# ANTIMICROBIAL ACTIVITY, SPECTROSCOPIC AND THERMAL CHARACTERISATION OF OIL FROMAFRICAN OIL BEAN (PENTACLETHRA MACROPHYLLA) SEED

**Oyedeji O. A.* Odeyemi B. A. and Koleosho A.O.**
Department of Science Laboratory Technology, Federal Polytechnic, Ilaro, Nigeria.
*Corresponding author email: olalekan.oyedeji@federalpolyilaro.edu.ng; +2348055186225

**ABSTRACT**
The application of vegetable oils is presently in the spotlight of the chemical industry, because of their widespread availability, low price, inherent biodegradability, and excellent environmental qualifications that include low ecotoxicity and toxicity toward humans. This paper reports the antimicrobial, spectroscopic and thermal properties of the seed oil of *Pentaclethra macrophylla*. The oil inhibited *Aspergillus flavus* but had no effects on *A. niger, Candida albican*, *Rhizopus oligosporus* and clinical isolates that included *Staphylococcus aureus*, *Streptococcusmutans, Salmonella typhimurium* and *Escherichia coli*. The differential scanning calorimetry (DSC*)* curve of the oil is characterized with melting and melting enthalpy at 35.52$^{o}$C, and -7.62 Jmol$^{-1}$, respectively with crystallization at 33.9 $^{o}$Cand an enthalpy of -3.25 Jmol$^{-1}$, with no further troughs or crests as signs of the oil homogeneity. The IR peaks obtained showed significant functional groups associated with unsaturation in vegetable oils, particularly those rich in linoleic acid (3008-3010 cm$^{-1}$), and the UV profile of the oil showed high absorption at 190-320 nm important for oils that shield against dangerous UV-A and B radiations. Oil of *P. macrophylla* if exploited could be useful industrially for the production of cosmetics and creams thereby relieving the cost of popular seed oils.

**Keywords: Antifungal, biodiesel, spectra characterisation, cosmetic, *Pentaclethra macrophylla,*underutilised seed oil.**

## INTRODUCTION

Nigeria, the fourth-largest producer of oil palm after Malaysia, Indonesia, and Thailand still imports vegetable oils to augment local production (Okolo, 2014). The production deficit for vegetable oils remains high at about 600,000 tons a year to satisfy the local demand for food and non-food uses. Vegetable oils are one of the most utilized substances in the world, and widely studied (Orsavova, Misurcova, Ambrozova, Vicha&Mlcek, 2015). They have a wide-spread uses that make them a vital material in the daily activity of man. They are consumed domestically during food preparations. They also have a regular use industrially where they are applied as antimicrobial, antioxidant agents and for other medicinal purposes (Pandini et al., 2018). Vegetable oils have the advantage of high biodegradation rates, and thus reducing environmental risks,as well as reduced burning risk due to their higher fire points than mineral oils (Ligadas, Ronda, Galia& Cadiz, 2013; Gomez, Abonia, Cadavid& Vargas, 2011). In Nigeria, the most commonly consumed oils are soya beans, palm and peanut oils (Kamal-Eldin, 2005). Several plant seeds and their characteristic oils have been relegated whereas they equally possess all necessities as the needed oils. Relegated plant seeds are termed 'lesser-known or underutilized seeds', and this is because of the little and or no information provided about them (Adewale, Rotimi, Rao, & Prasad, 2011; Zarinah, Maaruf, Nazaruddin, Wong, &Xuebing, 2014). Overutilization and dependence on popular seed oils have led to high prices and scarcity in most parts of the world in addition to problems associated with their consumption and availability if not immediately resolved. There is, therefore, need to research into underutilized plants seeds which can be used alternatively to the conventionally used oils.

*Pentaclethra macrophylla* (African oil been seed) is a big leguminous arboreal plant that belongs to the subfamily Mimosoideae (Agbogidi, 2010). It is mostly found in the southern and middle belt regions of Nigeria, coastal parts of the west and central Africa. The mature dispersed seeds are collected and sold in markets. The seeds have been used to complement protein (Enujiugha&Agbede, 2000), source of oils for cooking, candle and soap making (Tico, 2005). However, little is known about the spectroscopic, thermal and antimicrobial activities of the seed oil of *P. macrophylla*.

This study, therefore, was aimed at characterising the oil for the said properties so as to encourage its cultivation and further usage for industrial purposes if found suitable, thus providing additional income for the peasants.

