ORIGINAL PAPER

Nig. J. Pharm. Res. 2015, 1, pp. 74-83
ISSN 0189-8434

Phytochemical and Anti-Fungal Activity of Crude Extracts, Fractions and Isolated Triterpenoid from *Ficus Thonningii* Blume

*Morenike E Coker[1A-F], Olufemi E. Adeleke[1ACEF], Muyiwa Ogegbo[2BCF]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria.
[2]Institute of Health Research and Policy, London Metropolitan University, United Kingdom.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The importance of plants in human health cannot be overemphasized as plants have provided a good source of anti-infective agents in the fight against microbial infections. *Ficus thonningii* Blume (Moraceae), a plant used ethnomedicinally in West Africa for the treatment of some microbial infections was studied for its antifungal potentials.
Objective: To investigate the phytochemical and *in vitro* antifungal activity of the extracts and isolated compound from leaf and stem bark of *Ficus thonningii*.
Materials and Methods: Determination of phytochemical composition of *Ficus thonningii* leaf and stem bark and evaluation of the anti-fungal activity of the leaf and stem bark extracts using agar-diffusion technique. The Minimum Inhibitory Concentrations (MIC) of the crude extracts, fractions and isolated compound were determined by agar-dilution. Bioassay-guided fractionation of the crude extracts was carried out using column chromatography.
Results: The phytochemical screening revealed the presence of alkaloids, cardenolides, terpenoids, saponins, tannins and flavonoids. Crude extracts of *F. thonningii* demonstrated inhibitory activity on moulds and yeast but none against the dermatophytes (*Trichophyton* and *Microsporium* species). Hexane leaf extract was the most active inhibiting all the strains of *Aspergillus niger*, *Candida albicans*, *Penicillum chrysogenum* and *Rhizopus nigricans* with an MIC range of 20-312 µg/mL. The MIC of the isolated triterpenoid (10 – 78µg/mL) compared favourably with that of the standard drug, tioconazole, used (10 - 20 µg/mL).
Conclusion: The study showed that *Ficus thonningii* Blume is a good source of various phytochemicals including antimicrobial compounds that showed the potential of being developed into a drug for treating relevant fungal infections.

Keywords: Ficus thonningii, Antifungal activity, Triterpenoid, Phytochemical screening

INTRODUCTION

Naturally occurring antimicrobial agents have been derived from plants, animal tissues, or microorganisms. Medicinal plants are potential antimicrobial crude drugs and also a source for natural compounds that can serve as new anti infective agents (Newman *et al.*, 2003). *Ficus thonningii* Blume is a dicotyledonous plant that can grow as a shrub, sub-shrub or tree. It is a medium sized, low branching tree which can grow up to 40 ft high. The tree grows perennially and is evergreen with a rounded to spreading and dense crown. *F. thonningii* is widely distributed in the tropics and sub tropics. It can be found in West Tropical Africa in Benin, Cote D'ivoire, Ghana, Niger, Nigeria, Mali, Senegal and Sierra Leone, in Southern Africa in Botswana, Swaziland, South Africa, in North East Tropical Africa in Ethiopia (Keay *et al*., 1964). The whole plant exudes copious milky latex. The leavesare simple, glossy, dark green, thin and papery. The fruits occur singly or in pairs and is often recognised by the milky juice (Schmidt *et al*., 2002). The flowers of *Ficus thonningii* are unisexual, pollinated by small wasps.The nutritional profile of *Ficus thonningii* has shown that the leaves are a good source of protein ranging from 18.7-20.5 g/100 g dry matter (Tegbe *et al*., 2006), and also contain high levels of calcium (18.7-20.5 mg/100 g dry weight), potassium (910-1250 mg/100 g dry weight) and magnesium, 260-357.2 mg/100 g dry weight (Bamikole and Ikhatua, 2010). *F. thonningii* has been proved to be a standing reserve feed for rabbits during the dry season and also used as forage for ruminants (Jokthan *et al*., 2003; Bamikole and Ikhatua, 2010). The leaves are cooked as

vegetable by the Fulani and Igede people of Nigeria (Locket *et al*., 2000; Igoli *et al.*, 2002).

