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Antimicrobial activity of Extracts and Fractions of the Leaves of *Ficus thonningii* (Blume) and *Ficus natalensis* (Hochst) on Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) infections constitute a global health threat with high mortality and morbidity rates; a problem aggravated by antimicrobial resistance. *Ficus thonningii* and *Ficus natalensis* have been used in ethnomedicine for the treatment of bacterial infections. Successive extraction was carried out with hexane, ethyl acetate, and methanol using a soxhlet apparatus. The crude extracts obtained were screened for the presence of phytochemicals using standard methods. Vacuum liquid chromatography (VLC) of the extracts was carried out. Clinical isolates were verified with standard biochemical assays and antibiogram was determined using the disc diffusion method. The agar well diffusion method was used to determine the antibacterial activity of the extracts and fractions. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined by microbroth dilution method. Bactericidal kinetics was determined using the viable count technique. Phytochemicals present in both plants include saponins, tannins, flavonoids, cardiac glycosides, terpenoids, steroids, alkaloids, and anthraquinones.

Extracts and fractions exhibited appreciable activity on the test organisms. The ethyl acetate extract and hexane extract of *F. thonningii* and *F. natalensis* respectively produced larger zones of inhibition than the other extracts. The MICs of the crude extracts from both plants ranged from 1.56 mg/mL to 50 mg/mL while the MBCs were between 12.5 mg/mL and >100 mg/mL. The fractions on the other hand exhibited lower MICs and MBCs; with MICs ranging between ≤ 0.039 mg/mL and 3.125 mg/mL and MBCs between 1.525 mg/mL and 12.5 mg/mL. Bactericidal kinetics showed a total kill at 24 hours at 10 mg/mL and 5 mg/mL for both fractions.

INTRODUCTION

Staphylococcus aureus are frequently implicated in invasive infections, skin and soft tissue infections (SSTs), resulting in death in some cases (Kourtis *et al.*, 2019). MRSA is a leading cause of bacteremia, nosocomial, bone, and wound infections globally with a significant mortality and morbidity rate (Lee *et al.*, 2013; Hassoun *et al.*, 2017; Mikkaichi *et al.*, 2019).

Use of plants for medical purposes is as old as mankind. Plants have been reliable sources of treatment and prophylaxis. Ethnomedicinal information passed from generation to generation

has provided scientists with hints on plants that could be explored in the development of therapeutic agents (Atanasov *et al.*, 2015; Ezekwesili-Ofilu and Okaka, 2019). Medicinal plants are plants whose part(s) contain substances with therapeutic characteristics and can be used as precursors for drug synthesis (Ezekwesili-Ofilu and Okaka, 2019). With the advent and spread of antimicrobial resistance, there has been a growing interest in researching the therapeutic potency of various medicinal plants. Medicinal plants are paving a new approach to improved medicine and are emerging as alternative therapeutic options (Ali *et al.*, 2016). About a quarter of all Food and Drug Administration (FDA) and/or the European Medical Agency (EMA) approved drugs are plant based and over the last 20 years, about a third of FDA approved drugs are based on natural products and their derivatives (Thomford *et al.*, 2018).

The genus *Ficus* of the family *Moraceae* (Mulberry) is commonly referred to as figs. It is one of the most widely distributed genera of the family (Lansky and Paavilainen, 2011); having about 750 species (Chaudhary *et al.*, 2012). *Ficus* spp are very useful as they are used in traditional medicine, as food plants, ornamental trees, religious plants, fodder, fuel wood, hedges or enclosures (Yinxian *et al.*, 2014). There has been an increasing interest in the genus *Ficus* because of its chemical composition and potential health benefits (Yadav *et al.*, 2015; Tkachenko *et al.*, 2016). *F. thonningii* is also known as the bark cloth fig or strangler fig (English), *Chediya* (Hausa) and *Odan* (Yoruba). It is an evergreen tree (about 6-21m tall) with a dense rounded to spreading crown (Omoriegbe *et al.*, 2015) mainly distributed in the upland forests of tropical and sub-tropical Africa (Dangarembizi *et al.*, 2012). It has been reported that the leaves are used to treat diarrhoea, gonorrhoea, diabetes mellitus, wounds, gingivitis, schistosomiasis, malaria, liver disorders, disease conditions associated with jaundice, bronchitis and urinary tract infections (Ahur *et al.*, 2010; Dangarembizi *et al.*, 2012; Falade *et al.*, 2014).

F. natalensis commonly referred to as natal fig (English), *ogunke* (Yoruba) is a small tree

native to Southern and Western Africa. It is a monoecious evergreen epiphytic or terrestrial tree. Its leaves are 2.5-10 cm long and 1-8 cm wide (Burrows and Burrows, 2003; Oyen, 2011). In traditional medicine, the leaves are used in treating malaria, wounds, ulcers and warts (Oyen, 2011).

We therefore aimed to investigate the susceptibility of extracts and fractions of leaves of *F. thonningii* and *F. natalensis* on MRSA.

