ORIGINAL RESEARCH

# A comparison of antioxidant and Fourier Transform Infrared Spectroscopy (FTIR) analysis on extracts of *Syzygium guineenses* (Myrtaceae)

**Shonekan OO*[1], Otuka AC[1], Adeyemi DK[1] and Fatunsin OT[2]**

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Idi-araba campus, Lagos, Nigeria [2]Department of Chemistry, Faculty of Science, University of Lagos, Akoka, Nigeria

**Address for correspondence:**
Dr. (Mrs.) O.O. Shonekan
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Lagos
E-mail: oshonekan@unilag.edu.ng

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## ABSTRACT

**Background:** *Syzygium guineenses*, (the most common and abundant *specie i*n Nigeria) is a medicinal plant used by traditional practitioners in northern Nigeria for a variety of healing purposes.

**Objective:** The main objective of this project was to carry out a comparison of antioxidant activities and Fourier Transform Infrared Spectrophotometric (FTIR) analysis on both methanol and hexane leaf extracts of *S. guineenses*.

**Methods:** Phytochemical screening, Semi-quantitative DPPH (1,1-diphenyl-2-picrylhydrazyl)- dot blot assay and FTIR analysis were performed on both extracts to determine antioxidant activity and identify the functional groups present.

**Results:** Phytochemicals tested for, were observed to be more prominent in the methanol extract than hexane. The *in vitro* antioxidant assay also revealed a more intense yellow colour of inhibition in methanol extract than the hexane extract. The FTIR spectra revealed different characteristic peak values with various functional compounds in both extracts. The methanol extract displayed major peaks of absorption at 3341 $cm^{-1}$ (-OH) for alcohol, 1736 $cm^{-1}$ (C=O) carbonyl group, 1161.83 $cm^{-1}$, 1036.49 $cm^{-1}$ (C-O) of esters. Other absorption bands like 1452.25 $cm^{-1}$ and 1612.20 $cm^{-1}$ for alkenes were present in both extracts.

**Conclusion:** This result shows that the methanol extract of *S. guineenses* has a higher potential of phytochemicals, antioxidants and functional groups than the hexane extract.

**Keywords:** FTIR, *S. guineenses*, DPPH-Dot-blot assay, phytochemicals

## INTRODUCTION

Medicinal plant research has succeeded in creating an insatiable yearning of discovery for lead compounds of health benefit to humans. According to World Health Organisation (WHO)[1] around 21,000 plant species have the potential for being used as medicinal plants. Available WHO data states that over three-quarters of the worlds' population rely mainly on plants and their extracts for their health care needs[2]. Treatment with medicinal plants is considered very safe as there are minimal or no side effects. Identification of phytochemical compounds such as flavonoids, saponins etc which are known to be responsible for antioxidant, anticancer and antibacterial activities etc present in these medicinal plants provide information on the different functional groups they possess[3].

*Syzygium guineense*, (Family Myrtaceae) is a medicinal plant used to treat intestinal parasites infection in children. It is called 'Malmo' (in Hausa language), while in Western Nigeria it is called 'Adere' (Yoruba language). It usually grows in moist conditions, sometimes in water, along streams and even on rocky ground in high rainfall savannah. The leaves and bark of *S. guineenses* are locally used in the treatment of tuberculosis and cough while extracts of the stem, bark, leaves and seeds have shown antibacterial and antifungal activity[4]. The folk medical practitioners rely on us, scientists, to investigate the basis of their claim, in the traditional use of these plants.

The fruits are usually purple in colour and edible when mature with a single rounded seed. It is used in the preparation of beverage, vinegar and added to spirits for flavouring. The bark is used for tanning, dyeing and sometimes used to harden lateritic floors or to glaze pottery while the flowers are a source of nectar for honey bees. This investigation is to compare the phytochemicals present in hexane and methanol extracts of *S. guineenses* collected from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state and determine their functional groups using FTIR spectra analysis.

## METHODS

***Collection:*** Fresh leaves of *Syzygium guineenses* were collected from the garden of (International Institute of Tropical Agriculture) IITA Ibadan, Oyo state with the help of botanists (Ms. D. Bown and Mr O. Olukunle). A sample of the leaf was deposited at the herbarium in University of Lagos, Akoka and a voucher number was given LUH7238.