## MATERIALS AND METHODS
### Sample collection

The seeds of *P. macrophylla* were collected from the botanical garden of the Department of Science Laboratory Technology, The Federal Polytechnic, Ilaro between July and October, 2018 during which time it was most abundant. Seed samples were identified at Forestry Research Institute of Nigeria (FRIN) with voucher number FHI 111205.

**Extraction of seed oil**
The modified method of Nehdi, Khalil and Al-Resayes (2010)as previously reported by Oyedeji, Azeez and Osifade (2017) was used for oil extraction. Briefly, 200±1.0 g powdered seeds of *P. macrophylla* were extracted with 800 ml hexane (95% SCP, Surechem Products Ltd, Suffolk, England) with a Soxhlet extraction set-up for 12 hours. The hexane was recovered using a rotary vacuum evaporator (RE300 Bibby Scientific, Fisher Scientific, Loughborough, Leicestershire, UK) at reduced pressure while traces of n-hexane was expelled placing the oil extract in a water bath at 40$^{o}$C for 24 hours.

**Thermal analysis**
Thermal analysis of the oil was performed using a differential scanning calorimeter (DSC) (Metler Toledo, 2 Stare system, Metler Toledo, Columbus, USA) equipped with an FRS 6 measuring cell. 35±0.5 mg of the oil sample was measured into a 40 µl aluminium standard pan with restricted gas exchange in a self-generating atmosphere. The heating measurement was from about 30-250°C at 5K/min with nitrogen ($N_2$) as the purge gas in an inert atmosphere.

**Infra redspectroscopy characterisation**

The oil sample was analyzed by the potassium bromide (KBr) pellet method. 0.1% sample was mixed into 250 mg KBr powder, finely pulverized and kept in a pellet-forming die. The Fourier transform infrared (FTIR) spectrum was recorded in the wavelength range of 650 – 4000 cm$^{-1}$ (Agilent 630 FTIR, Agilent Technologies, Germany) by KBr pellet technique with a resolution and scanning speed of 8 cm$^{-1}$ and 2 mmsec$^{-1}$, respectively. Apodization was by the Happ-Genzel function with 32 background and sample scans each.

**UV-Visible spectroscopy**
The sample was diluted in isooctane (1:30, v/v), shaken and left in the dark for 10 minutes. The organic layer was afterwards removed for spectrophotometetric analysis. The spectrophotometer (ATICO double beam UV/VIS Spectrophotometer, Atico Medical Pvt Ltd., Haryana, India) was calibrated using isooctane as blank and the absorbance of the sample was recorded at varying wavelengths. The molecular absorption spectrum of *P. macrophylla* seedoil was recorded from 190 to 400 nm at 5 nm interval in a 1 cm quartz cuvette (Kruzlicova, Mocak, Katsoyannos&Lankmayr, 2008).

**Antimicrobial tests**
The antimicrobial property of the oil sample was tested by the paper disc-diffusion method against pathogenic bacterial isolates including two Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcusmutans*and two Gram-negative bacteria*, Salmonella typhimurium* and *Escherichia coli;* and as described by Helal et al. (2019) with a little modification.
Briefly, the bacteria isolates were grown on Czapecdox agar media at room temperature for seven days. Culture plates were flooded with 0.45% sterile saline solution and inoculum turbidity was adjusted to 0.5 McFarland standards. Inocula of *S. aureus, S. mutans, S. typhimurium* and *E. coli* plates were added to 4 and 2%, v/v of oil sample in ethylene glycol. Zones of inhibition were measured against 2 mg/ml gentamicin standard.

**Antifungal assay (Disc diffusion technique)**
Four strains of fungi; *Aspergillus niger, A. flavus, Candida albican* and *Rhizopus oligosporus*Disc diffusion method was performed. 100 µl of the inoculum was seeded on the plates containing Czapek Dox Agar (CDA) medium. The plates were allowed to dry for 2-5 minutes. 100 µl of the extract was loaded to the sterile discs of 6 mm diameter and placed on the test plates and incubated at 37°C for 72-96 hours. The diameter of the inhibition zones was measured in millimeter (mm). The plate was loaded with ketoconazole as control.

**RESULTS AND DISCUSSION**

The DSC curve for *P. macrophylla* seed oil as shown in Fig. 1 is characterized by an endothermic peak at 35.52°C with an enthalpy of -7.62 Jmol$^{-1}$. The peak is marked by an on-set and end-set temperatures of 33.90 and 40.52°C, respectively. This is followed by an endothermic decomposition peak at 41.43°C with an on-set and end-set temperature of 41.18 and 42.80 °C, respectively. The enthalpy of the reaction is -3.25 Jmol$^{-1}$. Melting and crystallization, two frequently used physical events to characterise thermal behaviour of oil samples, could either be endothermic or exothermic with respect to release of thermal enthalpy. Thermal curves are usually indicated by troughs and crests, and are for crystallization and melting of oils respectively (Tan & Man, 2000). The trough in Fig. 1 shows the crystallization of the oil sample at 35.52$^{o}$C, and no other troughs were observed because of the homogeneity of the sample. The DSC curve obtained from an oil sample is characteristic of the particular oil and could serve as a "fingerprint" of its authenticity (Tan & Man, 2002), geographical origin and variety (van Wetten, van Herwaardena, Splinter & van Ruth, 2004).

