FATTY ACIDS, PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITIES EVALUATION OF *FICUS THONNINGII* SEED EXTRACTS

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Abstract: *Ficus thonningii* is highly neglected and underutilized plant. It is a traditionally important plant used in the treatment of various diseases such as malaria, dysentery, diabetes, diarrhea, eye problem as well as wound healing. Its fruits are eaten mostly by goats and when fallen, the fruits are discarded. The aim of this research was to extract the oils from *ficus thonningii* seeds obtained from Nasarawa State using n-hexane and ethanol and to investigate the antimicrobial activities, fatty acids profiles and phytochemicals present in these extracts. Phytochemicals screening revealed the presence of saponins, tannins, alkaloids, and flavonoids but no glycosides and steroids were detected. The seed extracts, especially the ethanol extract, possessed high antimicrobial activities. The results obtained for these microbial sensitivities showed that the seed possess great antibacterial potentials. The study has also shown that the *Ficus thonningii* seed contains oil rich in unsaturated fatty acids and biologically active phytochemicals which could have medicinal uses.
Keywords: *ficus thonningii*, seed oils, fatty acids, phytochemicals, antimicrobial, seed extracts

Introduction

In recent years, a great number of researches have been carried out to investigate the potential use of plants and plant materials as sources of phamaceuticals. Plant-based medicines are extensively used in ethnomedicinal and ethnoveterinary practices especially in rural areas in Africa and other developing regions. This is because they are highly abundant and have proven to be very effective in the treatment and management of diseases at far less cost than conventional medicines (Dangarembizi *et al*., 2013), proven to be very therapeutic with far less side effects than their synthetic counterparts and have been emphasized for use in prevention of bacterial and fungal growth in food production and storage (Hemalatha, 2013).

Seeds are sources of edible oils as well as oils of industrial and pharmaceutical importance (Nzikou *et al*., 2010). Oils are useful in cosmetics and medicine especially with the rise in the practice of aromatherapy (Ugoeze *et al.*, 2014). The composition, yield, physical and chemical characteristics of an oil determines its usefulness and its most suitable application (Aluyor and Ori-Jesu, 2008) and no oil from a single source can be suitable for all purposes (Mohammed and Jorf-Thomas, 2003)

Ficus thonningii is used in therapeutics in Southern and Eastern African rural communities (Viol *et al*., 2016). The powdered stem-bark is used in wound healing and aqueous decoction of its bark is used in West Africa to manage cough and treat throat infections (Ndukwe *et al*., 2007), as influenza remedy, lactation stimulant and diarrhea medicine while its leaves are used to treat malaria and yellow fever (Danthu *et al*., 2002). The therapeutic properties of this plant result from the phytoconstituents of its different parts. Its leaves contain terpenoids, alkaloids, cardiac glycosides, saponins, flavonoids, anthraquinones and phlobatannins and other secondary metabolites such as lignins, lignans and active proteins such as ficin, which add to their pharmacological and biological activity (Dangarembizi *et al*., 2014). Essential oils which are comprised mainly of 6, 10, 14 trimethyl-2-pentadecanone, phytol, acorenone and β-gurjunene have also been isolated from its leaves (Ogunwande *et al*., 2008). The antimicrobial activities of the root (Enideg, 2008), leaf and stem bark (Ndukwe *et al*., 2007) of *Ficus thonningii* have been investigated. This study is aimed at investigating the phytochemicals, antimicrobial activities and the fatty acid profiles of the n-hexane and ethanol extracts of the *Ficus thonningii* seed.

Materials and Methods

Sample Source and Preparation

Mature fruits of *Ficus thonningii* were harvested directly from the tree beside Central Primary School Nasarawa, Nasarawa State Nigeria. The fruits were partially dried, broken and the seeds were manually removed and then air-dried for two weeks before being milled using a domestic blender. Extraction of the oil from seed was done immediately using freshly distilled n-hexane at 60 °C for 8 h in a soxhlet apparatus and the solvent was separated from the oil by distillation and finally by evaporation using water bath at about 60 °C.

Phytochemicals Screening

The phytochemical screening was carried out as described by Dosumu *et al.* (2014).

