INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

*ISSN 2278 – 0211 (Online)*

# GC And FTIR Studies Of Hexane and Ethanol Extracts of Calophyllum Inophyllum, Nigeria

**David. O. Adekunle**
Assistant Lecturer, Department of Chemistry and Industrial Chemistry,
Bowen University Iwo, Nigeria
**Anointing O. Olanrewaju**
Student, Department of Chemistry and Industrial Chemistry,
Bowen University Iwo, Nigeria
**Adeshina I. Odugbemi**
Assistant Lecturer, Department of Biochemistry, Bowen University Iwo, Nigeria

***Abstract:***
*This study investigates the phytochemical constituents in ethanol and hexane seed extract of Calophyllum inophyllum. The seed extracts contains saponins, tannins, glycosides, flavonoids, and steroids and absence of alkaloids and anthraquinones.*
*The FTIR study shows various absorptions in the two extracts of Callophylum inophyllum. A strong absorption at 3265.3 $cm^{-1}$ corresponds to O-H stretch which was observed in the ethanol extract only. C=O stretch was observed in 1742.5 $cm^{-1}$ and 1701.5 $cm^{-1}$ in both extracts. Other strong absorptions were observed at 2853.3 $cm^{-1}$, 2929.7 $cm^{-1}$, 2922.2$cm^{-1}$, 2976.3 $cm^{-1}$, 1578.5 $cm^{-1}$, 1459.3 $cm^{-1}$, 1347.4 $cm^{-1}$, 1235.6 $cm^{-1}$ and 1312.0 $cm^{-1}$ in both extracts. 1407.1$cm^{-1}$, 1449.9$cm^{-1}$ and 1116.3$cm^{-1}$ in the ethanol extract only. 3006.1 $cm^{-1}$, 1654.9$cm^{-1}$, 1625.1 $cm^{-1}$, and1377.3 $cm^{-1}$ in hexane extract only. 1079.1 $cm^{-1}$, 1097.7 $cm^{-1}$,1118.2 $cm^{-1}$ and 1159.2 $cm^{-1}$ in both extracts. Other absorptions observed are 1039.9 $cm^{-1}$,855.2 $cm^{-1}$, 721.2 $cm^{-1}$, 928.1 $cm^{-1}$, 885.2 $cm^{-1}$, and 697.0 $cm^{-1}$.*
*Gas Chromatography analysis identified the presence of eight fatty acids in Calophyllum inophyllum seed extract namely: Lauric (3.19%), Pentadecanoic (0.3%), Stearic (8.03%), Oleic (17.63%), Linoleic (16.67%), Arachnidic (0.2%), Behenic (0.05%), Lignoceric (0.04%) and absence of Tridecanoic, Myristic, Palmitic, Tricosnoic.*
*This study concludes that the seed extracts of Calophylluminophyllum contain large amounts of phytochemicals and beneficial fatty acids with a possibility of wide range application taking advantage of the biological activities such as antimicrobial, antiviral, cytotoxic, analgesic and against other disorders in favor of public health.*

***Keywords:*** *Fatty acids, FTIR, GC, linoleic acid, oleic acid*

## 1. Introduction/Background

Calophyllum innophyllum is an evergreen tree (also known as “Alexandrian Laurel”) widely dispersed throughout the tropics and popular for its timber and seed oil products. The plant tolerates harsh environmental conditions of acidity, salinity, drought and a wide temperature range, requiring little maintenance (Friday and Okano, 2006; Hathurusingha and Ashwath 2007). Among its many economic uses is the high commercial demand for Calophyllum innophyllum seed oil from pharmaceutical industry.