MATERIALS AND METHODS

Plant collection and preparation. The leaves of *Ficus thonningii* and *Ficus natalensis* were collected from Arulogun junction, Ojoo, and the Botanical Garden, University of Ibadan, and were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan with voucher numbers FHI: 112235 for *Ficus natalensis* and FHI: 112351 for *Ficus thonningii*. The leaves were air-dried under shade at about 29°C in October. The dried leaves were pulverised, weighed and stored in airtight containers.

Plant Extraction and Fractionation. Pulverised leaves (1050g of *F. thonningii* and 650g of *F. natalensis*) were successively extracted with solvents of varying polarity (hexane, ethyl acetate and methanol) using a Soxhlet apparatus (GSC International, Inc. MO, USA). The crude extracts were evaporated to dryness using a rotary vacuum evaporator (Heidolph Laborota, Germany), weighed and stored in a freezer at 4°C for further analysis. Ethyl acetate extract for *F. thonningii* (40g) and hexane extract for *F. natalensis* (12g) were subjected to vacuum liquid chromatography (VLC). VLC was carried out using a modified procedure as described: the extracts were separately absorbed with silica gel (60-200 mesh size) and allowed to dry. Filter paper (Whatman no 1) was placed on the internal surface of the Buchner funnel, after which silica gel was poured into the funnel. A layer of cotton wool was placed on top of the silica gel, the dried extract was loaded onto the funnel and a filter paper was placed over the extract. Hexane, hexane/chloroform gradient, chloroform, chloroform/ethyl acetate gradient, ethyl acetate,

ethyl acetate/methanol gradient and methanol were used as eluents. Thirteen fractions each were collected for both plants. On the basis of TLC profiling, fractions were pooled based on their TLC profiles to yield seven fractions (FTE 1-7) for *F. thonningii* and six fractions (FNH 1-6) for *F. natalensis*. Antimicrobial assay was carried out to determine active fractions.

Phytochemical screening. The crude extracts obtained from both plants were screened for the presence of secondary metabolites according to standard procedures (Vinoth, 2012).

Test organisms. Clinical isolates of MRSA from different sources (wounds, blood, and urine) were obtained from the Medical Microbiology Laboratory, University College Hospital, Ibadan. All isolates were screened for purity and identified by biochemical tests. Isolates positive for both slide and tube coagulase test were identified as *S. aureus*. Methicillin-resistance was determined using cefoxitin disc according to Clinical Laboratory Standards Institute (CLSI, 2018). Isolates were maintained in slants of nutrient agar at 4°C. *S. aureus* strain ATCC 29213 was used as a reference. Antibiotic sensitivity pattern of clinical isolates was determined by standard disc diffusion method according to the standards prescribed by Clinical and Laboratory Standards Institute (CLSI, 2018).

Gram staining. A sterile wire-loop was used to transfer a colony of microbial culture to a loopful of water on a clean glass slide. A thin smear was made on the glass slide and air dried. The smear was heat fixed and stained with crystal violet for 30 seconds and rinsed with distilled water, Lugol's iodine was added as a mordant for 60 seconds and rinsed off. Ethanol (95%) was added for 45 seconds to decolorize and the smear was counterstained with safranin for 30 seconds and rinsed with distilled water. The slide was air-dried, a drop of immersion oil was added and the slide was viewed under the microscope at x100 objective lens.

Selective plating on blood agar. Blood agar plates were prepared by mixing 1 mL of freshly collected human blood with 19 mL of molten

sterile nutrient agar at 45°C. The mixture was poured and allowed to set. The surface was dried and streaked with the 18 h broth culture of the organism and incubated at 24 h at 37°C. The plates were observed for isolated, round golden yellow colonies with surrounding haemolysis.

Catalase test. A sterile wireloop was used to pick a colony of the isolate to make a smear on a glass slide containing 2-3 drops of 3% hydrogen peroxide. Production of gas bubbles indicated a positive reaction.

Slide coagulase test. On a clean grease free glass slide, part of a colony was emulsified and 10 µL of plasma was added and was observed for the presence of agglutination which indicates a positive reaction.

Tube coagulase test. Isolates were incubated in Mueller Hinton broth at 37 °C for 24 h. In a sterile glass test tube, 0.2ml of the overnight broth culture was added to 0.5ml citrated human plasma and mixed gently. The test tubes were incubated at 37 °C and observed for coagulation at the 1 h, 2 h, 4 h and 24 h. Formation of a firm opaque clot which remains in the place when the tube is tilted on its side was considered positive.

In vitro antimicrobial screening of plant extracts. Antimicrobial screening was conducted using agar well diffusion as described by Coker and Onu, 2019. The extracts were dissolved in methanol to give varying concentrations. Bacterial suspension adjusted to 0.5 McFarland standard was used to inoculate Mueller Hinton plates with the aid of sterile cotton tipped applicator. Equidistant wells of about 8 mm were bored with a cork borer in the set agar. The holes were filled with the appropriate concentration (25 mg/mL and 100 mg/mL) of the prepared extracts. The plates were left on the bench for an hour to allow the extracts diffuse into the agar before incubating at 37°C for 24 h. Zones of inhibition were measured after the incubation period from which the antibacterial activity of the crude extracts against the test organisms was determined. Vancomycin (30 µg/mL) was used as standard drug control (positive control) and methanol as negative control. The experiment

was done in duplicates.