***Extraction:*** Fresh leaves (3.0kg) of *Syzygium guineenses* were dried under shade for two weeks and pulverized into powder form with a local mill. The dried powdered leaves (1kg) of *Syzygium guineenses* were extracted with hexane and methanol solvents (5L) respectively for 3 days. The extracts were filtered separately using Whatman filter paper every 24 hours. The methanol filtrates were concentrated at 40°C under reduced pressure with Rotar Vapor B-480 (BUCHI) while hexane extract was allowed to evaporate in the fume cupboard. Dried methanol (47.18g) and hexane (28g) extracts were kept in the refrigerator prior to phytochemical screening and DPPH Dot blot assay.

### *Phytochemical Screening*

The phytochemical analysis of the extracts was carried out using a standard method from Trease and Evans 2002[5]. The screening method of analysis for alkaloids, saponins, reducing sugar, tannins, flavonoids, cardiac glycosides and proteins were employed to identify the constituents in each extract of the plant.

### *Antioxidant assay*

The qualitative antioxidant analysis was determined using Semi-quantitative 1, 1-diphenyl-2-picrylhydrazyl DPPH Dot Blot Assay. 50ml of 0.2% DPPH solution in methanol was freshly prepared and kept in the refrigerator for use. Both extracts were subjected to DPPH Dot blot analysis using Vitamin C as standard according to the modified method of Chang 2007[6].

*Semi-quantitative DPPH-Dot Blot Assay*

This method is also called the Dot blot assay. The dot blot method described by Chang 2007[6] was employed. A grid with 1.0cm$^2$ spacing was lightly drawn in pencil on a pre-coated TLC sheet. The grid of TLC sheet was labeled with name of extract and control on the horizontal axis and concentration of spot by extract and control on the vertical axis. Each of the two extracts and control were spotted into the boxes of the TLC plate in labeled amount (2, 4, 6 and 8 drops). The concentration and volume of extract spotted was deliberately kept the same so that all the spots were of the same surface area for fair comparison of any colour changes. After spotting, with various concentrations of the extract and control (Vitamin C) on a TLC plate, the spots were air-dried then sprayed

with 0.2% DPPH solution and kept in the dark for 2 hours. The appearance and intensity of yellow spots was used to determine the extract with the highest antioxidant activity. Photographs were taken and shown in the results (Figure 1).

***Fourier Transform Infrared Spectrophotometry (FTIR)***

The hexane and methanol extracts of *S. guineenses* were analyzed using FTIR Alpha Bruker Model at a range of 3500cm$^{-1}$ to 500cm$^{-1}$ in the Department of Chemistry, University of Lagos; about 0.2g of the air-dried sample was dissolved in hexane and methanol in a 10ml volumetric flask respectively. 2 drops of each extract were applied on the ATR-diamond surface,

## RESULTS

***Phytochemical Screening***

The results of the phytochemical screening of *S. guineenses* extracts are given below in Table 1. The table reveals the presence of saponins, flavonoids and tannins only in the methanol extract.

**Table 1:** Phytochemical analysis on methanol and hexane extracts of *S. guineenses*.

| S/N | PHYTO-CHEMICAL | METHANOL EXTRACT | HEXANE EXTRACT |
|---|---|---|---|
| 1. | Alkaloids | + | + |
| 2. | Flavonoids | + | - |
| 3 | Saponins | ++ | - |
| 4 | Reducing sugars | + | + |
| 5 | Cardiac glycosides | + | + |
| 6 | Proteins | - | - |
| 7 | Tannins | ++ | - |

**Key: ++ Highly Present; + Present; - Absent**

***DPPH Dot Blot Assay***

The appearance of a white colour spot against purple background after spraying the TLC plate with DPPH reagent is of much screening value for the evaluation of antioxidant potential of the extracts. The intensity of white colour for ME (methanol extract) was more visible than HE (hexane extract) in Figure 1 below.