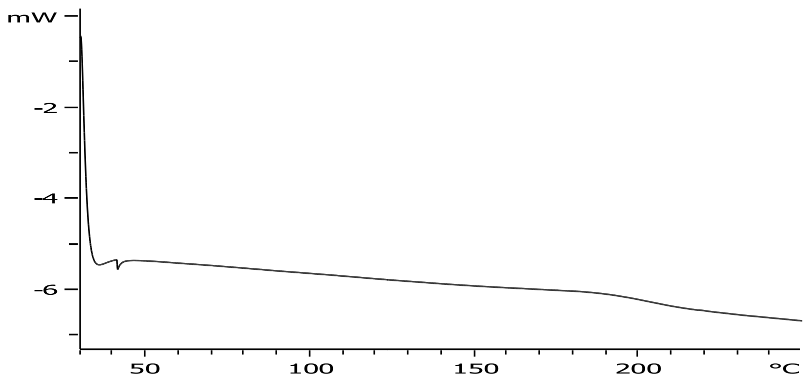


Fig. 1: DSC curve of *P. macrophylla* seed oil measured from 30-250$^{o}$C, heating 5°C/min.

The IR spectrum was used to identify the functional groups in the oil based on the bands obtained through stretching and bending vibrations in the region of IR radiation. Different bands were obtained due to the shifts in the infrared spectra. The IR spectrum of the seed oil showed a series of bands with different intensities. The wavenumber was observed within the region of 3008.0to 715.6 cm$^{-1}$.

The interval of 3100 to 2800 cm$^{-1}$ from the IR spectrum shows the absorption bands in the vicinity of frequencies 3008.0, 2918.5 and 2847.7 cm$^{-1}$. These absorptions are characteristic of symmetrical and asymmetrical vibrations (C-H) of the $CH_3$ and $CH_2$ aliphatic groups from the alkyl of the triglycerides, which are found in large quantities in vegetable oils. The first being 3008 cm$^{-1}$ indicating C-H of terminal alkene (C-H). Studies have shown that absorption near 3010 cm$^{-1}$represents a significant index of the degree of unsaturated oils that is characteristic of vegetable oils, richness in lionoleic acid, and could be used for authenticity purposes (Alexa, Dragomirescu, Pop, Jianu& Dragos 2009; Gomez et al., 2011). At peak 1581.1 cm$^{-1}$, the presence of an amine group (N-H) is indicated. The peak at 1461.1 cm$^{-1}$ shows the presence of a methylene group similar to those of rapseed, corn, olive and peanut oils (Shi, Liu, Li & Qin, 2017). The vibration at 1745 cm$^{-1}$ is characteristic of carbonyl group (C=O) in esters. Saturated esters bands (C-C(=O)-O) usually occur between 1230-1160 cm$^{-1}$, and in this study the vibration occured at 1162 cm$^{-1}$. This vibration has been linked with the presence of palmitic acid. The vibrations below 1162 cm$^{-1}$ reveal the presence of unsaturated esters (Kachela, Matwijczuk, Przywara, Kraszkiewicza&Koszela, 2018) Oil samples bearing similar IR characteristics with the vegetable oil under investigation have been used as dielectric coolant in distribution transformers, and also in the production of biodiesel (Gomez et al., 2011; Georgieva, Petkov, Todorov &Denev, 2018).