Determination of MIC and MBC. Minimum inhibitory concentration (MIC) of the extracts and fractions was determined by the microdilution broth method described by Kuete *et al.* (2008). The extracts and fractions were serially diluted in Mueller Hinton broth (MHB) to obtain a concentration range between 0.391-100 mg/mL (extracts) and 0.391-12.5 mg/mL (fractions). Each well contained 100 μ L of each concentration, 95 μ L of MHB and 5 μ L of bacterial suspension adjusted to 0.5 McFarland standard. Wells containing 195 μ L of MHB and 5 μ L of bacterial suspension served as fertility control. Vancomycin was used as the control drug. The plates were covered and incubated at 37 °C for 24 h. After which about 50 μ L of tetrazolium salt (2 mg/mL) was added to each of the wells at 37 °C for 30 minutes. A purple coloration indicated the presence of viable bacteria. The lowest concentration that prevented this change was taken as the MIC. To determine the MBC, wells showing no visible growth were streaked on Mueller Hinton agar plate using a sterile loop and incubated at 37 °C for 24 h. The lowest concentration showing no bacterial growth was taken as the MBC.

Bactericidal kinetics. Bactericidal kinetics was carried out on the most active fraction from both plants using a modified procedure described by Idowu and Idowu (2014). Overnight culture of the most sensitive isolate was made in 5 mL of nutrient broth. 0.1 mL of the culture was used to inoculate 3.9 mL of nutrient broth containing 1 mL of fraction at varying concentrations (10 mg/mL, 5 mg/mL, 2.5 mg/mL). Serial dilutions was made from this mixture containing culture, broth and fraction and 0.1 mL of the 10⁻³ dilutions was used to inoculate a freshly prepared nutrient agar plate at time intervals 0, 0.5, 1, 2, 4, 6 and 24 h. Plates were incubated at 37 °C for 24 h. Colony count was determined and a graph of log of CFU/mL was plotted against time. A control broth was set up without any added extract.

RESULTS

Qualitative phytochemical screening of extracts of both plants (Table 2) showed the

presence of alkaloids, saponins, tannins, flavonoids, cardiac glycosides, terpenoids, steroids and anthraquinones in at least one of the extracts. Despite exhibiting multi-drug resistance as shown in Table 1, isolates proved susceptible to the crude extracts and fractions of both plants. Zones of inhibition ranging from 10 mm to 20 mm across were recorded from the crude extracts (Table 3). However, a few isolates had zones of <10mm or showed no zone of inhibition. The highest zone of inhibition recorded was 20mm at 100 mg/mL for the hexane extract of *F. natalensis* and the ethyl acetate extract for *F. thonningii*. The MIC recorded of the crude extracts from both plants ranged between 1.5625mg/mL and 50mg/mL while the MBC ranged between 12.5 mg/mL and >100mg/mL (Table 4). The ethyl acetate extract of *F. thonningii* had the lowest MIC values, the MIC values ranged between 3.125 mg/mL to 25 mg/mL while the MBC of the isolates were either at 25 mg/mL or 50 mg/mL. Hexane extract of *F. natalensis* had lower MICs ranging between 1.5625mg/mL and 25mg/mL. For most isolates, the MBC was either 4X the MIC or 6X the MIC.

Fractionating the most active extracts of both plants yielded fractions with lower MIC and MBC values than the crude extracts (Tables 5 and 6). The MICs of the fractions ranged between \leq 0.039 mg/mL and 3.125 mg/mL and MBCs between 1.525 mg/mL and 12.5 mg/mL. Figs 2 and 3 show the bactericidal action of fractions FTE3 and FNH6 respectively. Initially, growth was observed in both fractions between 0 and 1 hour, after which killing progressed slowly over a period of 24 h. Across all concentrations for both fractions significant reduction was observed after 4 h. A total kill was observed at concentrations 10 mg/mL and 5 mg/mL for both fractions after 24 h of exposure. At 2.5 mg/mL, a few growth colonies were observed after 24 h of exposure for both fractions, notwithstanding, the initial growth observed at 0 hour had greatly reduced. The control broth showed progressive increase in growth colonies over the 24 h time frame.

Comparing the activity of extracts and fractions from both plants, the activity of extracts

and fractions from *F. natalensis* showed better activity on the isolates.

DISCUSSION

MRSA is a common nosocomial pathogen, which is gradually being associated with community associated infections with significant mortality and morbidity (Osinupebi *et al.*, 2018; Lakhundi and Zhang, 2018; Mikkaichi *et al.*, 2019). According to Lee *et al.* (2004), MRSA is usually resistant to commonly used antibiotics such as aminoglycosides, macrolides, chloramphenicol, fluroquinolones, cephalosporins, ampicillin-sulbactam, carbapenems, beta lactams and derivatives.

