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Full Length Research Paper

Phytochemical and antimicrobial screening of the crude petroleum spirit and methanol extracts of the stem bark, leaves and roots of *Ficus thoningii* (blume)

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Ficus thoningii **which has some traditional medicinal uses was investigated. Phytochemical screening of the stem bark, leaves and roots gave positive results for carbohydrates, glycosides, saponins and alkaloids. Antimicrobial screening of the crude petroleum spirit and methanol extracts showed activity against** ***Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Providencia stauti*** **and** ***Bacillus subtilis*** **but no activity was observed against** ***Salmonella typhi*****. The crude petroleum spirit extracts of the leaves and stem bark of the plant had minimum inhibitory concentrations at 50 mg/ml while the roots had no minimum inhibitory concentration at the test concentration. The crude methanol extracts of the various plant parts showed minimum inhibitory concentration at 50 mg/ml on all the pathogens tested for.**

Key words: Moraceae, *Ficus thonningii,* clinical isolates, *in vitro* studies.

INTRODUCTION

There are about twenty four (24) species of *Ficus* indigenous to South Africa and the fruits of most of them are edible although not so palatable as *Ficus caracal* L. Monkeys and many species of birds delight in eating them. The fruits are often infested by insects to a degree and this makes them disagreeable to the human palate (Watt and Breyer, 1962). *Ficus thoningii* belongs to the Moraceae family. It is widely distributed in upland forests, open grasslands, riverines and rocky areas. It is found in the savannahs. Soil requirements occur on a variety of soils but prefer light, dried and well drained soils with neutral reaction to acid. It is propagated by cutting and seed dispersal by birds and animals. It is used as a live fence with the intention of using the leaves as mulch or green manure, and also for producing shade or fodder. It has the ability to store water and conserve soil. The stem bark is used to treat colds, sore throat, diarrhea, wounds and to stimulate lactation (Watt and Breyer, 1962). It has other sacred ceremonial purposes. In Tanzania the root of *F. thoningii* is an indigenous galactogogue (Brenan and Greenway, 1949; Watt and Breyer, 1962. Although the pinkish abundant latex is said to yield no catouchouc but it is used in West Africa as a bird lime (Watt and Breyer, 1962). The fruit is edible and is used in alcohol production (Watt and Breyer, 1962). The powdered bark is used on wounds and a decoction of the bark is used for cough and throat infections in West Africa (Watt and Breyer, 1962). In Tanzania the bark is used as an influenza remedy and the bark and root bark for stimulating lactation. The bark is also used in Tanzania and Belgian Congo for making bark-cloth (Brenan and Greenway, 1949). The leaves of various species of *Ficus* commonly referred to as wild figs are chewed and applied to a poison-arrow wound, which is then sucked. The latex of various species of *Ficus* species has been used in folk medicine and the benefits have generally been ascribed to its antihelmintic action.

MATERIALS AND METHODS

Sample collection

The plant was collected in February 2005 from Kakuri in Kaduna State, Nigeria. It was identified by a taxonomist-Mall Musa Abdullahi of the Herbarium of the Biological Sciences Department, Ahmadu

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Bello University, Zaria, Nigeria and a voucher specimen Number 7084 deposited there. The leaves and roots were separated from the whole plant and the stem bark was carefully peeled from the stem.

Extraction procedure

The samples were air-dried and pounded in a wooden mortar. The pounded samples were weighed and defatted using petroleum spirit 60 – 80°C in a soxhlet extractor. The residues were then respectively and exhaustively extracted using redistilled methanol. The extracted solutions were concentrated *in vacuo* using a rotary evaporator.

Phytochemical analysis of the plant materials

The various plant parts (root, stem bark and leaves) were screened for plant metabolites using the pulverized materials respectively. Standard techniques of Brain and Turner (1975) were employed in the phytochemical screening. These metabolites include carbohydrates, soluble starch, glycosides, steroids, unsaturated steroids, aglycones, tannins, saponins, flavonoids, titerpenes and alkaloids.

Antimicrobial screening test

Antimicrobial analyses were carried out using the agar diffusion method of Barry and Thornsberry (1985) and Bauer et al. (1966). Fresh and pure clinical isolates of *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Providencia stauti and Bacillus subtillis* collected from the Department of Microbiology, Ahmadu Bello University, Zaria were used for the test. Nutrient agar weighing 11.5 g was dissolved in a conical flask containing 500 ml of distilled water. The mouth was plugged with cotton wool and boiled for complete dissolution. This was sterilized using an autoclave maintained at 121°C for 15 min. The media was allowed to cool to 45°C before it was dispensed into sterilized Petri dishes after which they were allowed to gel and finally stored in a refrigerator until needed. The cooled media was aseptically coated with the test organism after which bores (10 mm in diameter) were aseptically made into them using sterilized cork borer. The crude plant extract of known concentration was introduced into the well using a sterilized syringe. The Petri dishes were incubated at 37°C for 24 h and the experiment was duplicated. At the expiration of the time the plates were examined for inhibition zones and the observed zones were measured and recorded in millimeters.

