PHYTOCHEMICAL AND ANTI-TYPHOID POTENTIAL OF *TERMINALIA AVICENNIOIDES*

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Abstract

The phytochemical screening and the anti-typhoid potential of *Terminalia avicennioides* leaf extract was investigated in other to verify its ethnomedicinal claim in the treatment of typhoid fever.Thin layer chromatography (TLC), was used to determine the presence of the phytochemical constituent and the anti-typhoid activity of methanol leaf extract, Acetone leaf extract, Petroleum ether leaf extract and methanol solube fraction of *Terminalia avicennioides* was tested against *Salmonella typhi* collected from Ahmadu Bello University (ABUTH) Shika, Zaria, using the Agar well diffusion method. The phytochemical screening carried out on the methanol leaf extract using thin layer chromatography (TLC) revealed that phenolic compounds and terpenoids were present and alkaloid absent. The anti-typhoid activity of *T. avicennioides* leaf extracts against *Salmonella typhi* showed significant inhibitory activity against *Salmonella typhi* with the methanol soluble fraction showing the highest zone of inhibition (31±0.62) mm. The Minimum Inhibitory Concentration (MIC) value obtained was 6.25 mg/ml while Minimum Bactericidal Concentration (MBC) was 12.5 mg/ml. It can be concluded that the leaves of *Terminalia avicennioides* possess anti-typhoid activity.

Keywords: *Terminalia avicennioides*, Phytochemical, Chromatography, Typhoid, Ethnomedicinal

1.0 Background of the Study

Terminalia avicennioides Guill. and Perr. (Combretaceae) is a tropical herb common in North central vegetation of Nigeria. It is locally called *bushel* (Hausa), *Pace* (Nape), *Adin* (Yoruba) and *Edo* (Igbo) (Aliyu *et al*., 2018). It is a tree with yellowish brown hard and durable wood (Bur kill, 1985). In ethnomedicine, the plant is known to be active against trypanosomes (Bulus *et al*., 2008). Extracts of the stem bark and roots of *T. avicennioides* are used for the treatment of bacterial infections such as diarrhea and gastrointestinal disorders (Abdullahi *et al*., 2001), bloody sputum (tuberculosis) and cough in humans (Mann *et al*., 2007). The stem bark of *T. avicennioides* have been found to be fungicidal against the dermatophytic *Epidermophyton floccosum*, *Microsporum gypseum* and *Trichophyton mentagrophytes* and fungistatic against *Candida albicans* (Baba-Moussa *et al*., 1999).

Extracts of the stem bark of *T. avicennioides* contain large quantities of saponins and tannins (Baba-Moussa *et al*., 1999; Chiroma *et al*., 2018), which might be responsible for the good antifungal effects.

Typhoid fever is an acute illness associated with fever that is most often caused by the bacteria *Salmonella typhi*. It can also be caused by *Salmonella paratyphi*, a related bacteria that usually leads to a less severe illness. It is known as an enteric fever transmitted through ingestion of food or drink that is contaminated by the faeces or urine of infected persons (Muhammad *et al*., 2021). Each year the disease affects at least 16 million persons world-wide, most of whom reside in the developing countries of Southeast Asia and Africa (CDC, 2003).The treatment of typhoid fever is done by the use of antibiotics, but increasing resistance rates to the primary agents used (ampicillin, chloramphenicol, co-trimoxazole as well as quinolones) have been associated with complications and increased severity of illness (Anyanwu and Okoye, 2017).

2.0 MATERIALS AND METHODS

2.1 Collection and identification of plant

The plant, *Terminalia avicennioides*, (Guill and Perr) was obtained from Tashan Fulani village in Zaria Local Government of Kaduna State. The plant's identity was confirmed at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria with a voucher number 901452.