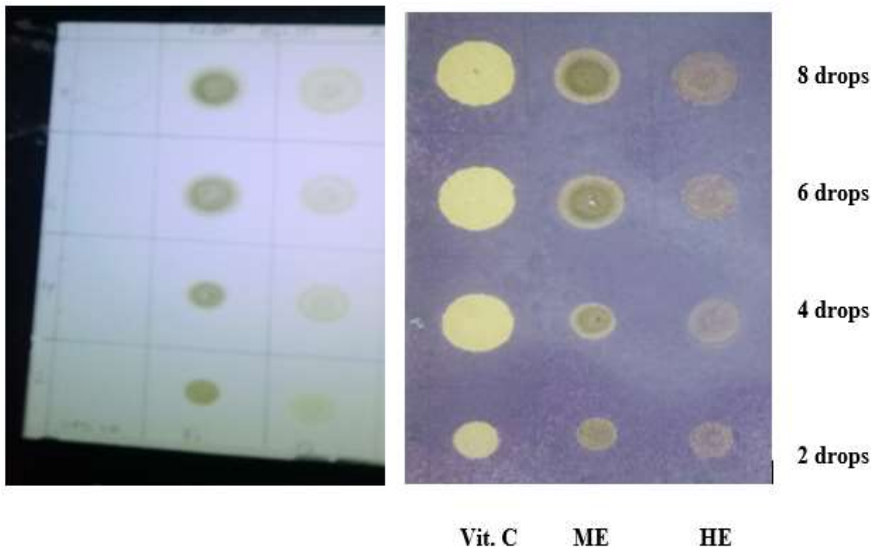


**Figure 1 :** DPPH Dot blot assay: spotting of Ascorbic acid (Vit. C STD), Methanol extract and Hexane extract sprayed with DPPH (before and after spraying) .

This research work revealed the different components present in both extracts of *S. guineenses.* and their functional groups (like alcohols, esters etc). The data on the peak values and the functional groups (obtained by FTIR analysis) present in both extracts of *S. guineenses* are presented in Table 2 while the FTIR spectrum are shown in Figure 2.

**Table 2:** FTIR spectral data of methanol and hexane extracts of *S. guineenses*

| ***Syzygium guineenses* leaf extract** | **Peak values** | **Functional groups** |
|---|---|---|
| **Methanol extract** | 3362.77 | OH Group of alcohols |
| | 2922.93 | CH Alkane |
| | 2852.69 | CH Alkane |
| | 1736.53 | CO Carbonyl group |
| | 1710.70 | CO Carbonyl group of esters |
| | 1612.20 | CC Alkene |
| | 1452.25 | CH bending |
| | 1377.02 | CO stretching of alcohols |
| | 1242.22 | CO esters |
| | 1161.83 | CO esters |
| | 1036.49 | CO esters |
| **N-hexane extract** | 2918.77 | CH Stretching |
| | 2850.03 | CH Stretching |
| | 1736.23 | CO Carbonyl group |
| | 1710.92 | CO Carbonyl |
| | 1619.51 | CC Alkene |
| | 1461.56 | CH Bending |
| | 1452.43 | CH Bending |
| | 1377.02 | CH bending |
| | 1242.73 | CO Esters |
| | 1169.04 | CO Esters |
| | 1092.14 | |
| | 1006.92 | |

## DISCUSSION

Phytochemical analysis is a screening method carried out on plant extracts to identify the active metabolites present in the plants. These medically bioactive constituents are referred to as phytochemicals. They are found in all medicinal plants and play a major role in their uses. Phytochemicals are responsible for the physical, chemical and biological properties of the plant which they are found in. In this project, extraction was carried out using methanol (polar) solvent
which would easily extract the hydrophilic substances to be detected during analysis, while the n-hexane (non-polar) solvent would reveal the hydrophobic constituents. The phytochemical screening revealed the presence of alkaloids, reducing sugars and cardiac glycosides, higher in the methanol extract than the hexane extract. Flavonoids are known to elicit a wide range of therapeutic activities such as antihypertensive, antirheumatic agent, etc[6,7]. The absence of flavonoids in the hexane extract might limit the solvent choice of hexane in the extraction of active medicinal ingredients from *S. guineenses*. The results show that only reducing sugars, alkaloids and cardiac glycosides were present in both methanol and hexane extracts. Higher extraction of saponins and tannins was also observed in the methanol extract of *S. guineenses*. Saponins are glycosides with foam like characteristics, they have an aglycone unit, known to be heat stable and inhibit cholesterol absorption from the intestinal lumen in experimental animals[6]. Tannins are

polyphenolic compounds with anti-carcinogenic, anti-mutagenic and anti-oxidising properties. Tannins are known for stabilizing blood pressure and used in herbal tea as well as in wine preparation[8]. Hence, they are useful in pharmaceutical industries as a source for nutraceutical beverages etc.