F. thonningii is one of the indigenous plants of West Africa used in Nigeria for ethomedical purposes some of which suggest its antimicrobial potentials. The leaves and fruits of the plant are used to treat bronchitis and urinary tract infections (Iwu, 1993). The leaves are used in the treatment of ringworm, thrush, scabies and athlete's foot rot (Alawa *et al*., 2002; Moshi *et al*., 2009). The bark is used for the treatment of influenza (Kokwaro, 1976) and also, the bark has been found useful in the treatment of wounds, sore throat, diarrhoea and cold (Watt and Breyer, 1962). Decoctions of leaves of *F. thonningii* are used for treating wounds in Angola. The wounds are washed with the leaf extract. In Nigeria, the fresh leaves of *F. thonningii* are grounded with potash and applied to affected parts to treat lumbago while the leaves are burned in hot ash to decolorize, and rubbed on dislocated limbs (Bhat *et al.*, 1990). In Central Africa, Congo Brazaville, the bark is used for treating diarrhoea, cysts, skin diseases and ulcers (Teklehaymanot and Gidday, 2007). The latex is used in traditional medicine for treating fever, tooth decay and ringworm (Alawa *et al*., 2002; Arbonnier, 2004). Apart from its use in ethnomedicine, *F. thonningii* is an ornamental tree used in improving agroforestic system. The bark is used for making bark cloth and the leaves as fodder.

Onwkaeme and Udoh (2000) identified the presence of starch grains, lignin, calcium oxalate crystals and suberin in *F. thonningii*. *F. thonningii* has been found to be a good source of protein and fat and an excellent source of calcium, iron, copper and zinc.

The anti-inflammatory activity of the ethanol leaf extract of *F. thonningii* has been reported (Otimenyin *et al*., 2004). Ethomedically, fresh leaves of *F. thonningii* have been used to treat lumbago (Bhat *et al*., 1990)

Many skin diseases such as ringworm and tinea caused by fungi exist frequently in tropical and semi tropical regions. Fungi generally live in the dead top layer of skin cells. They can also be found in moist areas of the body such as between the toes, and the groin. Fungal infection can penetrate into the cells causing itching, blistering and scaling (Chuang *et al*., 2007). The use of the plant parts of *F. thonningii* in the treatment of skin diseases, ringworm, thrush, scabies and athlete's foot rot suggests that the plant may contain some antifungal components.

With the continuous use of antibiotics, microorganisms have become increasingly resistant, coupled with the emergence of new infectious diseases thus making the existing conventional antimicrobial drugs unreliable in the treatment of diseases. Hence, the need to search for new and potent anti-infective agents with little or no side effects than the orthodox drugs.

The present study provides the evidence that the leaves and stem bark of *F. thonningii* possess some bioactive substances with anti-fungal properties.

MATERIALS AND METHODS

Plant collection and preparation

The leaves and stem bark of *F. thonningii*`were collected from Olodo village in Ibadan and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan. Herbarium sample was deposited at FRIN with voucher number (FRIN 1106898). The plant materials were dried, pulverized and weighed.

Phytochemical screening

The powdered plant parts were screened for secondary metabolites using standard procedures (Sofowora, 1993), as described below:

Test for Alkaloids

One gram of the dried powdered leaves and stem bark was heated with 5 mL of 0.1N HCL. The filtrate was divided into two portions. To the first portion was added five drops of Dragendorff's reagent while Wagner's reagent was added to the second portion drop-wise. Formation of reddish brown precipitate showed the presence of alkaloid.

Cardiac glycosides (Keller Killiani test)

One gram of the powdered sample was heated for 5 minutes with 10 mL of 80% v/v ethanol and then filtered. To the filtrate was added an equal volume of water and a few drops of lead acetate. The filtrate was then extracted with chloroform followed by evaporation and collection of residue to which was added 3 mL of ferric chloride reagent (0.3 mL of 10%v/v ferric chloride in 50 mL glacial acetic acid) in a test tube. Concentrated sulphuric acid, 2mL, was carefully poured down the tube. Brown ring and green coloration at interface showed the presence of cardenolides.

Terpenoids (Salkowski test)

To 0.5 g of the sample was added 2 mL of chloroform. Concentrated sulphuric acid (3 mL) was added carefully to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids.

Anthraquinones (Bontrager's test)

To 1 g of the powdered sample was added 3 mL of 0.1N HCL and the mixture was heated and filtered. The filtrate was extracted with an equal volume of chloroform. To the chloroform layer an equal volume of 10 % v/v of ammonia solution was added. The colour changes were noted.