The genus Calophyllum is a large group of tropical trees of up to 200 different species (Oliver-Bever, 1986) but Calophyllum innophyllum is the most abundant and has a rich reserve of secondary metabolites like triterpenes and steroids, benzopyrans, xanthones, coumarins and flavonoids (Govindachari et al., 1967; Sartori et al., 1999; Ito et al., 2003; Ma et al., 2004). Due to its rich phytochemicals, studies have shown that Calophyllum innophyllum has medicinal properties ranging from antimalaria (Hay et al., 2004), antibacterial (Yimdjo et al., 2004), to anticancer (Itoigawa et al., 2001). The leaves are used in treating migraine, dermatosis, urticaria (hives) and eczema (Prabakaran and Britto., 2012). The oil extracted from the seeds is claimed to be analgesic and is used for the treatment of leprous neuritis, rheumatism and gout. The oil also has the ability to accelerate wound healing and render the skin healthy (Akter et al., 2017). Moreover, there are reports of isolated xanthones and coumarins having anti-HIV-1 properties (Laure et al., 2008; Patil et al., 1993), enabled through inhibition of HIV-1 reverse transcriptase (McKee et al., 1998; Sekino et al., 2004). Furthermore, the rising demand for renewable energy makes Calophyllum innophyllum a target for potential biodiesel product because of its oil content.

For its diversity, the kernel of Calophyllum innophyllum is rich in lipids. Five kilograms of oil may be extracted from a hundred kilograms of nuts (Friday and Okano, 2006). It has been reported that fatty acid composition may influence the quality of the resultant biodiesel (Ramos *et al.*, 2009). In addition, the presence unsaturated fatty acids like

omega-3 and omega-6 may give credence to anti-inflammatory properties of Calophyllum innophyllum (Nowak, 2010). Calophyllum inophyllum is noted to have healing properties. The natives in New Caledonia and in Samoa utilize these leaves for treating skin inflammations, leg ulcers and wounds (Quisumbing., 1951).Calophyllum inophyllum has been used for treatment of various diseases such as eye diseases, rheumatism, and inflammation. The leaves are used in migraine, dermatosis, urticaria (hives) and eczema and are more affordable than purchasing modern pharmaceuticals (Prabakaran and Britto., 2012). This study gives insights to the medicinal uses, functional groups and phytochemical constituents of seed extracts of Calophyllum inophyllum from Nigeria.

Interestingly, neither the fatty acid composition nor the phytochemical properties of the Nigerian species of Calophyllum innophyllum has been reported.

## 2. Materials and Methods

*2.1. Plant Collection, Preparation and Extraction*

The matured seeds of Calophyllum inophyllum (Balltree) were obtained from Ademola Ishola hall, Bowen University, Iwo, Osun state, Nigeria, during the month of March, 2018. The seeds obtained were cleaned, deshelled, pulverized and stored in dark airtight container. Approximately 200g of Calophyllum inophyllum seeds were collected and blended. This is to ensure powdered plant materials have maximum surface area contact with solvents for an efficient extraction.

The powdered seed was soaked in hexane for 24 hours at room temperature. It was noted that plant extracts should not be stored in solvent for long periods, because this will increase the risk of decomposition of extract constituents. After 24 hours, the mixture was concentrated; the extract was soaked in hexane for another 24 hours and weighed. The hexane crude extract was filtered and solvent was evaporated under reduced pressure via a rotary evaporator. The steps were repeated using Ethanol as a solvent.

## 3. Chemical Analysis

*3.1. Gas Chromatography (GC)*

Gas Chromatography (GC) of the samples were recorded at the Central laboratory Bowen University, Iwo using a 7890B Aligent Technologies Gas Chromatgraphy.

*3.2. FTIR Studies*

Fourier Transform Infrared spectra (FTIR) of the samples were recorded at Bowen University, Iwo, Central laboratory using a computerized Cary 630 spectrometer, Agilent technologies in the range of 4000-650/cm.

*3.3. Phytochemical Investigation of Hexane and Ethanol Extract of the Seeds of C.Inophyllum*

Phytochemical tests were studied on the extracts using standard procedure described by Sofowora, Trease and Evans and Harbone in 1996,1989 and 1973 respectively.