The FTIR spectra of both methanol and hexane extracts of *S. guineenses* are shown in Figure 2 below The FTIR spectrum was used to identify the functional groups of the active components present in both extracts based on the peak values in the region of IR radiation. he results of FTIR analysis confirmed the presence of alcohol, ester, alkane and carbonyl compounds.

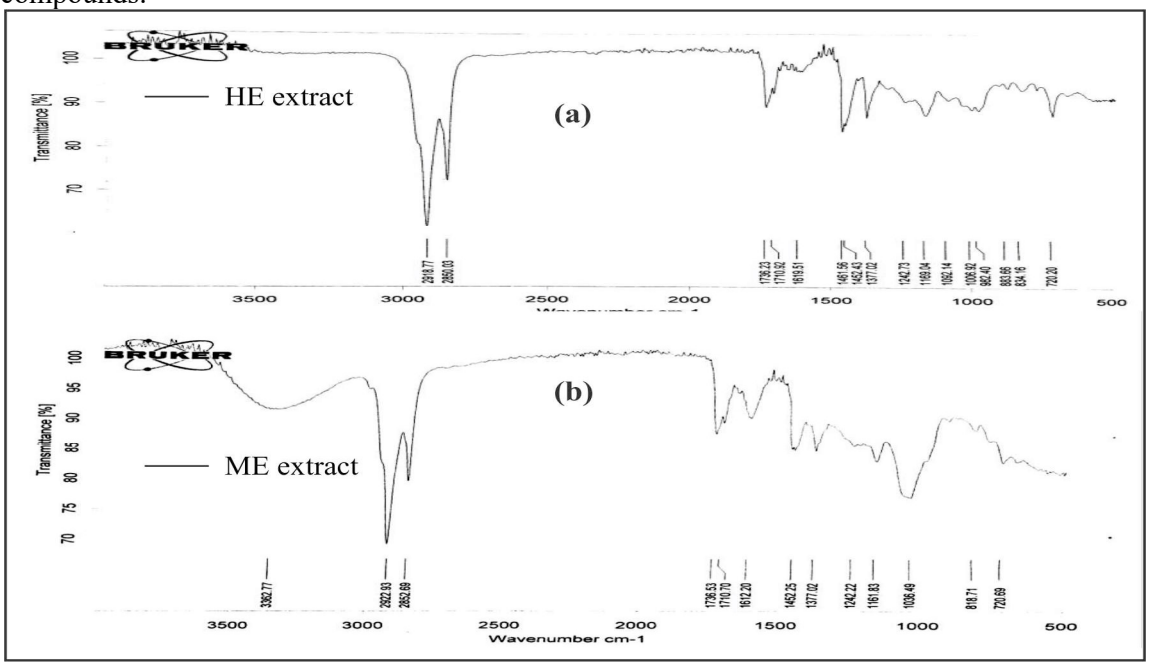


**Figure 2:** FTIR spectrum of (a) hexane extract, (b) methanol extract of *S. guineenses*

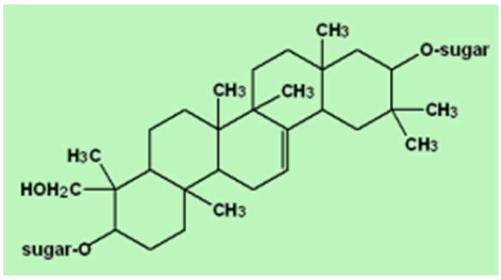


**Figure 3**: saponins

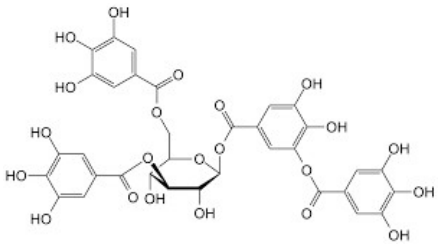


**Figure 4:** Tannins

The radical scavenging properties of methanol and hexane extracts of *S. guineenses* were determined using the DPPH dot blot method. Antioxidant molecules, which have the ability to scavenge free radicals, will change stable-purple coloured DPPH radical to a yellow colour non radical form.