Saponins

To 1 g of the powdered sample was added 20 mL of water followed by boiling and filtration. Two 5 mL portions of the filtrate were treated as follows:

The first 5 mL portion was shaken in a test tube noting the presence or absence of frothing.

To another 5 mL portion, 2.5 mL dilute HCL was added and boiled, noting the presence or absence of white particles.

Tannins

To 1 g of the powdered sample, 20 mL of water was added, boiled and filtered. The filtrate was adjusted to 20 mL with water. One millilitre of the adjusted solution was made up to 5 mL with water, and two drops of 0.1 % w/v ferric chloride solution were added.
Two drops of bromine water were added to another 1 mL of the adjusted solution. Deep blue colour with ferric chloride was positive for tannins.

Flavonoids

The powdered sample weighing 5 g was extracted with 10 mL of methanol and filtered. To the filtrate was added small quantity of magnesium powder and three drops of concentrated HCL. A red coloration was positive for flavonoids.

Plant Extraction and purification

Gradient extraction of 5kg each of powdered leaf and stem bark of plant was successively carried out separately with solvents of increasing polarity: hexane, chloroform and methanol using a Soxhlet apparatus. The filtrates obtained were concentrated in a rotary evaporator, evaporated to dryness and stored at 4 ^{0}C for further analysis.
Hexane leaf extract (5g) was subjected to column chromatography on silica gel 60 (70-230 mesh) using hexane, hexane-ethyl acetate gradients and methanol as eluents. Twelve fractions (HLF 01-12), were obtained. Methanol leaf extract (5g) was chromatographed using dichloromethane-ethyl acetate gradients, ethyl acetate, ethyl acetate-methanol gradients and methanol as eluents. Twelve fractions (MLF 01-12) were obtained. Hexane-dichloromethane gradients, ethyl acetate, ethyl acetate-methanol gradients and methanol were used for the elution of hexane stem bark extract (3g), six fractions (HSF 01-06), were obtained. Methanol stem bark extract (4.06g) was eluted with the same solvents used for the hexane fraction and six fractions (MSF 01-06) were obtained.
On the basis of analytic TLC, fractions were pooled together and antimicrobial assay was carried out to determine active fractions. The fractions upon microbial assay were subjected to further purification using column chromatography. A white compound (EC.HL02) was isolated from HLF 07 and was purified by recrystallisation to produce white crystals.

Identification and characterisation of isolated compound

Aluminium sheet pre-coated with silica gel 60 *F*254 nm (Merck) was used for thin layer chromatography. The spots were visualized using both ultraviolet light and a mixture of 0.5% vanillin and 10% sulphuric acid spray. NMR spectra were recorded on a Bruker Avance 300 at 300MHz (1H) and Bruker Avance 600 MHz (^{1}H) and 150 MHz (^{13}C) with the residual solvent peaks as internal references. The structure of the compound was confirmed by comparing with reference data from available literature.

Microorganisms

Pure cultures of these fungi were used: *Aspergillus niger* (3), *Candida albicans* (3), *Penicillium chrysogenum* (2), *Rhizopus nigricans* (1), *Trychophyton rubrum* (1) *Microsporum canis* (1) and *Trichophyton mentagrophyte* (1). All the fungal strains were maintained on Saboraud dextrose agar at 4 $^\circ$C and sub-cultured in tryptone soy broth prior to every antimicrobial test. The fungi were laboratory stock cultures obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Department of Veterinary Microbiology and Parasitology, University of Ibadan and University of Wolverhampton, United Kingdom. Two standard strains were used; *Aspergillus niger* III NCTC 772, *Penicillum chrysogenum* II NCIB 67.

Determination of Antifungal Activity

Agar-well diffusion was used to determine the antifungal activity of the isolates, each of which was prepared as 10^{-2} dilution (equivalent to 1.5 x 10^6 cfu/mL) from where 0.1mL was used to prepare seeded plate cultures. Equidistant wells were punched in the culture medium using a sterile cork-borer of 6mm diameter. Each well was filled with graded concentrations of the extract (3.125 to 50.00mg/mL). The plates were incubated at 28^{0}C for 24 hours to 5 days and the antifungal activity was assessed by measuring the diameter of zone of inhibition and comparing the zone of growth inhibition with that produced by the standard antibiotic tioconazole.

