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

**Ndukwe, I. G.*,** Bello, A. I., Habila, J. D. and John, C.

Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

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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 thoningii*, 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 latex 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
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 subtilis 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 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, flavor-noids, 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. pneu-

| 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 |

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