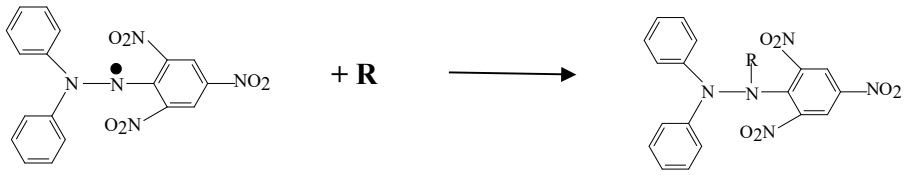


**Figure 5:** DPPH Radical

The colour change depicts the antioxidant potential of different extracts and indicates the strong antioxidant capacity of both extracts as compared to the positive control, Vitamin C. The methanol extract showed a more intense yellow colour than the hexane extract. Radical scavenging ability may help to explain the alleged beneficial health effects of *S. guineenses*, as reactive oxygen species (ROS) /reactive nitrogen species (RNS) or peroxide radical species add to the pathology of multiple human disorders [9]. Antioxidants serve as the defensive factor against free radicals in the body. It has been reported that the methanol extract of *S. guineenses* possesses antioxidant properties [10] and anti-venom properties [12].

Fourier Transform Infrared Spectroscopy (FTIR) is a high-resolution analytical tool used to identify the chemical functional groups in an organic molecule. The FTIR spectra of the methanol extract (ME) of *S. guineenses* showed characteristic bands for a hydroxyl group, O-H stretching vibrations of alcohol at 3362.77cm$^{-1}$ present in methanol extract of *S. guineenses* and absent in hexane extract (figure 2). Strong absorption bands at 1736 cm$^{-1}$ and 2922 cm$^{-1}$, 2918cm$^{-1}$ showed the presence of C=O stretching and C-H stretching in sp3 hybridized carbon, respectively in both extracts. On the other hand, medium absorption bands at 1036cm$^{-1}$, 1006cm$^{-1}$ indicates C-O stretching of an ester in both extracts. This may be due to the presence of phytochemicals like alkaloids, phenols, reducing sugars, tested for in table 1. This is in accordance with Visveshwari *et. al.* [11]. Others at 2922cm$^{-1}$, 2852cm$^{-1}$, 1710cm$^{-1}$, 1612cm$^{-1}$ , 1452cm$^{-1}$, indicate the presence of functional groups such as alkanes (C-H stretch), carboxylic acids (C=O stretch), alkenes (C=C stretch), aromatics (C-C stretch), carboxylic acids, esters, ethers (C-O stretch). The hexane extract (HE) of *S. guineenses* exhibited a characteristic band at 2850cm$^{-1}$ for C-H group, for carbonyl C=O group at 1736cm$^{-1}$,1710cm$^{-1}$ and C-O at 1169 -1006cm$^{-1}$. These may be the absorption peaks for monosaccharides, low molecular weight carbohydrates revealed in the presence of saponins from phytochemical screening.

## CONCLUSION

This study revealed the presence of various bioactive compounds in both extracts of (methanol and hexane) *Syzygium guineenses,* however alkaloids, reducing sugars and cardiac glycosides were highly present in the methanol extract than the hexane extract. FTIR spectrum also revealed a strong characteristic peak of –OH group (hydrogen bonding capacity) present in the methanol extract only while the antioxidant activity was found to be higher in the methanol extract than the hexane. The methanol extract of *S. guineenses* has a higher therapeutic potential than the hexane and as such, it is recommended as a plant of phytopharmaceutical importance

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