*3.4. Qualitative Analysis on Phytochemical Constituents*

- Test for Alkaloids: From each extracts, one (1) ml was measured and was added to 2ml of dilute $H_2SO_4$, it was then filtered. 1ml of Meyer's reagent was added. A white or creamy precipitate indicates the presence of alkaloids which was absent.
- Test for Saponins: 1ml of each extracts was added to 2ml of distilled water and it was vigorously shaken for 15 minutes. Formation of 1cm foam confirmed the presence of saponins.
- Test for tannins: 1ml of each extracts was placed in a test tube and few drops of 5% ferric chloride solution ($FeCl_3$) was added and observed for bluish-black coloration, which showed the presence of tannins.
- Test for Glycosides: 1ml of extracts was added to 3ml of chloroform ($CHCl_3$) and 2ml of 10% ammonia ($NH_3$) was also added. A pink coloration represented the presence of Glycosides.
- Test for Anthraquinone: 1ml of each extract was added to 5ml of $CHCl_3$ and vigorously agitated for 5 minutes, it was then filtered. 2ml of 10% ammonia solution was added to the filtered solution and the mixture was shaken. A bright pink color proves the presence of Anthraquinone which was absent.

## 4. Results

Qualitative analysis carried out for the hexane and ethanol extract of the *C. inophyllum* seeds is summarized in the table below.

| Phytochemicals | Hexane | Ethanol |
|---|---|---|
| Alkaloids | _ | _ |
| Saponins | + | + |
| Tannins | + | + |
| Glycosides | _ | + |
| Anthraquinone | _ | _ |
| Flavonoids | + | _ |
| Steroids | + | + |

*Table 1: Results of the Qualitative Analysis on Phytochemical Constituents*
*Key: +: Present --: Absent*

The FTIR results of the hexane and ethanol extracts showing the peak values, bond stretch and functional groups.

| Hexane | | | Ethanol | | |
|---|---|---|---|---|---|
| **Peak Values (Cm-1)** | **Bond Stretch** | **Functional Groups** | **Peak Values (Cm-1)** | **Bond Stretch** | **Functional Groups** |
| 3006.1 | =C-H stretch | Alkene | 3265.3 | O-H | Phenol |
| 2853.3 | C-H stretch | Alkane | 2929.7 | C-H stretch | Alkane |
| 2922.2 | C-H Stretch | Alkane | 2976.3 | C-H stretch | Alkane |
| 1742.5 | C=O stretch | Carbonyl | 1701.5 | C=O stretch | Carbonyl |
| 1654.9 | C=C stretch | Alkene | 1578.5 | C=C stretch | Aromatic |
| 1625.1 | C=C stretch | Alkene | 1407.1 | C-H bending | Alkane |
| 1347.4 | C-N stretch | Amine | 1449.9 | C-H bending | Alkane |
| 1235.6 | C-N stretch | Amine | 1312.0 | C-N stretch | Amine |
| 1459.3 | C=C Stretch | Aromatic | 1039.9 | C-F | Alkyl halide |
| 855.2 | =CH bending | Alkene | 1079.1 | C-O Stretch | Ether |
| 1377.3 | N-O stretch | Nitro=, | 1116.3 | C-H bending | Alkane |
| 721.2 | =C-H stretch | Alkene | 928.1 | =C-H stretch | Alkene |
| 1097.7 | C-O stretch | Ether | 885.2 | =C-H Stretch | Alkene |
| 1118.2 | C-O stretch | Ether | | | |
| 697.0 | C-Cl Stretch | Alkyl halide | | | |
| 1159.2 | C-O stretch | Ether | | | |

*Table 2: FTIR Result of Hexane and Ethanol Seeds Extract of C. Inophyllum*

Results of the GC analysis showing the peak area and the percentage composition of the various fatty acids pressent in the hexane extract.