2.2 Extraction of the Leaves of *Terminalia avicennioides*

The leaves were dried under shade and milled into powder. The powdered sample (200g) was macerated with 1000ml of 70% Methanol, acetone and petroleum

ether rsepectively using maceration techniques (Tiwari *et al*., 2011) and drained after 48 hrs. The petroleum ether marc was further partitioned with methanol in a separating funnel to get methanol soluble fraction to remove the non-polar fraction. The marc was further macerated with 70% methanol for 24 hours to get the polar fraction (Methanol soluble fraction).

2.3 Preliminary phytochemical screening using Thin layer chromatography (TLC)

Thin Layer Chromatography (TLC) technique was used to determine the type of phytochemicals present in the leaves of the plant using standard Laboratory methods. The chromatograms were developed at room temperature and visualized under daylight and U.V light at both 254 nm and 366 nm. The location of the separated compounds were ascertained and marked. The components were analysed by calculating the retention factor (Rf) values of the spots. The different chromatograms developed were sprayed with sulphuric acid (a general spraying reagent), dragendorff spray (For the detection of alkaloid), p-anisaldehyde spray (For the detection of terpenoids) and ferric chloride spray (For the detection of phenolic compounds).

2.4 Test Organisms

The organism *Salmonella typhi,* was obtained from the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital, Zaria for the susceptibility tests. The organism was used after the identity was confirmed at the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria. The stock culture was maintained on agar slants at 4^{o}C in the refrigerator. Active culture for experiments were prepared by transferring a loopful of cells from the stock culture to sterile bottles of Nutrient broth and then incubated for 24 hours at 37^{0}C.

2.5 Antimicrobial Susceptibility tests

The Susceptibility of the test organism to the extract was evaluated using the agar well diffusion method. Suspension of organisms were adjusted to 0.5 McFarland turbidity standard (Abdulsalami *et al.*, 2019). The inoculum was swabbed on the surface of sterile Mueller-Hinton agar plate, it was allowed for 10 to 15 min to pre-diffuse. Wells were made in the agar using 8mm sterile cork borer and sealed with one drop of melted Mueller-Hinton agar. Different concentrations of the extract from 12.5mg/ml to 100mg/ml were used .Using a sterile syringe, 0.1ml of the dissolved extracts were introduced into the wells made on the agar plates. The plates were then allowed to stay for one hour at room temperature for effective diffusion to take place and then incubated at 37^{0}C for 24hours.The diameters of the zones of inhibition were measured in millimeter with a transparent ruler and recorded. This procedure was repeated three times and average diameters were recorded. A positive control (Ciprofloxacin disc 5µ, oxoid) was used while 0.1ml distilled water dispensed into one of the wells served as the negative control.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extract was determined using the Broth dilution method. Nutrient broth was prepared according to the manufacturer's instructions. It was dispensed into ten test tubes followed by the dilution of the extract in the nutrient broth to obtain concentration of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.12mg/ml, 1.56mg/ml, 0.78mg/ml, 0.39mg/ml, 0.19mg/ml. using a syringe, 1ml of the test organism adjusted to 0.5 McFarland standard was inoculated into each test tube and mixed thoroughly on a vortex mixer and then incubated at 37^{0}C for 24hours. The test tubes were observed for turbidity. The tube with the lowest concentration of the extract showing a clear solution was considered as the MIC (Coyle, 2005).

2.7 Determination of Minimum Bactericidal Concentration (MBC)

The content of the test tubes examined for MIC which showed no growth (turbidity) were sub cultured into sterile nutrient agar plates by dipping a sterile wire loop and streaking the surface of the agar plates and then incubated at 37^{0}C for 24hours after which they were checked for growth. The plate with the lowest concentration of the extract without growth was regarded as the MBC. (NCCLS, 2000).

3.0 RESULTS

3.1 Chromatographic Analysis of the Leaves of *T. avicennioides*

The developed chromatogram revealed seven spots after spraying with sulphuric acid (A general spray),greenish black spots and a red colored spot after spraying with ferric chloride spray and p-anisaldehyde

spray respectively and one spot when viewed under U.V light (254nm).

