



Phytoconstituents evaluation and antimicrobial efficacy of the crude flavonoids and saponins rootbark extracts of *Terminalia avicennioides* and *Ficus polita*

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ABSTRACT

Introduction: Plant is a friend to man in survival as it gives him food, shelter and medicine beyond the ages of human civilization. This paper evaluates the phytochemical constituents and the antimicrobial activities undertaken on *Terminalia avicennioides* and *Ficus polita*.

Methods: Phytochemical screening was conducted on the root extracts according to the standard procedures. The hole-in-plate disc diffusion technique was used to determine the antimicrobial activities of the crude saponins and crude flavonoids against the tested microorganisms used in this study.

Results: The results revealed the presence of alkaloids flavonoids, saponins, sterols, phlobatannins and terpenoids. The antimicrobial activities presented as diameter of inhibition zones showed high activity value of 34.70 ± 0.57 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at a concentration of 100 mg/mL and by crude flavonoids portion of *T. avicennioides* while the least activity was shown by crude saponins portion of *F. polita* at a concentration of 25 mg/mL with value of 9.67 ± 0.58 mm against *Shigella dysenteriae*. The crude flavonoids inhibited the growth of *Candida albicans* at all concentrations while resistances were found towards the crude saponins portion of both plants. Hence, flavonoids extractives from the two plants appeared to be more effective than the saponins against the tested microorganisms.

Conclusion: These findings justify their potential use as drug-plant against bacterial-related infections in African traditional medical system and also suggest a possible insight for the isolation of bioactive chemotherapeutic agents from *T. avicennioides*.

Implication for health policy/practice/research/medical education:

The phytochemical evaluation of the rootbark extracts of *T. avicennioides* and *F. polita* revealed the presence of alkaloids flavonoids, saponins, sterols, phlobatannins and triterpenes. Anthraquinones was absent in both extracts. The extractives exhibited tremendous activities on the test microbes; however, crude saponins portion was inactive against *C. albicans*. These results are not unrelated to the phytochemicals present in the extracts. The results are indicative of the possible use of these plants for preparation of new drugs.

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Introduction

Ethnomedicine is a multi-disciplinary complex system which involves the use of plants, spirituality and the natural environment and has been the source of healing people for millennia (1). The use of plants for medicinal

purposes dates back to the Vedic period (10th-12th BC). However, up to a few decades ago herbal medicines were replaced by synthetic medicines due to their fast-relative effects. More so, side effects posed by allopathic medicines are reverting the global trend towards green medicine.

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According to the World Health Organization (WHO), an investigation into the safety, properties and effectiveness of medicinal plants should be continued, bearing in mind that they stand the chance of unfolding variety of potent drugs especially antimicrobial compounds. Being the largest producer of herbal medicines, India is considered the world botanical garden (2).

The threat of rapid development of drug-resistance by pathogenic organisms has led to the emergence of new concepts in drug therapy which is the 'combination therapy'. This therapeutic approach to treatment specifically favours the use of crude natural products which are thought to have many active ingredients working in synergy to exert the observed pharmacological effect (3). However, the variation of the constituents in herbal preparations could be due factors like to genetic, cultural, and environmental; have made the use of these medicines more challenging than it would have been (4). The global campaigns for the search of new bioactive agents with fewer side effects and greater activities have led to the screening for bioactive compounds in many medicinal plants of the Tropics (5) especially *Terminalia* species (Combretaceae) and *Ficus polita*. The selection of these species was based on its application in traditional medicine in Africa and other parts of the world for the treatment of microbial infections. Therefore, studies into medicinal plants with antimicrobial properties are expected to enhance and validate the use of such plants against bacteria related infections. The active principles of many drugs found in plants are due to secondary metabolites (6). Hence, basic phytochemical investigation of the extracts for their main phytochemicals is very important. *T. avicennioides* Guill. & Perr. family: Combretaceae, are locally called in various Nigerian languages as *Kpace* in Nupe, *Kpayi* in Gwari, *Baushe* in Hausa, *Igiodan* in Yoruba, and *Edo* in Igbo; have been reported to possess antimicrobial activities (7-11).