Determination of Minimum Inhibitory Concentration (MIC)

The microdilution assay was carried out to determine the MIC of the extracts, fractions and isolated compound of *F. thonningii* (Kuete *et al.,* 2008). The crude extracts, fractions and isolated compound were dissolved in di-methyl sulfoxide (DMSO) and the solution obtained was added to sterile Mueller Hinton Broth (MHB) to obtain a stock concentration of 625µg/ mL which was then serially diluted two-fold to obtain concentration ranges of 5-625 µg/mL. Each concentration in 100 µL volume was put into the wells of the microtitre plates containing 95 µL of MHB and 5 µL of inoculums (standardized at 1.5 ×10^6 cfu/ mL by adjusting the optical density to 0.1 at 600 nm on SHIMADZU UV-120-01 spectrophotometer, Kuete *et al.*, 2008). Wells containing 195 µL of MHB and 5 µL of standard inoculum served as fertility control. Tioconazole was used as the control drug. Each plate was covered with a sterile plate sealer, agitated to mix the contents of the

wells using a plate shaker and incubation was carried out at 28 ^{0}C for 24 hours to 5 days. The assay was repeated thrice. The MIC of samples was detected following addition of 40 µL of 0.2 mg/mL of *p*-iodonitrotetrazolium chloride to contents of wells and incubated at 37 ^{0}C for 30 minutes. A colour change from the yellow to pink indicated the presence of viable microorganisms. The lowest sample concentration that prevented this change, showing complete inhibition of microbial growth was taken as the MIC.

Statistical Analysis

The results of the experiment in determining the zones of growth inhibition are given as Mean ± Standard Deviation. The results given are the average of 3 determinations.

Chromatographic separation

Fractionation of crude extracts was carried out using column chromatography. Spectroscopic techniques using UV spectrometry, High Performance Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR) were used to elucidate the structure of the isolated compound.

RESULTS AND DISCUSSION

The qualitative phytochemical analysis of leaf and stem bark of *Ficus thonningii* revealed the presence of alkaloids, cardenolides, terpenoids, saponins, tannins and flavonoids. The pharmacological activities of most medicinal plants have been found to be related to the type of secondary metabolites they contain (Edeoga *et al*., 2005). Tannins are reported to be toxic to bacteria, yeasts, filamentous fungi and viruses (Scalbert, 1991; Patel *et al*., 2014). Tannins inactivate microbial adhesions, enzyme and cell envelope proteins and may complex with polysaccharides (Cowan, 1999). Tannin concentrations in *F. thonningii* leaves have been estimated to be about 90mgl/100mg dry matter (Bamikole *et al*., 2004) *In vitro*, flavonoids have been found to be effective against a wide array of microorganisms (Bennet and Walgrove, 1994) complexing with extracellular and soluble proteins as well as bacterial cells (Cowan, 1999). Terpenes and terpenoids are active against bacteria, fungi, viruses and protozoa distrupting microbial cell membranes (Cowan, 1999). Essential oils possess strong antimicrobial properties and have been reported to inhibit fungi and bacteria (Chaurasia and Vyas, 1997). Alkaloids are used in small quantities and some have shown antimicrobial properties. The presence of alkaloids tannins, flavonoids and terpenoids in plant parts of *F. thonningii* could account for its antimicrobial activity (Cowan, 1999; Kuete *et al*., 2011).

The chemical structure of the isolated compound was established using spectroscopic analysis, especially NMR spectra in conjuction with COSY (HMQC) and comparison with published information. The compound was identified to be a triterpenoid.

Hexane and methanol leaf and stem bark extracts of *Ficus thonningii* demonstrated appreciable antimicrobial activity against all the yeasts and moulds used except the dermatophytes (*Trychophyton rubrum*, *Microsporum canis* and *Trichophyton mentagrophyte),* as shown in Table 1. Hexane leaf extract inhibited all the strains of *A. niger, C. albicans, P. chrysogenum and R. nigricans* with zones of growth inhibition ranging from 10 – 18 mm. The chloroform extracts exhibited the least inhibition on the fungal strains showing no inhibitory effect on some strains of *A. niger* and *C. albicans*. The antifungal activities of hexane and methanol stem bark extracts were similar to that of the leaf extracts with the leaf extracts showing slightly better activity. The susceptibility pattern of each fungal strain to the extracts of leaf and stem bark of plant is shown in Figs. 1 and 2. Although most of the organisms were susceptible to the leaf and stem bark extracts, *Penicillum chrysogenum* was the most susceptible.