Phytochemicals occur naturally in medicinal plants; they are primary (examples, proteins, chlorophyll, common sugar) and secondary compounds (terpenoids, alkaloids, saponins, flavonoids) occurring naturally in plants (Wadood *et al.*, 2013; Mudasar *et al.*, 2016). Alkaloids, saponins, tannins, anthraquinones, and flavonoids are known to have activity against several pathogens (Usman *et al.*, 2009; Shalini and Sampathkumar, 2012). Alkaloids have been known to show good antibacterial activity against Gram positive bacteria (Karou *et al.*, 2005; Mabhiza *et al.*, 2016; Gurrapu and Mamidala, 2017). Terpenoids have been isolated from various plants and they have shown *in vitro* antimicrobial activities against both Gram positive and Gram negative bacteria (Ludwiczuk *et al.*, 2016). The phytochemical screening of *F. thonningii* leaves reported by Coker *et al.* (2015) supports the phytochemical screening reported in this study. While the methanol extract contained all phytochemicals tested for, the ethyl acetate and hexane extract lacked some of the phytochemicals. Usman *et al.* (2009) had a similar finding; some of the phytochemicals present in the methanol crude extract were absent in the n-butanol and residual aqueous extracts of *F. thonningii* stem bark.

The antibacterial activity of the extracts can be attributed to the presence of phytochemicals present in the extracts. Phytochemicals such as flavonoids, tannins, alkaloids, terpenoids etc have been associated with combating antimicrobial resistance in organisms (Neog *et*

al., 2013; Debalke *et al.*, 2018). *F. thonningii* has been proven to have antibacterial activity on both Gram positive and Gram negative pathogens (Usman *et al.*, 2009). It was however expected that the methanol extract in this study will exhibit a better activity based on the various phytochemicals it contained, however, it showed the least activity with some of isolates having no zone of inhibition. This observation is similar to reports by Usman *et al.* (2009). They reported better activity with the n-butanol and aqueous extracts of *F. thonningii* stem bark than with the methanol extract despite the methanol extract indicating presence of more phytochemicals than the other extracts. The findings on the antibacterial activity of the crude extracts of *F. thonningii* infer that the bioactive components responsible for the activity on MRSA are moderately polar. The appreciable antibacterial activity observed with the hexane extract is in line with the activity observed with the petroleum ether extract (Ajaib *et al.*, 2016). However, the zones of inhibition observed against *S. aureus* are wider compared to the diameter of zones of inhibition observed in this study. Comparable zones of inhibition for hexane, ethyl acetate and methanol extracts were reported by Coker and Oaikhena (2020). In comparison to the inactivity of the hexane, ethyl acetate and methanol extracts of *F. natalensis* on MRSA reported by Sheyin *et al.* (2018), the extracts in this study exhibited better activity against MRSA.

The MICs of the crude extracts of *F. thonningii* and *F. natalensis* were between 1.56 mg/mL to 50 mg/mL while the MBCs ranged from 12.5 mg/mL and >100 mg/mL. We observed lower MICs (≤ 0.039 mg/mL and 3.125 mg/mL) and MBCs (1.525 mg/mL and 12.5 mg/mL) for active fractions, implying that the fractions contained more active constituents than the crude extracts. Usman *et al.* (2009) reported MIC as low as 5 mg/mL of methanol stem bark of *F. thonningii* on *S. aureus* and Ajaib *et al.* (2016) recorded an MIC as low as 0.625 mg/mL of *F. natalensis* on *S. aureus*. Sheyin *et al.* (2018) recorded no MIC of *F. natalensis* on MRSA. It has been proposed that plant extracts with MIC values ≤ 0.5 mg/mL are strong inhibitors, those

with MIC between 0.6 mg/mL and 1.5 mg/mL are moderate inhibitors and extracts with MICs ≥ 1.6 mg/mL are weak inhibitors (Aligiannis et al., 2001). Based on this proposition on MIC threshold, the inhibitory activity of the fractions is better than that of the crude extracts, this implies that with further purification of the active fractions, fractions with lower MICs and strong inhibitory activity can be obtained.

Bactericidal kinetics showed a concentration dependent activity of the fractions against MRSA. The bactericidal kinetics of the fractions suggested that the mechanism of action of the extracts may be cidal rather than static or inhibitory.

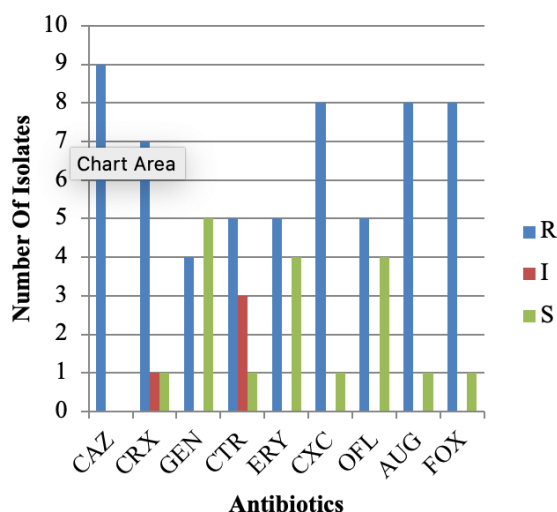


Figure 1: Graph showing the sensitivity of the test organisms to different antibiotics. CAZ- ceftazidime, CRX- cefuroxime, GEN- gentamicin, CTR- ceftriaxone, ERY- Erythromycin, CXC- cloxacillin, OFL- ofloxacin, AUG- augmentin, FOX- ceftiofuran. R- resistant, I- intermediate, S- sensitive.