Ficus polita Vahl. is a tropical evergreen shrub or small tree of the family Moraceae, usually grow up to 15 m and reaching up to 40 m tall. The leaves are occasionally harvested from the wild for food. Like most other *Ficus* species, the fruits are sometimes eaten as aphrodisiac and stimulant. *Ficus polita* is known as Hartblaarvy, Heart-leaved fig, polish fig, rubber plant, wild rubber fig, or wild rubber tree. It is locally called "durumi" in Hausa. The fruit and young leaf are chewed for dyspepsia (12), infusions from the bark and roots are utilized in the management of infectious diseases, abdominal pain, dyspepsia and diarrhoea like many of the species from this family (12-14).

In our continuous search for medicinal plants with anti-infective potency from this part of the Tropics especially those with antimicrobial effects, the authors carried out a survey on these plants with the view of finding the most active for further studies. In this regard, this study was

designed to evaluate the phytochemical constituents and the antimicrobial activities undertaken on *T. avicennioides* and *F. polita*.

Materials and Methods

Plant collection

Fresh root barks of *T. avicennioides* Guill. & Perr. and *F. polita* Vahl. were collected respectively from Ayetoro-Gbedde, Ijumu Local Government Area, Kogi State and Goniri Village, Bama Local Government Area, Borno State, Nigeria. The samples were identified by a Taxonomist, and the plant samples were deposited in herbarium of Research Laboratory in the Department of Chemistry, University of Maiduguri, Nigeria.

Sample preparation and extraction

The root barks samples were dried under shade with intermittent weighing until a constant weight was obtained. The samples were pulverized using wooden mortar and pestle. The powdered materials (400 g) were extracted with the mixture of two solvents, 80% ethanol and 20% distilled water using reflux apparatus. The extracts were filtered and concentrated under reduced pressure and temperature.

Separation of crude saponins and flavonoids

The method of Won et al (15) was adopted. Twenty grams of the crude extracts of each sample were dissolved in n-hexane, the insoluble residue was then suspended in distilled water and diethyl ether added to it. The distilled water fraction was then partitioned with n-butanol. The water portion was then discarded. The n-butanol fraction was then treated with 1% KOH and separated to afford the first n-butanol fraction (saponins). To the residual 1% KOH portion was added conc. HCl and then further partitioned with n-butanol until exhaustion to obtain the second n-butanol fraction (flavonoids) as schematically presented in Figure 1.

Phytochemical evaluation

Phytochemical evaluations of the root bark extracts of *T. avicennioides* and *F. polita* was independently carried using conventional phytochemical methods as described earlier by several authors. The tests for steroidal nucleus (Salkowski's test) and terpenoids were described by (16), free and combined anthraquinones, tannins and phlobatannins tests were as described by (17), test for saponins by (18), alkaloids by (19) and flavonoids by (17,19,20).

Antimicrobial evaluation

Test microorganisms

Five pathogenic microorganisms namely: *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Staphylococcus* and *Candida albicans* were clinical