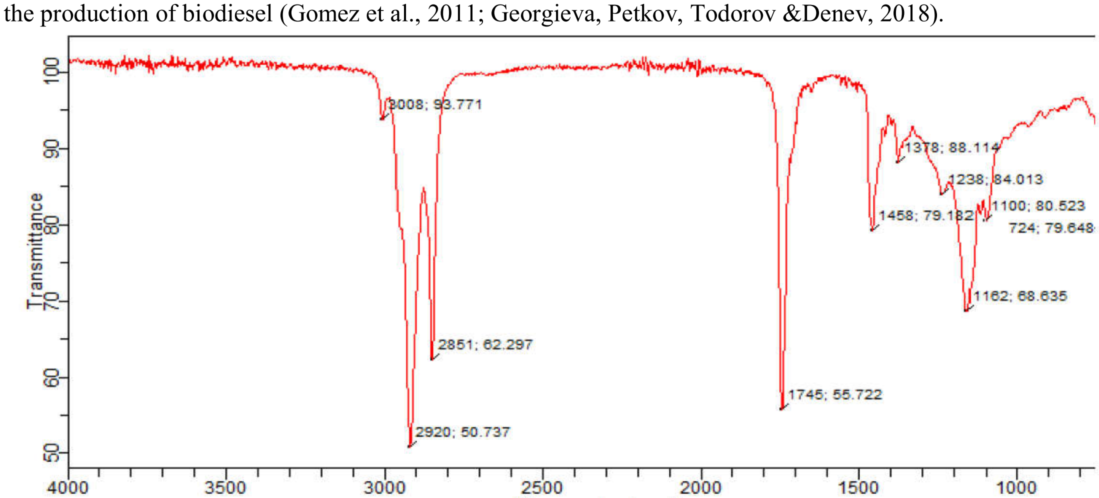


Fig. 2: An IR spectra of the oil of *P. macrophylla*

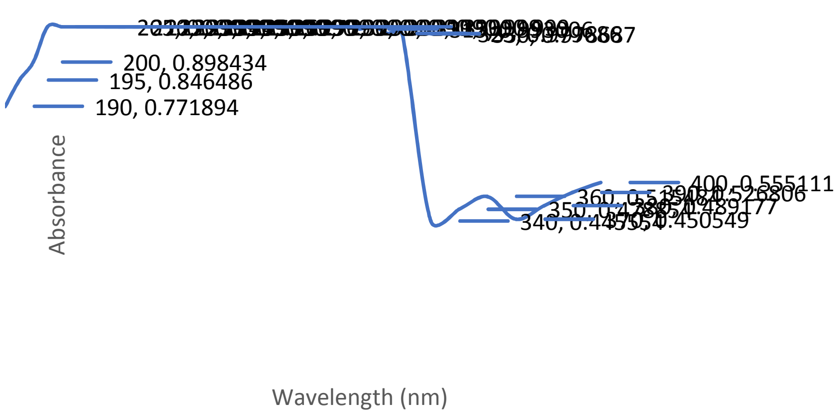


Fig. 3: UV spectral profile of the oil of *P. macrophylla*

The oil of *P. macrophylla* showed high absorption values at 190 and 390 nm, and these values are in the UV region. Oils with absorption in this region (190-390 nm) are known to shield against UV.-A and UV-B radiations that cause skin damage since UV-C is known to be completely absorbed by the atmosphere, the oil, therefore, could be useful in the cosmetic industry (Kumar &Viswanathan, 2013; Xu, Zhang, Xing, Mothibe& Zhu, 2013). Chicken oil used as skin softener in some areas of India are reported to have absorption in the region (Kumar & Viswanathan, 2013) consistent with that of *P. macrophylla* oil. UV absorption profile, like IR spectra is useful in the monitoring of oils during cooking and processing at production facilities, and more generally within the food industry (Dinovitser, Valchev& Abbott 2017). The aforementioned processes affect the fatty acids content of oils, and could therefore be used to monitor the changes that accompany each processing method.

The antimicrobial property of *P. macrophylla* measured on basis of the zones of inhibition had activity against only *A. flavus*, a fungus at 20 mm; and did not show any activity against the pathogenic bacterial isolates (*S. aureus*, *S.mutans, S. typhimurium* and *E. coli;*) and the remaining fungi (*A. flavus, C. albican* and *R. oligosporus*). The observations from this study are however in contrast to the report of Ugbogu and Akukwe (2008) that *P. macrophylla* oil inhibited the growth clinical isolates including *E. coli, S. aureus* and *S. epidermidis*.

## CONCLUSIONS

The oil of *P. macrophylla* seed from its UV spectra data will be good to shield against dangerous ultraviolet radiation thus making it appropriate for use in formulating sun-screening pomades. The oil according to the IR spectra showed a significant index of unsaturated fatty acids that protect against cardio-vascular related diseases. Its activity against *A. flavus* makes a good candidate in the formulation of *A. flavus* creams and medications. The commercial production of the oil of *P. macrophylla* is recommended as an income earner for the peasants, and this will ultimately safe the country its hard earned foreign exchange spent in the importation vegetable oils. Finally, commercial cultivation and exploitation of *P. macrophylla* for its oil will expand the Nation's production base and provide jobs for the youths.

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