CONCLUSIONS

F. thoningii and *F. natalensis* have shown potent antimicrobial activity against MRSA. The secondary metabolites contained in the plants may be responsible for the observed antibacterial activity. The activity of the extracts and fractions on the test isolates despite being resistant to multiple antibiotics showed that the plants have the potential to be alternative options for treatment of drug-resistant bacterial infections and could serve as precursors for production of drugs against MRSA infections.

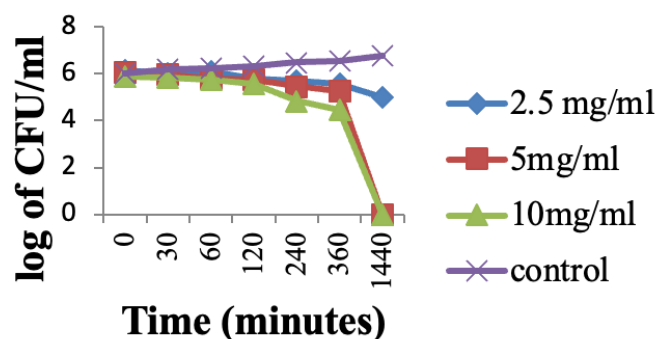


Figure 2: Bactericidal kinetics of ethyl acetate fraction FTE 3 on MRSA I showing gradual kill in response to the fraction concentrations over 24 hours.

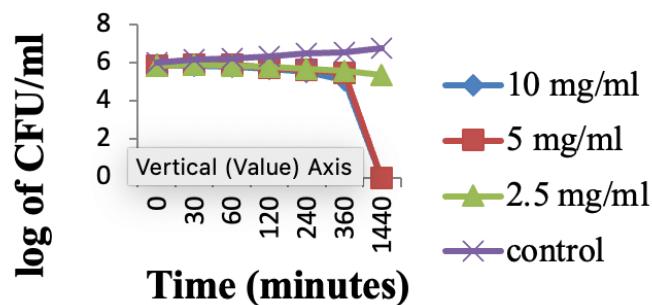


Figure 3: Bactericidal kinetics of hexane fraction FNH5 on MRSA I showing gradual kill of the organism in response to the fraction concentrations over 24 hours.

Table 1 Antibiotic sensitivity patterns of the test organisms

Isolates	CAZ*	CRX	GEN	CTR	ERY	CXC	OFL	AUG	FOX
MRSA I	R	R	R	R	R	R	R	R	R
MRSA II	R	R	S	I	S	R	S	R	R
MRSA III	R	R	R	R	R	R	R	R	R
MRSA IV	R	R	S	I	S	R	S	R	R
MRSA V	R	I	S	I	S	R	S	R	R
MRSA VI	R	R	R	R	R	R	R	R	R
MRSA VII	R	R	S	R	R	R	R	R	R
MRSA VIII	R	R	R	R	R	R	R	R	R
<i>S. aureus</i> ATCC 29213	R	S	S	S	S	S	S	S	S

* CAZ- ceftazidime, CRX- cefuroxime, GEN- gentamicin, CTR- ceftriaxone, ERY- Erythromycin, CXC- cloxacillin, OFL- ofloxacin, AUG- augmentin, FOX- ceftioxin.

R- resistant, I- intermediate, S- sensitive.

Table 2 Qualitative phytochemical screening of leaf extracts of *Ficus thonningii* and *Ficus natalensis*

Phytochemical	Hexane extract		Ethyl acetate extract		Methanol extract	
	Ft	Fn	Ft	Fn	Ft	Fn
Saponins	-	-	++	-	+	+
Tannins	-	-	+	-	++	+
Flavonoids	+	-	++	+	++	+
Cardiac glycosides	+	-	-	-	++	+
Terpenoids	++	++	+	+	+	+
Steroids	-	++	+	+	+	+
Alkaloids	+	-	-	+	+	+
Anthraquinones	++	+	+	+	+	+

* Ft; *Ficus thonningii*, Fn; *Ficus natalensis*, -; absence of phytochemical; +; presence of phytochemical, ++; abundance of phytochemical.

Table 3 Antibacterial activity of crude extracts of *Ficus thonningii* and *Ficus natalensis* on test isolates