| Fatty acids | Peak Area 0.0313% Standard | Extract + Hexane | |
|---|---|---|---|
| | | P/A | % |
| Lauric | 4.63 | 47.26 | 3.19 |
| Tridecanoic | 12.60 | - | - |
| Myristic | 186.27 | - | - |
| Pentadecanoic | 3.82 | 3.21 | 0.3 |
| Palmitic | 130.82 | - | - |
| Stearic | 66.69 | 1710.45 | 8.03 |
| Oleic | 66.93 | 3770.57 | 17.63 |
| Linoleic | 81.16 | 4323.79 | 16.67 |
| Arachnidic | 198.84 | 112.28 | 0.2 |
| Behenic | 209.34 | 32.74 | 0.05 |
| Tricosnoic | 219.63 | - | - |
| Lignoceric | 229.82 | 31.23 | 0.04 |

*Table 3: GC Result of Hexane Seeds Extract of C. Inophyllum*

**5. Discussion**

Phytochemistry, generally termed "plant chemistry", is a study of chemical constituents that are naturally occurring in plants. The term secondary metabolites are referring to the phytochemicals that are synthesized by specialized plants cells in a trace quantity via secondary metabolism pathway. Each plant family, genus and species produces characteristic phytochemicals. Secondary metabolites have no obvious function in a plant's primary metabolism as well as in photosynthesis and reproduction. They may possess an ecological role, as pollination attractants, represent chemical adaptions to environmental stresses, or coordinate the development of the whole plant defense mechanisms. For instance, plants produce toxic phytoalexins to kill pathogenic microbes and insects (Monika *et.al*., 2008). A wide variety of active phytochemicals have been identified and found to possess a broad range of biological activities such as antimicrobial, antiviral, antioxidative, anti-malarial and antitumor properties (Su *et al.,* 2008). Table 1 shows the results of the phytochemical screening of the plant seeds which revealed the presence of saponins, tannins, glycosides, flavonoids and steroids and the absence alkaloids and anthraquinones.

Characteristics of saponins includes formation of foams in aqueous solution, cholesterol binding properties (Sodipo and Akiniyi., 2000).

Tannins have antibacterial and antihelminthic properties (Hedberg *et al*., 1983). Tannins are reported to have physiological effects such as anti-irritant, antisecretolytic, antimicrobial and antiparasitic effects. Plants that contains tannins are used to treat diarrhea, inflammations of mouth and throat and slightly injured skins (Westendarp., 2006).

Flavonoid indicates naturally occurring phenolic compounds, with beneficial effects such as antioxidant and neutralizing free radicals in the human diet. Flavonoids have the ability to react with extracellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria (Cowan., 2002).

Steroids are antioxidants and they are active against bacterial activity (Okwu., 2001).

Glycosides will mostly remain inactive until they are hydrolysed to release an aglycone (phenols, terpenes, steroids and quinines) that has the active effect.

**6. Fourer Transform Infrared Sprectroscopy**

The FTIR results of the hexane and ethanol extracts showing the peak values, bond stretch and functional groupsis shown in Table 2. Various absorptions were observed in the two extracts of *Callophylum inophyllum*. The strong absorption at 3265.3 $cm^{-1}$ corresponds to O-H stretch which was observed in the ethanol extract only. C=O stretch was observed in 1742.5 $cm^{-1}$ and 1701.5 $cm^{-1}$ in both extracts. The absorption at 1377.3 $cm^{-1}$ corresponds to N-O stretch which was observed in the hexane extract only =C-H of alkenes was observed in 3006.1 $cm^{-1}$ in hexane extract only. The strong absorption at 2853.3 $cm^{-1}$, 2929.7 $cm^{-1}$, 2922.2$cm^{-1}$ and 2976.3 $cm^{-1}$ corresponds to the C-H stretch of alkanes found in both extracts. C-H bending was observed in 1407.1 $cm^{-1}$, 1449.9 $cm^{-1}$ and 1116.3 $cm^{-1}$ in the ethanol extract only. C=C stretch of aromatics was observed in1578.5 $cm^{-1}$ and 1459.3 $cm^{-1}$ in both extracts. C=C stretch of alkenes were observed in 1654.9 $cm^{-1}$ and 1625.1 $cm^{-1}$ in hexane extract only. C-N stretch of amines was observed in 1347.4 $cm^{-1}$, 1235.6 $cm^{-1}$ and 1312.0 $cm^{-1}$ in both extracts. C-O stretch of ether was observed in 1079.1 $cm^{-1}$, 1097.7 $cm^{-1}$,1118.2 $cm^{-1}$ and 1159.2 $cm^{-1}$ in both extracts. =CH bending of alkenes was observed in 855.2 $cm^{-1}$ in hexane extract only. =CH stretch of alkenes was observed in 721.2 $cm^{-1}$, 928.1 $cm^{-1}$, 885.2 $cm^{-1}$ in both extracts. CF and C-Cl stretch of alkyl halides were observed in 10399 $cm^{-1}$ and 697.0 $cm^{-1}$ respectively in both extracts.