Table 3.1: Chromatographic resolution of Methanolic Extract of the Leaves of *T. avicennioides*

DETECTING AGENT	OBSERVATION	Rf Value	INFERENCE
Day Light	No visible spot		-
U.V Light	One red spot		-
Spraying reagent			
Sulphuric spray	Seven colored spots	0.2, 0.29, 0.38, 0.5, 0.67, 0.71, 0.75	Detection of compounds
Ferric chloride	Greenish black colored spots	0.55, 0.71	Phenolic compounds present
p-anisaldehyde	Red colored spot	0.71	Terpenoids present
Dragendorff	No change in color	-	Alkaloid absent

3.2 Anti-typhoid studies on the leaves of *T. avicennioides*

The evaluation of the anti-typhoid activity shows that the methanol soluble fraction exhibited the highest zone of inhibition (31 ± 0.62) mm followed by the Methanol extract (crude) then the acetone extract (18± 0.88)mm (Table 2). The pet ether extract only showed the zone of inhibition (17± 0.58) mm at a concentration of 100mg/ml.

Table 2: Zone of inhibition (mm) at different concentration of plant (*T. avicennioides)* extract

EXTRACT	Zone of Inhibition (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Methanol extract	21± 0.44	16± 0.62	16±0.44	15±0.44
Acetone Extract	18±0.88	17±0.71	17±0.82	15±0.62
Methanol soluble extract	31±0.62	24±0.94	22±0.62	18±0.71
Petroleum ether	17 ± 0.58	-	-	-
Ciprofloxacin disc (5µ)	32±0.58			

4.0 DISCUSSION

Plants have been used for the management of a wide range of diseases for a long time in form of decoction and infusion, and this may be due to the presence of a variety of secondary metabolites that are said to be responsible for their medicinal properties. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and detection of plant constituents. The phytochemical analysis shows that the methanol leaf extract of *T. avicennioides* contains secondary metabolites such as phenolic compouds and terpenoids. This is consistent with the report of Chiroma *et al*. (2018) except for the presence of alkaloids. Terpenoids have antiseptic, anti-inflammatory and antimicrobial properties. They constitute the active constituents of a number of medicinal plants and are of current interest for their potential as future drugs (Evans, 2008; Upadhyay *et al*., 2014). Phenolic compounds such as tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo *et al*., 1991; Upadhyay *et al*., 2014). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al*., 1998).

The extract produced varying zones of growth inhibition against the test organism. The largest zone of inhibition recorded for *S. typhi*, was found in methanol soluble fraction at a concentration of 100mg/ml indicating the highest degree of activity against the organism. The methanol crude extract also showed a significant activity at a concentration of 100mg/ml followed by acetone extract. The pet ether fraction exhibited a zone of inhibition only at a concentration of 100mg/ml. The zones of inhibition decreased as the concentration of the plant extracts were reduced. It can be deduced that the bioactive constituents in the Methanol soluble fraction were responsible for the anti-typhoid activity which could be comparable to that of the antibiotic (ciprofloxacin) used in the study, although the latter exhibited a wider zone of inhibition, increasing the concentration of the plant extract might be more effective against *S. typhi*. The MIC recorded was 6.25mg/ml which suggests that at this concentration, the growth of the organism was inhibited. The MBC was 12.5mg/ml which implies that the organism was killed at this concentration. Minimum inhibitory concentrations (MICs) are considered the 'gold standard' for determining the susceptibility of organism to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. MICs are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation and the minimum bacteria concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic free media (Jennifer, 2001).

5.0 Conclusion

Studies on the methanol leaf extract of T. *avicennioides* revealed that it contains secondary metabolites that tend to be responsible for the observed anti-typhoid activity, buttressing the claim for its use in folkloric medicine in the treatment of enteric infection. Further research on the screening of the secondary metabolites of these medicinal plants for biological and pharmacological studies will be necessary as well as the isolation of active compounds and their structural elucidation for the maximal use of the medicinal plants.

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