The antimicrobial activity of bioactive fractions and the isolated triterpenoid from leaf and stem bark of *Ficus thonningii* on *C. albicans* is presented in Table 2. The plant extracts, fractions, and isolated triterpenoid inhibited *C. albicans*, an opportunistic dimorphic fungus responsible for a variety of human diseases ranging from superficial skin lesions to disseminate infection (Cruz *et al.*, 2007). The MIC values of hexane and methanol crude extracts, fractions and the isolated compound are presented in Tables 3 and 4. Hexane and methanol leaf fractions showed better antimicrobial activity on the fungal strains than the crude extracts while the isolated triterpenoid from hexane leaf fraction demonstrated the highest inhibitory effect on the organisms. The fractions and isolated compound showed an appreciable inhibitory effect on *A. niger*. *Aspergillus* species have emerged as an important cause of life-threatening infections in immune-compromised patients. The MIC of the isolated compound for *A. niger* was 10µg/mL which was the same for tioconazole, the standard drug used. The MIC of hexane leaf fractions for *A. niger* (20-39 µg/mL) was lower than that of *C. albicans* (78-156 µg/mL). Kuete *et al*. (2011) reported that the crude extract and fractions from the root of *Ficus polita* had activity on *C. albicans* with MIC which ranged from 32-128 µg/mL while the MIC of the isolated compounds ranged from 128-512 µg/mL. There have been no validated criteria for MIC end points of plant extracts in *in vitro* testing. Researchers have suggested threshold MIC of plant extracts. Aligiannis *et al*. (2001), proposed that the

plant extracts with MIC values up to 0.5 mg/mL be regarded as strong inhibitors, between 0.6-1.5 mg/mL as moderate inhibitors and above 1.6 mg/mL as weak inhibitors. Kuete (2010) considered the threshold MIC of plant extracts with significant activity as 100 µg/mL. These reports when considered along with results from present study are supportive of suggesting *F. thonningii* as a promising plant with significant therapeutic activity.

Hexane leaf and stem bark extracts, methanol leaf and stem bark extracts inhibited all the three strains of *A. niger* used for the study, thus making the plant a potential crude drug in the treatment of infections caused by *C. albicans* and *A. niger*. Antifungal activities of *Ficus chlamydocarpa, Ficus cordata* and *Ficus ovata* have also been reported (Kuete *et al*., 2008; Kuete *et al*., 2009).
Oyelana *et al*. (2011), reported that the leaf extracts of *F. thonningii* at 25 and 50mg/mL had activity on some fungi like *A. niger, A. flavus, Fusarium oxysporium, Fusarium solanum* and *Rhizopus stolonifer*. In ethnomedicine, the leaves of *Ficus thonningii* are used in the treatment of infectious diseases like ringworm, scabies, thrush and athletes foot rot thus, the results of this study provide evidence for the use of extracts and compounds from *Ficus thonningii* against fungal microbial infections.

Acknowledgements

This work was partially sponsored by a grant from MacArthur Foundation Grant, University of Ibadan. The authors are grateful to the School of Applied Sciences, University of Wolverhampton, U.K. and Institute of Health Research and Policy, London Metropolitan University, U.K. for their financial assistance and infrastructural facilities in carrying out this research. The assistance rendered by Prof Kelvin Chan and Prof Annie Bligh is gratefully acknowledged.

Table 1:Anti-fungal activity of crude extracts (6.25 mg/mL) of *Ficus thonningii* against the fungal strains

Extract	Zone of Inhibition (mm)											
	***A .niger* I**	***A. niger* II**	***A.niger* III**	***C.albic* I**	***C.albic* II**	***C.albic* III**	***P. chryso* I**	***P.chryso*II**	***R. nigric***	***T.rubrum***	***M.canis***	***T.mentag***
HLE	10±0.58	12±0.00	12±1.15	13±2.33	12±0.00	10±0.00	18±1.15	12±0.00	10±1.76	-	-	-
CLE	-	9±00	-	-	8±1.00	10±0.00	15±1.00	12±0.58	9±0.58	-	-	-
MLE	10±0.00	11±0.58	10±0.00	10±1.15	-	10±1.53	12±1.00	10±0.00	11±1.15	-	-	-
HSE	10±1.15	13±1.15	10±1.00	-	10±0.00	10±0.00	14±0.58	12±0.58	10±2.00	-	-	-
CSE	-	9±0.00	-	-	10±0.00	-	14±1.00	-	-	-	-	-
MSE	10±0.00	11±0.58	-	10±0.00	10±2.65	10±0.00	14±0.00	12±2.65	10±0.00	-	-	-
Tioc (40µg)	24±1.00	25±0.00	25±0.00	22±1.15	22±1.00	-	24±0.00	24±0.00	22±0.00	14±0.00	-	18±1.00