Isolates	Hexane extract						Ethyl acetate extract						Methanol extract				Van 30 (µg/mL)		
	100 mg/mL		50 mg/mL		25 mg/mL		100 mg/mL		50 mg/mL		25 mg/mL		100 mg/mL		50 mg/mL			25 mg/mL	
	Ft*	Fn	Ft	Fn	Ft	Fn	Ft	Fn	Ft	Fn	Ft	Fn	Ft	Fn	Ft	Fn		Ft	Fn
	Zones of inhibition (mm)																		
MRSA I	15	20	13	18	12	12	20	18	18	14	10	12	15	12	12	12	10	10	12
MRSA II	13	18	10	12	-	10	16	14	14	10	10	-	13	12	12	10	10	-	14
MRSA III	10	14	10	12	10	10	14	12	12	10	-	10	14	12	10	-	-	-	10
MRSA IV	14	14	12	12	10	10	16	14	12	10	10	-	-	-	-	-	-	-	10
MRSA V	14	18	12	16	-	14	15	12	10	10	10	10	-	10	-	10	-	10	12
MRSA VI	12	16	12	14	10	10	12	16	10	12	10	12	10	12	10	12	10	10	14
MRSA VII	12	12	10	12	10	12	10	12	10	12	10	12	10	12	10	12	-	12	13
MRSA VIII	15	12	12	-	10	-	10	12	10	10	-	10	10	10	10	10	-	-	14
<i>S. aureus</i> ATCC 29213	14	16	12	14	10	12	14	12	14	10	12	10	10	12	10	12	-	10	14

* Ft; *F. thonningii*, Fn; *F. natalensis*, Van- vancomycin MRSA; methicillin-resistant *Staphylococcus aureus* -; no zone of inhibition

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude extracts of *Ficus thonningii* and *Ficus natalensis* on test isolates on test isolates.

Isolate	Hexane Extract				Ethyl Acetate Extract				Methanol Extract			
	MIC (mg/mL)		MBC (mg/mL)		MIC (mg/mL)		MBC (mg/mL)		MIC (mg/mL)		MBC (mg/mL)	
	FT	FN	FT	FN	FT	FN	FT	FN	FT	FN	FT	FN
MRSA I *	12.5	3.125	100	50	12.5	6.25	25	25	25	3.125	>100	50
MRSA II	12.5	25	100	100	12.5	25	50	50	50	12.5	>100	>100
MRSA III	50	1.5625	>100	25	25	12.5	50	50	25	12.5	>100	50
MRSA IV	25	25	100	50	25	25	25	50	25	25	>100	>100
MRSA V	50	12.5	>100	50	25	25	50	50	50	25	>100	>100
MRSA VI	25	25	>100	100	25	25	100	50	50	12.5	>100	100
MRSA VII	100	25	-	100	25	25	50	50	50	12.5	>100	100
MRSA VIII	50	1.5625	>100	25	12.5	12.5	25	50	12.5	6.25	100	50
<i>S. aureus</i> ATCC 29213	3.125	1.5625	6.25	25	3.125	3.125	25	25	6.25	12.5	25	50

* MRSA; methicillin-resistant *Staphylococcus aureus*, -; no zone of inhibition, Ft; *F. thonningii*, Fn; *F. natalensis*

Table 5 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of active fractions obtained from VLC fractionation of crude ethyl acetate extract of *Ficus thonningii* on selected isolates.

Isolates	FTE 1*		FTE 2		FTE 3		FTE7	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
MRSA I	1.5625	6.25	0.781	≤3.125	≤0.391	≤3.125	0.781	≤3.125
MRSA II	3.125	12.5	3.125	6.25	3.125	6.25	3.125	6.25
MRSA III	3.125	12.5	1.5625	6.25	3.125	6.25	3.125	6.25
MRSA IV	1.5625	6.25	0.781	≤3.125	0.781	≤3.125	1.5625	≤3.125
MRSA VIII	3.125	12.5	3.125	12.5	3.125	6.25	3.125	6.25
<i>S. aureus</i> ATCC 29213	0.781	1.5625	0.781	1.5625	3.125	6.25	0.781	1.5625

*FTE; *F. thonningii* ethyl acetate fraction, MRS; Methicillin-resistant *Staphylococci*, MRSA- methicillin-resistant *Staphylococcus aureus*

Table 6. MIC and MBC of active fractions obtained from VLC fractionation of crude hexane extract of *Ficus natalensis* on selected isolates.

Isolates	FNH4 *		FNH 5		FNH6	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
MRSA I	0.781	3.125	≤0.391	1.5625	0.781	3.125
MRSA II	1.5625	3.125	1.5625	6.25	1.5625	6.25
MRSA III	1.5625	6.25	≤0.391	1.5625	3.125	6.25
MRSA IV	0.781	3.125	≤0.391	1.5625	1.5625	6.25
MRSA VIII	3.125	6.25	3.125	6.25	3.125	6.25
<i>S. aureus</i> ATCC 29213	0.781	1.5625	0.781	1.5625	3.125	6.25

* FNH; *F. natalensis* hexane fraction, MRSA- methicillin- resistant *Staphylococcus aureus*