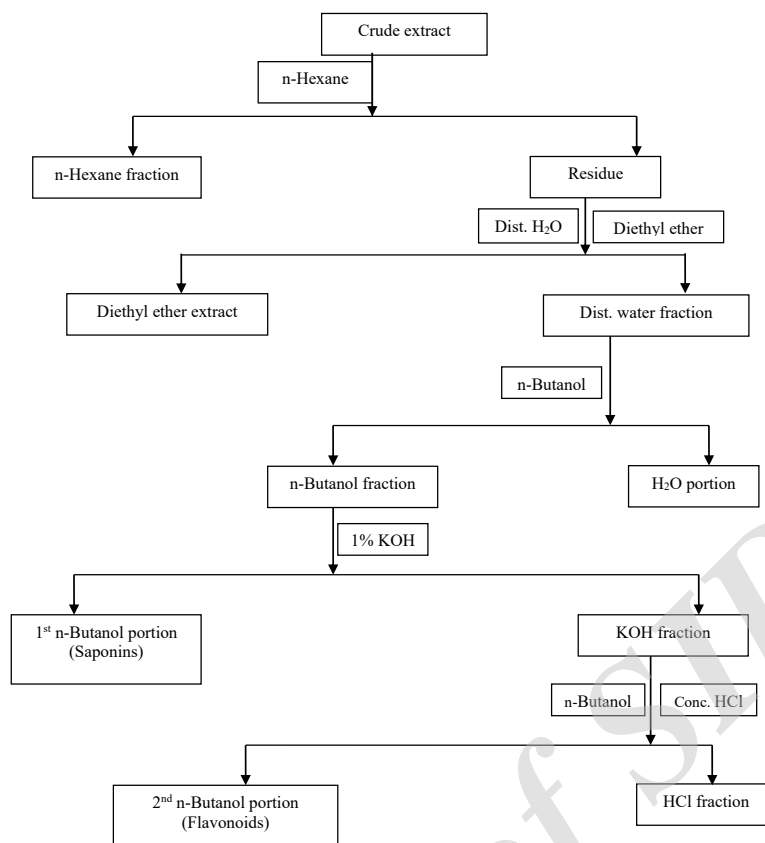


Figure 1. Fractionation of crude saponins and flavonoids (15).

isolates obtained from the Department of Microbiology and Department of Veterinary Medicine, University of Maiduguri.

Screening for antimicrobial activity

The root barks extracts of *T. avicennioides* and *F. polita* were subjected to preliminary antimicrobial evaluation on the mentioned strains using the hole-in-plate disc diffusion technique as described by Forbes et al (21) and adopted by Usman et al (22). Wells were bored on the media using 6 mm cork borer and were filled with 0.2 mL aliquots of various concentrations of the extracts (100 mg/mL, 50 mg/mL and 25 mg/mL equivalent to 20, 10 and 5 mg/hole respectively). The agar plates were then kept in an incubator at 37°C for 24 hours. After incubation, diameters of inhibition zone for each extract were measured in millimeters using a transparent metre rule. Each extract was tested in triplicates.

Results

The results of phytochemical contents of the crude extracts of *T. avicennioides* and *F. polita* are presented in Table 1 while the antimicrobial effects of the crude saponins and crude flavonoids fraction of the root bark extracts of the two-plant species are shown in Table 2.

The Phytochemical screening of both extracts revealed the presence of flavonoids, terpenoids, phlobatannins

and tannins. *T. avicennioides* contained alkaloids and saponins. None of the extracts showed the presence of anthraquinones (Table 1).

Antimicrobial evaluation

The results of antimicrobial studies showed that the crude saponins appeared to be less active compared to the crude flavonoids at all the dosages against the test microorganisms except against *S. aureus* where the crude saponins portion of *T. avicennioides* was more effective than crude flavonoids with DIZ values of 34.70 ± 0.58 and 30.70 ± 0.58 mm, respectively at the highest dosage of 20 mg/hole as shown in Table 2. Moreover, *T. avicennioides* was more effective than *F. polita*.

Table 1. Phytochemical evaluation of the root bark crude extracts of *Terminalia avicennioides* and *Ficus polita*

| Constituents | <i>T. avicennioides</i> | <i>F. polita</i> |
|-------------------------|-------------------------|------------------|
| Alkaloids | + | - |
| Flavonoids | + | + |
| Phlobatannins | + | + |
| Free Anthraquinones | - | - |
| Combined Anthraquinones | - | - |
| Tannins | + | + |
| Terpenoids | + | + |
| Saponins | + | - |

Table 2. Antimicrobial susceptibility pattern of the root bark extracts of *Terminalia avicennioides* and *Ficus polita* against some microorganisms