Note: The zones of inhibition are measured in mm and are an average of triplicate results

Key

HLE : Hexane Leaf Extract
CLE : Chloroform Leaf Extract
CSE : Chloroform Stem bark Extract
MLE : Methanol Leaf Extract
HSE : Hexane Stem bark Extract
MSE : Methanol Stem bark Extract
- : No inhibition

A.niger: *Aspergillus niger*, P.chryso: *Penicillium chrysogenum*, R.nigric: *Rhizopus nigricans*, T. mentag: *Trichophyton mentagrophyte*, T.rubrum*: Trichophyton rubrum*, M.canis*: Microsporum canis*

Table 2: Antimicrobial activity of bioactive fractions and isolated triterpenoid from leaf and stem bark of *F. thonningii* on *Candida albicans*

Bioactive Fraction	Conc. mg/mL	Zone of inhibition(mm)
HLF 04	0.625	10
HLF 07	0.625	12
HLF 11	0.625	11
MLF01	0.625	11
MLF06	0.625	11
MLF07	0.625	10
MLF11	0.625	11
HSF 02	0.625	10
HSF 03	0.625	10
MSF 01	0.625	10
MSF 06	0.625	10
EC.HL02	0.625	17
Tioco 40µg		24

Key:
HLF: Hexane leaf fraction
MLF: Methanol leaf fraction
HSF: Hexane stem bark fraction
MSF: Methanol stem bark fraction
EC.HL02.: Code for isolated triterpenoid
Tioco: Tioconazole

Table 3: The Minimum Inhibitory Concentration (MIC) in µg/mL of hexane crude extract, fractions and EC.HL02 against fungal strains

Hexane Sample	MIC (µg/mL)	
	Candida albicans	Aspergillus niger
HLF Crude	312	78
HLF 04	156	39
07	78	20
11	156	39
EC.HL02	78	10
Tioconazole	20	10

Key:

HL: Hexane leaf
HLF: Hexane leaf fraction
EC.HL02: Code for isolated compound

Table 4: Minimum Inhibitory Concentration (MIC) µg/mL of methanol crude extract and fractions against fungal strains

Methanol Sample	MIC(µg/mL)	
	Candida albicans	*Aspergillus niger*
ML Crude	625	156
MLF 01	156	78
06	156	39
07	312	78
11	156	39
Tioconazole	20	10

KEY:
ML: Methanol leaf
MLF: Methanol leaf fraction

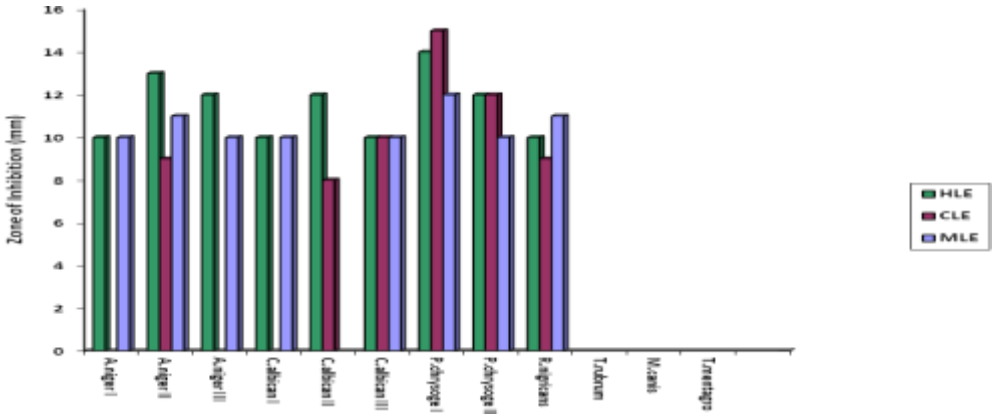

Fig 1: Antifungal activity of *F. thonningii* leaf extract (6.25 mg/mL) against the fungal strains