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REFERENCES

- Ahur, V. M., Madubunyi, I., Adenkola, A. Y., Udem, S. C. 2010. The effect of acetyl acetate extract of *Ficus thonningii* (Blume) leaves on erythrocyte osmotic fragility and haematological parameters in acetaminophen-treated rats. *Comparative Clinical Pathology*.10:1107–1111.
- Ajaib M., Almas, M., Khan, K. M., Perveen, S. and Shah, S. 2016. Phytochemical screening, antimicrobial and antioxidant activities of *Ficus natalensis*. *Journal of chemical society of Pakistan*, 38(2): 345-350
- Ali, S., Nasreen, S., Safeer, S., Andleeb, S., Ejaz, M. and Shakir, H. A. 2016. Medicinal plants as therapeutic agents for cancer treatment. *Punjab University Journal of Zoology*, 31(2): 295-305.
- Aliagiannis, 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.
- Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H., Rollinger, J. M., Schuster, D., Breuss, J. M., Bochkov, V., Mihovilovic, M. D., Kopp, B., Bauer, R., Dirsch, V. M. and Stuppner, H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnology Advances*. 33(8): 1582-1614.
- Burrows, J. and Burrows, S. 2003. *Figs of southern and south-central Africa*, Umdaus Press. Hatfield, South Africa.
- Chaudhary, L. B., Sudhakar, J. V., Kumar, A., Bajpai, O., Tiwari, R., and Murthy, G. V S. 2012. Synopsis of the Genus *Ficus* L. (Moraceae) in India. *Taiwania* 57 (2):193–216.
- Clinical and Laboratory Standards Institute (CLSI) 2018. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI supplement M100S.
- Coker, M. E., Adeleke, O. E. and Ogebo, M. 2015. Phytochemical and anti-fungal activity of crude extracts, fractions and isolated triterpenoid from *Ficus thonningii* Blume. *Nigerian Journal of Pharmaceutical Research*, 11(1): 74–83.
- Coker, M. E. and Oaikhen, A. O. 2020. Antimicrobial activity of the crude extracts and fractions of *Ficus thonningii* (Blume) on Isolates from urinary tract infections. *Journal of Medicinally Active Plants* 9(4): 310-322.
- Coker, M. E. and Onu, E. C. 2019. Antibacterial and antiadherence properties of the leaves of *Ficus thonningii* Blume on *Acinetobacter baumannii*. *Nigerian Journal of Pharmaceutical Research*, 15 (2): 193-204.
- Dangarembizi R., Erlwanger K. H., Moyo, D., Chivandi, E. 2012. Phytochemistry, pharmacology and ethnomedicinal uses of *Ficus thonningii* (Blume *Moraceae*): a review. *African Journal of Traditional Complementary Alternative Medicine* 10: 203-212.
- Debalke, D., Mastewal, B., Amebaye, K., Muluken, Y. 2018. Assessments of Antibacterial Effects of Aqueous-Ethanollic Extracts of *Sida rhombifolia*'s Aerial Part. *The Scientific World Journal Article ID* 8429809, 8 pages
- Ezekwesili-Ofili, J. O., and Okaka A. N. C. 2019. Herbal Medicines in African Traditional Medicine. *Herbal Medicine*, Philip F. Builders, IntechOpen. 10:191-214
- Falade, M. O., Akinboye, D. O., Gbotosho, G. O., Ajaiyeoba, E. O., Happi, T. C., Abiodun, O. O., Oduola, A. M. J. 2014. In vitro and In vivo Antimalarial Activity of *Ficus thonningii* Blume (*Moraceae*) and *Lophira*

- alata* Banks (*Ochnaceae*), Identified from the Ethnomedicine of the Nigerian Middle Belt. *Journal of Parasitology Research*, vol. 2014, Article ID 972853, 6 pages.
- Gurrapu, S., Mamidala, E. 2017. In vitro antibacterial activity of alkaloids isolated from leaves of *Eclipta alba* against human pathogenic bacteria. *Pharmacognosy Journal*, 9(4):573-7.
- Hassoun, A., Linden, P.K. and Friedman, B. 2017. Incidence, prevalence, and management of MRSA bacteremia across patient populations—a review of recent developments in MRSA management and treatment. *Critical Care* 21: 211.
- Idowu, P. A. and Idowu, O. S. 2014. In-vitro bactericidal kinetics of chlorhexidine gluconate disinfectant/antiseptic formulations containing different additives. *African Journal of Biomedical Research*, 17: 37-42.
- Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simporé, J., Colizzi, V., Traore, A. S. 2005. Antibacterial activity of alkaloids from *Sida Acuta*. *African Journal of Biotechnology* 5 (2):195–200.
- Kourtis, A. P., Hatfield, K., Baggs, J., Mu, Y., See, I., Epton, E., Nadle, J., Kainer, M. A., Dumyati, G., Petit, S., Ray, S. M., Emerging Infections Program MRSA author group, Ham, D., Capers, C., Ewing, H., Coffin, N., McDonald, L. C., Jernigan, J., and Cardo, D. 2019. Vital Signs: Epidemiology and recent trends in Methicillin-Resistant and in Methicillin-Susceptible *Staphylococcus aureus* bloodstream infections - United States. *MMWR. Morbidity and mortality weekly report*, 68(9), 214–219.
- Kuete, V., Ngameni, B., Simo, C. C. F., Tankeu, R. K., Tchaleu, N. B., Meyer, J. J. M., Lall, N., Kuate, J. R. 2008. Antimicrobial activity of the crude extracts and compounds from *Ficus chlamydocarpa* and *Ficus cordata* (*Moraceae*). *Journal of Ethnopharmacology*, 120: 17 - 24.
- Lakhundi, S., and Zhang, K. 2018. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. *Clinical Microbiology Reviews* 31:e00020-18.
- Lansky, E. P., Paavilainen, H. M. 2011. Traditional herbal medicines for modern times. *Figs: The genus Ficus*. CRC Press, Taylor and Francis Group. 6000 Broken Sound Parkway NW, Suite 300. Page 383.
- Lee, B. Y., Singh, A., David, M. Z., Bartsch, S. M., Slayton, R. B., Huang, S. S. 2013. The economic burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). *Clinical Microbiology Infections*. vol 19. pp 528–536.
- Lee, J. H., Jeong, J., Park, Y., Choi, S., Kim, Y., Chae, J., Moon, J., Park, H., Kim, S., Eo, S. 2004. Evaluation of the Methicillin-Resistant *Staphylococcus aureus* (MRSA)-screen latex agglutination test for detection of MRSA of animal origin. *Journal of Clinical Microbiology*, 42 (6):2780–82.
- Ludwiczuk, A., Skalicka-Woźniak, K., Georgiev, M. I. 2016. Terpenoids. *Pharmacognosy*. Pp. 233-266.
- Mabhiza, D., Chitemerere, T. and Mukanganyama, S. 2016. Antibacterial properties of alkaloid extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *International Journal of Medicinal Chemistry*, vol. 2016, Article ID 6304163, 7 pages
- Mikkaichi, T., Yeaman, M. R., Hoffmann, A., & MRSA Systems Immunobiology Group 2019. Identifying determinants of persistent MRSA bacteremia using mathematical modeling. *PLoS computational biology*, 15(7), e1007087
- Mudasar A., Kaloo, Z. A., Ganai, B. A., Ganaie, H. A., and Singh, S. 2016. Phytochemical screening of *Meconopsis aculeata* Royle an important medicinal plant of Kashmir Himalaya: A Perspective. *Research Journal of Phytochemistry*, 10: 1-9.
- Neog, P., Choudhury, S., Bhattacharjee, A and Chetia, P. 2013. “A review on ethnomedicinal plants and their active secondary metabolites in antibacterial drug