**7. Fatty Acids**

From the GC analysis of the hexane extract in Table 3 the results of the GC analysis showing the peak area and the percentage composition of the 12 fatty acids identified in varying proportionsare described below:

Lauric acid (3%): It is a 12 carbon antimicrobial fatty acid which has the ability to kill harmful pathogens like bacteria (Nakatsuji *et al*., 2009).

Pentadecanoic acid (0.3%): It is a 15 carbon fatty acid that is rare in nature (Rolf, 2002).

Stearic acid (8.03%): It is an 18 carbon fatty acid that has found many applications ranging from its use in cosmetic products, as food additives, in soaps and detergents, lubricants. It has the ability to react with some metals and form products of great values such as reaction with calcium to for calcium stearate, which functions as a release agent, with zinc to form a lubricant known as zinc stearatje (Tsenga *et al*., 1999).

Oleic acid (17.63%): It is an 18 carbon atom that is present in the fat of all foods and is also produced from essential fatty acids in normal human liver cells and fat cells. It has the highest percentage of fatty acids found in *Calophyllum inophyllum* hexane extract. It reduces blood pressure, increases fat burning to help with weight loss, protects cells from free radical damage, may also prevent type 2 diabetes (Vassilious *et al*., 2009).

Linoleic acid (16.67%): Linoleic acid is an essential fatty acid, a carboxylic acid, a polyunsaturated omega-6 fatty acid whose low levels indicate dietary insufficiency, which can lead to a variety of symptoms such as Eczema-like skin eruptions, loss of hair (Skolinik *et al*., 1977) liver degradation, behavioural disturbances, kidney degeneration, increased thirst, frequent infections, poor wound healing, sterility, miscarriage, arthralgia, cardiovascular disease, growth retardation. (Skolinik *et al*., 1977, Emily L.G., and Rajani K., 2017). It has a physiological role in maintaining the water permeability barrier of the skin as a constituent of acylglycosyl ceramides, it also gives rise to arachidonic acid, which is a major precursor of a series of bioactive metabolites called eicosanoids, which regulate a lot of physiological processes (Thomas, 2016).

Arachidonic acid (0.2%): Arachidonic acid is not an essential fatty aid. It only becomes relevant if there is inability to convert linoleic acid to arachidonic acid (Rivers *et al*., 1975).

Behenic acid (0.05%): is a saturated fatty used for hair conditioners and moisturizers
Lignoceric acid or tetracosanoic acid (0.04%) is a 24 carbon fatty acid.

## 8. Conclusion

This study shows that *Calophylluminophyllum* hexane and ethanol seed extracts contain large amounts of phytochemicals with a possibility of wide range of biological activities such as antimicrobial, antiviral, cytotoxic, analgesic and against other disorders in favor of public health.

Gas Chromatography test carried out to identify the presence of fatty acids in *Calophyllum inophyllum* hexane seed extract discovered eight (8) fatty acids present namely: Lauric, Pentadecanoic, Stearic, Oleic, Linoleic, Arachnidic, Behenic, Lignoceric and absence of Tridecanoic, Myristic, Palmitic, Tricosnoic

It is recommended that further studies should be carried out using different solvents and other species in the *Clusisceae* family so as to obtain new and better results which may further be used in the production of drugs for diseases whose cure are yet to be found also aside the application in the production of biodiesel other applications especially in the production of cosmetics should also be researched into.

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