| Organisms/Plant species | Extract fractions | | | | | |
|-------------------------------|---|------------|------------|---|------------|------------|
| | Crude saponins | | | Crude flavonoids | | |
| | Concentrations/diameter of inhibition zone (mm) as Mean±SEM | | | Concentrations/diameter of inhibition zone (mm) as Mean±SEM | | |
| | 20 mg/hole | 10 mg/hole | 5 mg/hole | 20 mg/hole | 10 mg/hole | 5 mg/hole |
| <i>Staphylococcus aureus</i> | | | | | | |
| <i>T. avicennioides</i> | 34.70±0.58 | 30.70±0.57 | 22.30±0.58 | 30.70±0.58 | 24.30±0.58 | 18.70±0.58 |
| <i>F. polita</i> | 24.00±1.15 | 20.00±0.00 | 14.46±0.58 | 29.67±0.58 | 24.00±1.00 | 18.33±1.15 |
| <i>Shigella dysenteriae</i> | | | | | | |
| <i>T. avicennioides</i> | 19.70±0.58 | 15.70±0.58 | 11.33±0.58 | 24.70±0.58 | 21.30±0.58 | 14.30±0.58 |
| <i>F. polita</i> | 15.00±0.0 | 11.67±0.58 | 9.67±0.58 | 23.67±0.58 | 20.00±0.00 | 14.67±0.58 |
| <i>Salmonella Typhi</i> | | | | | | |
| <i>T. avicennioides</i> | 19.70±0.58 | 30.70±0.58 | 22.30±0.58 | 30.70±0.58 | 24.30±0.58 | 18.00±0.58 |
| <i>F. polita</i> | 16.0±0.00 | 12.67±0.58 | 11.00±0.00 | 27.67±0.58 | 22.33±2.80 | 16.33±0.58 |
| <i>Pseudomonas aeruginosa</i> | | | | | | |
| <i>T. avicennioides</i> | 18.00±1.00 | 13.30±0.57 | 11.00±0.00 | 34.70±0.58 | 28.70±0.58 | 20.00±0.00 |
| <i>F. polita</i> | 18.00±0.00 | 13.67±0.58 | 10.33±0.58 | 30.67±0.58 | 26.33±0.58 | 20.67±0.58 |
| <i>Candida albicans</i> | | | | | | |
| <i>T. avicennioides</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 22.30±0.58 | 18.30±0.58 | 13.00±0.00 |
| <i>F. polita</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 24.67±0.58 | 20.33±1.20 | 17.33±0.58 |

Data are mean of triplicate values (n = 3).

Discussion

The dependence on traditional medicine may be due to the perceived activities of medicinal plants. Phytochemical screening conducted on the root extracts (Table 1) revealed the presence of compounds which are known to exhibit therapeutic activities (23). These compounds are flavonoids, saponins, sterols and triterpenes. The presence of secondary metabolites in *T. avicennioides* is an indication that the root extract is of pharmacological importance and also justified their potential use as a drug by local people. The plants contained terpenes which are believed to be useful in the prevention and therapy of several diseases. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties (24). The presence of this compound probably justifies the use of the selected plants for the treatment of microbial infections. Flavonoids are phenolic substances known to be synthesized by plants in response to microbial infections and have been reported to exhibit antimicrobial properties against wide variety of microorganisms. The activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and also to complex with bacterial cell walls (25). In addition, sterols present in most of our plant samples, have been reported to have antibacterial properties (26) and are very important compounds especially due to their relationship with compounds like sex hormones (27). The absence of

alkaloids and saponins in *Ficus polita* Vahl in the present work is in contrast with the opinion of Gills (28) and Kasolo et al (29) who noted that saponins and alkaloids were two of the active constituents. Alkaloids are known to have antimicrobial properties possibly due to their ability to intercalate with DNA of the microorganisms. Also, the presence of saponins in *T. avicennioides* is in agreement with previous findings (30).

In the present study, the results of antimicrobial property of the 2 root bark extracts (Table 2) against tested microorganisms varied on species tested and. However, both root extracts showed inhibition zones against *S. typhi*, *S. dysenteriae*, *P. aeruginosa* and *S. aureus*, respectively. Nevertheless, resistances against *C. albicans* exhibited by both extracts do not indicate the absence of bioactive constituents, nor that the roots extracts were completely inactive; but rather resistance at the working concentration. It was noticed that *T. avicennioides* which showed the highest antibacterial activity could probably be due to the fact that the rate of the active constituents in the root extract is higher than that obtained in *F. polita*. Preliminary phytochemical screening of *T. avicennioides* roots showed that they possessed flavonoids, terpenes and saponins. Phytoconstituents such as flavonoids, triterpenes and saponins have been reported to inhibit bacterial growth and to be protective to plants against microbial infestations (30). The presence of saponins in this plant must have elicited direct antibacterial activity and suppression of bacterial virulence resulting to the

antimicrobial activity observed in this study (28).

Conclusion

The results of the present study offer a scientific basis for the use of the studied extracts in the treatment of infectious diseases. These plants species can be regarded as promising resources for drug development. However, further investigations are necessary in order to draw solid conclusions.

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Authors' contributions

All authors contributed to the study. AUK and OOI acquired data. AMF and HU prepared the draft. HU and IAA revised the manuscript critically for important intellectual content and HU submitted it. All read and confirmed the article ready for publication.

Conflict of interests

The authors declared no competing interests exist.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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