Determination of minimum inhibition concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined using the tube dilution method. A set of seven capped small test tubes were used for each extract against each organism. Nutrient broth was prepared and 1 ml of it was transferred into each of the test tubes. 1 ml of the solution of the extract with the highest concentration (50 mg/ml) that inhibited the growth of the organism was transferred into the first test tube and then diluted serially by a factor of two into the seven test tubes. A loop-full from the culture, which showed zone of inhibition was introduced into each of the test tubes, capped and left at room temperature for 72 h. The tubes were then examined for the presence or absence of growth of the microorganisms which was visualized by the level of turbidity of the solution in the test tube. The test tube containing the solution of lowest concentration of extract that produced a clear solution was taken and recorded as the MIC of the crude extract.

Table 1. Result of the Phytochemical analysis of *Ficus thoningii*.

Carbohydrates	
Mollish's test	Positive
Fehling's test for reducing sugars	Negative
Starch	
Fehlings solution test	Negative
Soluble Starch (I)	Negative
Soluble Starch (II)	Negative
Glycosides	
Anthracene Glycosides(Borntragers test)	Positive
Free Anthracene Derivatives	Negative
Saponins	
Lieberman-Buchard test for Triterpenes	Positive
Salkowski's test for Steroids	Positive
Froth test for unsaturated Saponins	Positive
Alkaloids	
Mayer's Reagent test	Positive
Dragendorff's Reagent test	Positive
Picric Acid Reagent test	Positive
Tannic Acid test	Positive

Determination of the minimum bactericidal concentration (MBC)

This was carried out to know if the organisms could be killed completely or their growths could only be inhibited by the crude extracts. Another set of plates of nutrient agar were prepared according to the manufacturers' standard and sterilized in an autoclave as earlier described. The microorganisms in the tubes of the MIC tests were re-activated. Emphasis was mostly paid to the tube that represented the MIC and the preceding tubes. The re-activation was done in a mixture of 0.5% egg lecithin and 3% Tween 80 solution in a test tube. The reactivated organisms were sub-cultured into appropriately labeled quadrants of the sterilized nutrient agar plates using wire loop into each test tube and streaking uniformly on the labeled quadrants with known crude extract concentration. This was then incubated for 24 h at 37°C after which they were observed for growths. The MBC was the quadrant with the lowest concentration of the extract without growth.

RESULTS AND DISCUSSION

The result of the phytochemical screening (Table 1) showed that *F. thoningii* contains carbohydrates, flavornoids, tannins, glycosides, saponins and alkaloids. These metabolites present in various parts are known to have varied pharmacological actions in man and animals. From the result of the antimicrobial screening as shown in Tables 2 and 3 the crude petroleum spirit and methanol extracts of the leaves of the plant are active on *S. aureus* and *P. stauti* respectively. It is only the crude methanol extract of the stem bark that was active on *E. coli.* While the crude petroleum spirit extract of the root was not active on any of the organisms tested the crude methanol extract of the root was active on *K. pneumo-*

Table 2. Zones of inhibition (mm) of the crude petroleum spirit extracts.

Test organism	Bark	Root	Leaves
K. pneumonia	00	00	00
S. aureus	20	00	11
E. coli	00	00	00
P. stauti	14	00	14
B. subtilis	13	00	00
S. typhi	00	00	00

Table 3. Zones of inhibition (mm) of the methanol extracts.

Test Organism	Bark	Root	Leaves
K. pneumonia	00	10	00
S. aureus	14	15	13
E. coli	15	00	00
P. stauti	11	16	15
B. subtilis	16	15	00
S. typhi	00	00	00

Table 4. MIC of the petroleum spirit extracts of the root, leaves and stem bark of *Ficus thoningii.*

Test organism	Type of extract	Concentrations (mg/ml)						
		50.00	25.00	12.50	6.00	3.00	1.50	0.75
E. coli	Root	+	+	++	++	++	+++	+++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++
S. aureus	Root	+	+	++	++	++	+++	+++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++
K. pneumoniae	Root	+	+	++	++	++	++	+++
	Leaves	+	+	++	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++
S. typhi	Root	+	+	++	++	++	+++	+++
	Leaves	-*	+	+	+	++	++	+++
	Bark	-*	+	+	+	++	++	+++
P. stauti	Root	+	+	++	++	++	+++	+++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-*	+	++	++	++	+++	+++
B. subtilis	Root	+	+	++	++	++	+++	+++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++