Antimicrobial Tests

The antimicrobial susceptibility test and Minimum Inhibitory Concentration tests were carried out by using the methods of Akter *et al.* (2014) methods with slight modifications

Antimicrobial activity

The Disc diffusion method was used. The sample solutions were prepared and infused into the paper discs at concentration of 100 μg/disc. Then the paper discs were placed onto nutrient agar media which have been inoculated with the test bacteria and incubated at 37 ^{o}C for 24 h. The diameter of the zone of inhibition observed around each disc was measured and recorded. Streptomycin (30 μg/disc) (Oxoid, UK) was used as standard.

Minimum Inhibitory Concentration (MIC)

Determination of MIC was carried out using the broth dilution method. 50mg of each extract was weighed and dissolved in 10mL of ethanol to get concentrations of 5mg/mL. Concentrations of 2.5, 1.25, 0.625, 0.313, and 0.156 mg/mL of the oils were made by serial dilution. Culture of each of the organisms was put into each tube, thoroughly vortexed and incubated at 37 ^{o}C for 24 h and the growth of bacteria in form of turbidity was observed. The test tube with the lowest dilution with no turbidity was marked the MIC.

GC-MS Analysis of the Oils

The n-hexane extract was trans-esterified by refluxing 1 g of it with 12 g of methanol containing few drops of concentrated sulphuric acid in a 250 mL round bottom flask equipped with reflux condenser heated to a temperature of about 60 °C. The methanolic $H_2SO_4$was poured into the flask containing the heated oil and the entire mixture was stirred for 1 hr. The product was allowed to cool to room temperature, poured into a separating funnel to settle down and the methyl esters (upper) layer was separated and collected for GC-MS analysis while the ethanol extract was collected for GC-MS analysis without trans-esterification.

Results and Discussion

Phytochemical Screening

Phytochemical screening of the ethanol extract showed the presence of saponins, tannins, alkaloids and flavonoids. Steroids and glycosides were not detected

Table 1: Qualitative phytochemicals analysis

Phytochemicals	Presence
Saponins	+
Tannins	+
Alkaloids	+
Flavonoids	+
Steroids	-
Glycosides	-

\+ detected - not detected

Okoronkwo *et al.* (2014) reported the presence of saponins, tannins, alkaloids and flavonoids in *Ficus sycomorus* seed. Saponins in aqueous solutions produce foams. They also bind cholesterol and coagulate (Kim *et al.*, 2003). Flavonoids and other phenolics are antioxidants, prevent cardiovascular diseases conditions and prevent allergies (Hooper *et al.*, 2008; Amic *et al.*, 2003).

Antimicrobial Activity

Table 2: Antimicrobial sensitivity test results showing zone of inhibition (in mm)

Microrganisms	N-hexane Extract (100 µg/disc)	Ethanol Extract (100 µg/disc)	Streptomycin (30µg/disc)
Streptococcus aureus	3	18	20
Basillus subtilis	3	17	20
Escherichia coli	0	14	15
Pseudomonas aeruginosa	0	12	16
Salmonella typhi	8	22	19
Klebsiella pneumonae	7	20	17

The result for the antimicrobial sensitivity test of the n-hexane and ethanolic extracts compared to Streptomycin as a standard is represented in Table 2. For both of the extracts, the highest sensitivities were recorded against *Salmonella typhi* followed by *Klebsiella pneumoniae*.. Generally, the ethanolic extract is more active against microorganisms than the n-hexane and the activity is comparable to the standard drug.

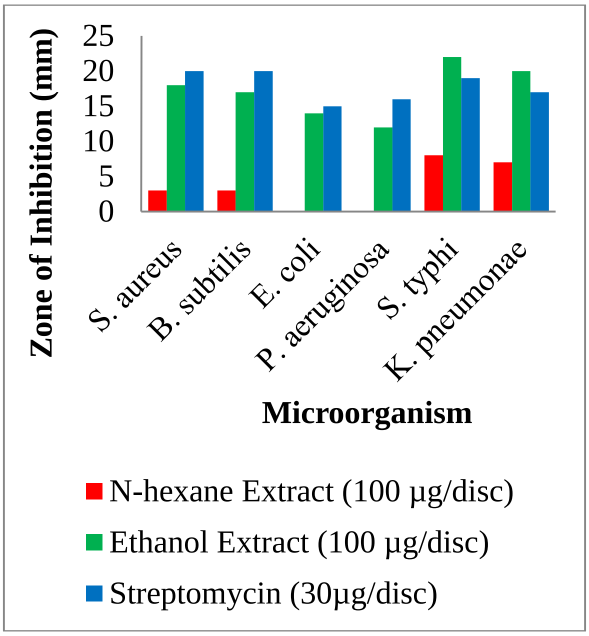

Fig 1: Antimicrobial activities of extracts compared to standard

The Minimum Inhibitory Concentration (MIC)