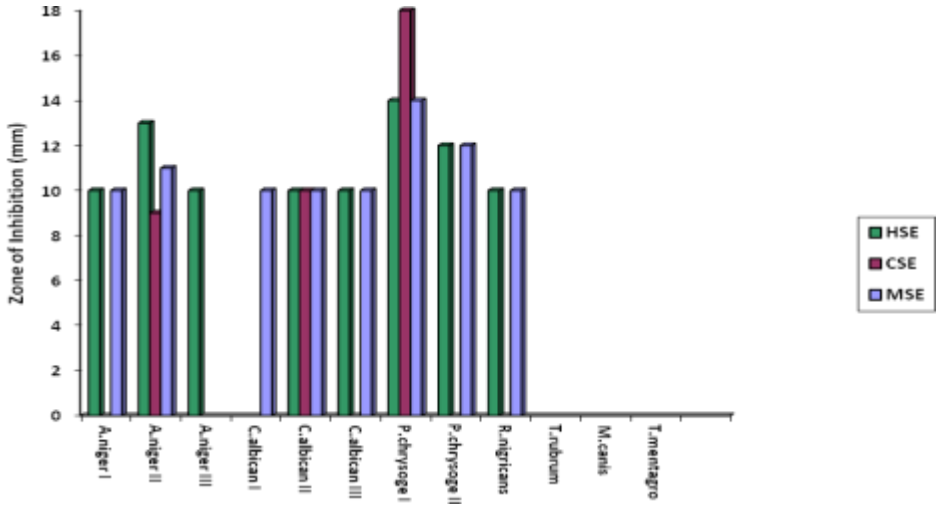

Fig 2: Antifungal activity of *F. thonningii* stem bark extract (6.25 mg/mL) against the fungal strains

REFERENCES

Alawa, J.P., Jokthan, G.E. and Akut, K. (2002). Ethno-veterinary medical practice for ruminants in the sub-humid zone of the Northern Nigeria. *Prev Vet Med.* 54: 79-90.

Aligiannis, N., Kalpotzakis, E., Mitaku, S. and Chinou, I.B. (2001). Composition and the antimicrobial activity of the essential oils of two *Origamum* species. *Journal of Agricultural and Food Chemistry.* 40: 4168-4170.

Arbonier, M.A. (2004). Trees, Shrubs and Lianas of West African Dry Zones, 2nd Ed. Netherlands: Margraf Publishers. Pp 412.

Bamikole, M.A., Ikhatua, Y.J., Arigbede, M.O., Babayemi, O.J. Etela, I. (2004). An evaluation of the acceptability as forage of some nutritive and anti-nutritive components of the dry matter degradation profiles of five species of *Ficus. Trop Animal Health Prod*. 36: 156-157.

Bennet, R.N. and Wallsgrove, R.M. (1994). Secondary metabolites in plant defence mechanisms. *Phytothe. Res*. 127: 617-633.

Bhat, R.B., Eterjere, E.O. and Oladipo, V.T. (1990). Ethnobotanical studies from Central Nigeria. *Economic Botany*, 44. 382-390.

Chaurasia, S.C. and Vyas, K.K.. (1977). *In vitro* effect of some volatile oil against *Phytophthtora parasitica* var *piperina. J. Res. Indian Med. Yoga Homeopath. 24-26.*

Chuang, P., Lee, C., Chou, J., Murugan, M., Shieh, B. and Chen, H. (2007). Antifungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. *Bioresource Technology.* 98: 232-236.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clin Microbiol. Rev*. 12(4): 564-582.

Cruz, M.C.S., Santos, P.O., Barbosa Jr., A.M., de Melo, D.L.F.M., Alviano, C.S. Antoniolli, A.R., Alviano, D.S. and Trindade, R.C. (2007). Antifungal activity of Brazillian medicinal plants involved in popular treatment of mycoses. *J ethnopharmacology.* 11: 409-412.

Edeoga, H.O, Okwu, D.E, Mbebo, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*. 4: 685-688.

Igoli, J.O., Tor-Anyin, T.A., Usman, S.S., Oluma, H.O.A. and Igoli, N.P. (2002). Folk medicines of the Benue valley of Nigeria. In: Recent progress in medicinal plants. Ethnomedicine and Pharmacognosy. Raleigh. NC. Science Technology Publishers. 7: 327-338.

Iwu, M.M. (1993). Catalogue of Major African Medicinal Plants In: Handbook of African Medicinal Plants, CRC Press Inc Florida. Pp. 48.

Keay, R.W., Onochie, C.F.A and Stanfield, D.P. (1974). Revised version of Nigerian trees. Pp 297-298.