-* = MIC, + = Slightly turbid, ++ = Moderately turbid, +++ = Highly turbid.

niae, S. aureus, P. stauti and B. subtilis. The petroleum spirit extract which is mainly a mixture of fatty acids showed a slightly lower degree of activity than the methanol extracts as in Tables 4 and 6. From Tables 5 and 7 it is seen that at a concentration of 50 mg/ml the stem bark extract of this plant can kill some of the organisms, while at the same concentration the petroleum spirit extracts only inhibited their growths as shown in Tables 4 and 6.

Table 5. MBC of petroleum spirit extracts of root, leaves and stem bark of *F. thoningii.*

Test organism	Type of extract	Concentration (mg/ml)						
		50.00	25.00	12.50	6.00	3.00	1.50	0.75
E. coli	Root	+	+	++	++	++	+++	+++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++
S. aureus	Root	+	+	++	++	++	+++	+++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++
K. pneumoniae	Root	+	+	++	++	++	++	+++
	Leaves	+	+	++	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++
S. typhi	Root	+	+	++	++	++	+++	+++
	Leaves	+	+	+	+	++	++	+++
	Bark	+	+	+	+	++	++	+++
P. stauti	Root	+	+	++	++	++	+++	+++
	Leaves	+	+	+	++	++	++	+++
	Bark	+	+	++	++	++	+++	+++
B. subtilis	Root	+	+	++	++	++	+++	+++
	Leaves	+	+	+	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++

-* = MIC, + = slightly turbid, ++ = moderately turbid, +++ = highly turbid.

Table 6. MIC of the methanol extracts of the root, leaves and stem bark of *Ficus Thoningii.*

Test organism	Type of extract	Concentration (mg/ml)						
		50.00	25.00	12.50	6.00	3.00	1.50	0.75
E. coli	Root	-	-	-	-*	+	+	++
	Leaves	-	-	-*	+	+	++	++
	Bark	-	-	-	-*	+	+	++
S. aureus	Root	-*	+	+	++	++	++	+++
	Leaves	-	-	-*	+	+	++	+++
	Bark	-	-	-	-*	+	+	++
K. pneumoniae	Root	-*	+	+	++	++	++	+++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-	-	-*	+	+	++	++
S. typhi	Root	-*	+	+	++	++	++	+++
	Leaves	-	-*	+	+	++	++	++
	Bark	-	-	-	-*	++	++	++
P. stauti	Root	-	-*	+	+	++	++	++
	Leaves	-	-*	+	+	++	++	++
	Bark	-	-	-	-*	+	+	++
B. subtilis	Root	-	-*	+	+	++	++	++
	Leaves	-	-*	+	+	++	++	++
	Bark	-	-	-*	+	+	++	++

-* = MIC, + = slightly turbid, ++ = moderately turbid, +++ = highly turbid.

Table 7. MIC of the methanol extracts of the root, leaves and stem bark of *F. Thoningii.*

Test organism	Type of extract	Concentration (mg/ml)						
		50.00	25.00	12.50	6.00	3.00	1.50	0.75
E. Coli	Root	-	-	-*	+	+	++	++
	Leaves	+	+	++	++	++	+++	+++
	Bark	-	-*	+	+	++	++	++
S. aureus	Root	-	-*	+	+	++	++	++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-	-*	+	+	++	++	++
K. pneumoniae	Root	-	-*	+	+	++	++	++
	Leaves	-*	+	+	++	++	++	++
	Bark	-	-*	+	+	++	++	++
S. typhi	Root	-	-*	+	+	++	++	++
	Leaves	-*	+	+	++	++	++	+++
	Bark	-*	+	+	++	++	++	+++
P. stauti	Root	-*	+	+	++	++	++	+++
	Leaves	-	-*	+	+	++	++	++
	Bark	-	+	+	+	++	++	++
B. Subtilis	Root	-*	+	+	++	++	++	+++
	Leaves	+	+	++	++	++	+++	+++
	Bark	-	-*	+	+	++	++	++

-* = MIC, + = slightly turbid, ++ = moderately turbid, +++ = highly turbid.

Conclusion

F. thoningii can be used in therapeutic preparations. The methanol extracts of the root, leaves and the stem bark are highly favoured in this regard and have proved to be more efficacious as is seen in this *in* vitro studies. These results agree with the claims by our ethno medicinal practitioners that the plant parts can be used in the treatment of colds, sore throats, diarrhea and wounds.

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