Table 3: MICs of the extracts

Microorganisms	N-hexane Extract MIC (mg/mL)	Ethanol Extract MIC (mg/mL)
Streptococcus aureus	50	0.625
Bacillus subtilis	50	0.625
Escherichia coli	50	0.625
Pseudomonas aeruginosa	50	0.625
Salmonella typhi	50	0.313
Klebsiella pneumonae	25	0.313

The minimum inhibitory concentration is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2001). The n-hexane extract which is mainly a mixture of fatty acids/oils showed low degree of activity compared to the ethanolic extract as shown in Table 3. At a concentration of 25 mg/mL the n-hexane extract of this seed can inhibit the growth of *Klebsiella pneumonae* among the organisms used. At 50 mg/mL, the n-hexane extract can kill all of the organisms. The ethanol extract which contains many other polar and non-polar compounds showed very high degree of activity especially against *Salmonella typhi* and *Klebsiella pneumonae.* It kills all the organisms at concentrations of 0.625 mg/mL.

Fatty Acids Content of the Seed

Table 4: Fatty acid contents of the n-hexane and ethanol extracts

Fatty acids	Saturation	Trans-esterified n-hexane Extract (%RA)	Ethanol Extract (%RA)
Palmitic acid	16:0	15	12.56
Stearic acid	18.0	5.85	0
Linoleic acid	18:2	37.5	0
Linolenic acid	18.3	41.6	87.44
Total saturated acids		20.85	12.56
Total unsaturated		79.1	87.44
Total fatty acids		99.95%	100%

%RA = Percent Relative Abundance

The fatty acid compositions of the seed extracts are presented in Table 4. The n-hexane extract contains two saturated fatty acids: palmitic acid (15%) and stearic acid (5.85%), while the ethanol extract contains only palmitic acid which is slightly lower in abundance than that of n-hexane extract (12.56%). The total unsaturated fatty acid in the n-hexane extract is 79.10% which comprised of linoleic (37.5%) and linolenic (41.6%) acid. This is higher than the 87.44% present in the ethanol extract which is made up of only linolenic acid; a polyunsaturated acid. Both extracts contain much higher unsaturated fatty acids. Eating diets rich in saturated fats and trans fats results in production of more low-density lipoproteins (LDL)-cholesterol in the body which increases heart diseases. According to Appel *et al.* (2005), in the setting of a healthful diet, monounsaturated fat can further lower blood pressure, improve lipid levels, and reduce estimated cardiovascular risk. Willett (1993) reported that partially hydrogenating vegetable oils to produce solid fats may have reduced the anticipated benefits of substituting these oils for highly saturated fats, and instead contributed to the occurrence of coronary heart disease (CHD). Hence, saturated fats in diet increase the risk of heart diseases. In the case of biodiesel production, fuels having highly saturated components like coconut oil methyl ester have higher cetane number resulting in reduction in the ignition delay period. A biodiesel fuel with more unsaturated fatty acid compositions like Jatropha oil methyl ester has more density and iodine number but less viscosity, heating value and cetane number (Puhan *et al.*, 2010). In general, cetane number, heat of combustion, melting point, and viscosity of fatty compounds increase with increasing chain length and decrease with increasing unsaturation (Knothe, 2005). This implies that oils rich in unsaturated fatty acids have more advantage when used in biodiesel production. Therefore, *Ficus thonningii* oil is healthy for consumption (for the heart) as well as for biodiesel production.

Conclusion

This research shows that the oil of *Ficus thonningii* seed have antibacterial and antifungal properties especially against *Klebsiella pneumonae* and *Salmonella typhi* and so can be explored for medicinal and therapeutic purposes. The presence of flavonoids and phenolics make the seed an excellent and natural antioxidant with health-promoting properties. One of the difficulties encountered when choosing a good antimicrobial food preservative is getting one that is strong but not poisonous. Given that the seed can be eaten and is naturally antimicrobial in nature, it can be uniquely used for food preservation. Most of the fatty acids in the seed are unsaturated. This makes it healthy for consumption and also suitable as a source of blend for biodiesel production.

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