeared at 3001.23 cm^{-1} for *C. inophyllum* and at 3004.52 cm^{-1} for *P. osun*. This peak representing unsaturation disappeared after the epoxidation with the appearance of a peak at 832 cm^{-1} in *C. inophyllum* and at 867 cm^{-1} in *P. osun* corresponding to the epoxy functional group. The epoxidized oils were separately treated with acetone as shown in Fig. 2 to produce acetonides, with the ketal peaks appearing at 1060 cm^{-1} , 1080 cm^{-1} , 1120 cm^{-1} , and 1200 cm^{-1} in the products.^{20,22}

Table 2
Antibacterial activities of oils and acetonides of *Calophyllum inophyllum* and *Pterocarpus osun*.

Organism	<i>C. inophyllum</i>	<i>P. osun</i>	ACI	APO
<i>Escherichia coli</i>	ND	ND	2.6	ND
<i>Bacillus subtilis</i>	2.0	ND	ND	ND
<i>Klebsiella pneumoniae</i>	ND	ND	ND	ND
<i>Pseudomonas putida</i>	ND	ND	ND	ND
<i>Staphylococcus aureus</i>	3.0	ND	ND	ND
<i>Flavobacterium</i>	ND	ND	ND	ND
<i>Salmonella typhi</i>	ND	ND	1.6	ND
<i>Pseudomonas aeruginosa</i>	ND	ND	3.2	ND

ACI = acetonide from *C. inophyllum*; APO = acetonide from *P. osun*; ND = no zone of inhibition.

3.3. Antibacterial activities of acetonides from *C. inophyllum* and *P. osun*

The use of a natural product and its modified forms as an antimicrobial agent is an important aspect of natural product chemistry with the prospect of developing and discovering new potent drugs. The synthesis of acetonides and their screening as a potential antibacterial agent is a positive move in this direction. The result of the antibacterial activities of the oils and acetonides of *C. inophyllum* and *P. osun* is presented in Table 2. The oil of *C. inophyllum* showed inhibition against *S. aureus* and *Bacillus* sp., whereas the oil of *P. osun* did not show any activity against the test organisms. The acetonides from *C. inophyllum* showed activity against *E. coli* (2.6 mm), *Salmonella typhi* (1.6 mm), and *P. aeruginosa* (3.2 mm), whereas its oil had inhibition against *Bacillus subtilis* (2.0 mm) and *S. aureus* (3.0 mm). Acetonides from *P. osun* did not show any activity against any of the tested organisms.

4. Discussion

The fatty acid composition of the oils of *C. inophyllum* and *P. osun* suggests the kind of application that can be given to them. The oil of *C. inophyllum* is rich in C18:1, which is similar to what has been reported for some conventional seed oils.^{23,24} Some of these conventional seed oils are edible and have also been used as industrial feedstock, which has increased their price in markets, and thus creating a competition between their use as food and industrial feedstock. This study has revealed *C. inophyllum*, an underutilized plant in Nigeria, as a fairly good source of oil that can be considered as a potential alternative for some of these conventional oils due to its high composition of C18:1 fatty acid. The fatty acid composition of *P. osun* seed oil can be compared with those reported for some vegetable oils.²⁵ Besides, the high unsaturation of these oils indicates the possibility of modification; the high unsaturation (C18:1 and C18:2 fatty acids) is also an indication of health and dietary importance.^{26,27}

Conventional seed oils have found application as raw materials in several industries either as starting materials for other products or as additives.^{28,29} Production of acetonides from these oils will be an important contribution to developing local oleochemical industries in Nigeria as these oils are underutilized with no specific use at present; they may be used in the production of other products apart from their present screening as possible antibacterial agents. The yield of the acetonide of *C. inophyllum* from its epoxidized oil was 91.70%, whereas that of *P. osun* was 92.10%.

The use of safe and environmentally friendly biomass as antibacterial agents plays a key role in health care as these have been used to treat diseases in developing countries such as Nigeria and has become a therapeutic modality, which has stood the test of time.³⁰ Today, several synthetic product prototypes have been developed by key actors in this field of research for commercial purposes, some of which are expensive for low-income earners in developing nations of the

world. The by-products of some of these synthetic pharmaceuticals have been determined to be emerging pollutants in water and hospital waste³¹; therefore, finding appropriate biobase products that will circumvent the short comings of synthetic products had been a challenge. Acetonides from the seed oils of *C. inophyllum* and *P. osun* are biobases, which were produced by simple reaction methods. Acetonide from *C. inophyllum* showed activities against Gram-negative organisms such as *E. coli*, *S. typhi*, and *P. aeruginosa*, whereas *P. osun* did not. *E. coli* is a Gram-negative bacterium, which causes gastroenteritis, urinary tract infections, and neonatal meningitis.³² *S. typhi* is a Gram-negative bacterium, which causes typhoid fever and is transmitted through food and water. Certain strains of *S. typhi* have been reported to have developed resistance to known drugs such as chloramphenicol, ampicillin, and trimethoprim,³³ thereby creating a need for the development of new drugs that will surmount their resistance. *P. aeruginosa* is also a Gram-negative bacterium that typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections.³⁴ Athanassiadis et al³⁵ and Batovska et al³⁶ have also reported the use of biomass as antimicrobial agents against similar organisms reported in this work. The antimicrobial activity of acetonides of *C. inophyllum* is also comparable with that of monocyclic and bicyclic β -lactams, whose activities were reported previously.³⁷ The oil of *C. inophyllum* was found to have activity against the growth of *S. aureus* unlike in the case of the essential oil and methanol extract from *Nepeta cataria*.³⁸ The ability of *C. inophyllum* acetonide to inhibit the growth of these pathogenic organisms suggested its potential as being an antibacterial agent.

In conclusion, in this study, oil was extracted from the seeds of *C. inophyllum* and *P. osun*, analyzed for fatty acid composition, and used in the synthesis of acetonides. The acetonides synthesized from these oils were finally screened for antimicrobial activities. The yield of oil from *C. inophyllum* ($41.96\% \pm 2.0\%$) and *P. osun* ($10.16\% \pm 0.5\%$) was fairly high. C18:1 was the most dominant fatty acid in *C. inophyllum* ($36.88\% \pm 0.20\%$), whereas C18:2 was the most dominant in *P. osun* ($38.97\% \pm 0.50\%$). Acetonides were produced from the oils with a yield of 91.70% for *C. inophyllum* and 92.10% for *P. osun*. The synthesis was monitored and confirmed using the PerkinElmer FTIR spectrophotometer. The acetonide from *C. inophyllum* inhibited the growth of organisms such as *E. coli*, *S. typhi*, and *P. aeruginosa*, whereas its oil inhibited the growth of *B. subtilis* and *S. aureus*. Acetonides from *P. osun* did not have any inhibition against the studied organisms.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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