Kokwaro, J.O, (1976). Medicinal Plants of East Africa, General Printers Ltd Kenya, pp 162.

Kuete, V. (2010). Potential of Cameroonian plants and derived products against microbial infections: A review. *Planta Med.* 76: 1-13.

Kuete, V., Nana, F., Ngameni, B., Mbaveng, A.T., Keumedjio, F. and Ngadjul, B.T. (2009). Antimicrobial activity of the crude extract, fractions and compounds from stem bark of *Ficus ovata* (Moraceae). *Journal of Ethnopharmacology*. 124: 556-561.

Kuete, V., Ngameni, B., Fotso Simo, C.C., Tankeu, R.K., Ngadjui, B.T., Meyer, J.J.M., Lall, N. and Kuiate, J.R. (2008). Antimicrobial activity of the crude extracts and compounds from *Ficus chlamydocarpa* and *Ficus cordata* (Moraceae). *Journal of Ethnopharmacology*. 120: 17-24.

Kuete, V., Kamga, J., Sanjo, P.L., Ngameni, B. Poumale, H.M., Ambassa, P. and Ngadjul, B.T. (2011). Antimicrobial activities of the methanol extract, fractions and compound from *Ficus polita* Vahl. (Moraceae). *BMC Complementary and Alternative Medicine*. 11: 6.

Locket, C.T., Carlvett, C.C. and Grivetti, L.E. (2000). Food-related behaviours during drought: A study of rural Fulani, North Eastern Nigeria. *Int J Food Sci Nutri.* 1: 91-107.

Moshi, M.J., Otieno, D.F., Mbabazi, P.K. and Weisheit, A. (2009). The ethnomedicine of the Haya people of Bugabo ward, Kagera region, North Western Tanzania. *J Ethnobiol. Ethnomed.* 5: 24.

Newman, D.J., Cragg, G.M., and Snader, K.M. (2003). Natural products as sources of new drugs over the period 1981-2002. *Journal of Natural Products* 66: 1022-1037.

Onwkaeme, D.N. and Udoh, F. (2000). Pharmacognostic and antidiarrhoeal studies of leaves of *Ficus thonningii, Nig J Nat Prod and Med.* 4: 27-29.

Otimenyin, S.O., Uguru, M.O. and Atang, B.L. (2004). Anti-inflammatory and analgesic activities of *Ficus thonningii* and *Pseudocedrela kotschyi* extracts. *Nig J Pharm Res.* 3 (1): 82-85.

Oyelana, O.A., Durugbo, E.U.,Olukanni, O.D., Ayodele, E.A., Aikuola, Z.O. and Adewole, A.I. (2011). Antimicrobial activity of Ficus leaf extracts on some fungal and bacterial isolates of *Dioscorea rotundata* from Southwest Nigeria. *J Bio Sci.* 11: 359-366.

Patel, P, Patel N; Patel D. Desai S; Meshram D (2014). Phytochemical analysis and antifungal activity of *Moringa Oleifera*. *International Journal of Pharmacy and Pharmaceutical Sciences.* 6: (5) 144 – 147.

Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*. 30: 3875-3883.

Schmidt, E., Lutter, M. and McCleland, W. (2002). Trees and shrubs of Mpumalanga and Kruger National park. Jacana, South Africa. Pp 80.

Sofowora. A. (1993). Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicine in Africa. Spectrum books Ltd, Sunshine House Ibadan, Nigeria. 2: 134-156.

Tegbe, T.S.B., Adeyinka, I.A., Baye, K.D. and Alawa, J.D. (2006). Evaluation of feeding graded levels of dried and milled *Ficus thonningii* leaveson growth performance, carcass characteristics and organ of weaner rabbits. *Pakistan J Nutr*. 5: 548-550.

Teklehaymanot, T. and Gidday, M. (2007). Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, North Western Ethiopia. *J. Ethnobiol. Ethnomed.* 3: 12.

Watt, J.M, and Breyer-Brandwijk, G.M. (1962). The medicinal and poisonous plants of Southern and Eastern Africa, 2nd Ed. E and S Livingstone Ltd, Edinburgh, London. Pp 773.

Address for correspondence:

Morenike E Coker
Department of Pharmaceutical Microbiology,
Faculty of Pharmacy, University of Ibadan, Nigeria.
E-mail:morencoker2002@yahoo.com

Conflict of Interest: None declared

Received: 25 May, 2015

Accepted: 18 July, 2015