- discovery research,” *Pleione*, vol. 7, pp. 413–423, 2013.
- Omoriege, H. and Oluyemisi, F. 2015. Phytochemical and pharmacognostic analysis of *Ficus thonningii* Blume leaves for monograph development. *International Journal of Basic and Applied Science* 4 (2):94–100
- Osinupebi, O. A., Osiyemi, J. A., Deji-Agboola, A. M., Akinduti, P. A., Ejilude, O., Makanjuola, S. O., Sunmola, N. O., Osiyemi, E. O. 2018. Prevalence of Methicillin-Resistant *Staphylococcus aureus* in Abeokuta, Nigeria. *South Asian Journal of Research in Microbiology*, 1(1): 1-8.
- Oyen, L. P. A. 2011. *Ficus natalensis* Hochst. Record from PROTA4U. Brink, M. & Achigan-Dako, E. G. (Editors). PROTA (Plant Resources of Tropical Africa / Resources végétales de l’Afrique tropicale), Wageningen, Netherlands. <<http://www.prota4u.org/search.asp>>.
- Shalini, S., Sampathkumar., P. 2012. Phytochemical screening and antimicrobial activity of plant extracts for disease management. *International Journal Current of Science*, pp: 209-218
- Sheyin, F.T., Ndukwe, G. I., Iyun, O. R. A., Anyam, J. V., Habila, J. D. 2018. Phytochemical and antimicrobial screening of crude extracts of Natal Fig (*Ficus natalensis* Kraus). *Journal of Applied Science and Environmental Management*. 22 (9): 1457 –1460
- Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Id, A. M. and Dzobo, K. 2018. Natural products for drug discovery in the 21st Century : Innovations for novel drug discovery. *International Journal of Molecular Science*, 9(1578): 1-29.
- Tkachenko, H., Buyun, L., Terech-Majewska, E. and Osadowski, Z. 2016. *In Vitro* antimicrobial activity of ethanolic extracts obtained from *Ficus* Spp. leaves against the fish pathogen *Aeromonas hydrophila*. *Archives of Polish Fisheries*, 24 (4):219–30.
- Usman, H., Abdulrahman, F. I., Usman, A. 2009. Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *African Journal of Traditional, Complementary and Alternative Medicine*, 6:289–295.
- Vinoth, B., Manivasagaperumal, R., and Balamurugan, S. 2012. “Phytochemical analysis and antibacterial activity of *Moringa oleifera* LAM.” *International Journal of Research in Biological Sciences* 2 (3): 98–102.
- Wadood, A., Ghufran, M., Jamal, S.B., Naeem, M., Khan, A. 2013. Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry and Analytical Biochemistry*, 2: 144.
- Yadav, R. K., Nandy, B. C., Maity, S., Sarkar, S., Saha, S. 2015. Phytochemistry, pharmacology, toxicology and clinical trial of *Ficus racemosa*. *Pharmacognosy Review*, 9(17): 73-80.
- Yinxian, S., Huabin, H., Youkai, X. U., Aizhong, L. 2014. An ethnobotanical study of the less known wild edible fig (genus *Ficus*) native to Xishuangbanna, Southwest China. *Journal of ethnobiology and ethnomedicine*, 10:68