



## **Trema orientalis Linn. Blume: Susceptibility Patterns of Selected Multiple Antibiotic Resistant Bacteria and Fungi to the Leaf Extracts**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Author POF designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AOO supervised the study. Author PA managed the literature searched. Author OA performed the statistical analysis, managed the analysis. All authors read and approved the final manuscript.

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### **ABSTRACT**

This study investigates the antifungal and antibacterial activities of extracts of *Trema orientalis* linn. Blume. The selected isolates used in the study includes *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Streptococcus faecalis*, *Shigella dysenteriae*, *Proteus mirabilis*, Haemolytic *Streptococcus viridian*, *Aspergillus niger*, *Candida albicans* ,and *Aspergillus flavus*. The antibiogram profile of the organism was obtained using the disc diffusion method. Tannin, flavonoid, Terpenoid, Saponin, and cardiac glycosides were found present in *T. orientalis*. The antibiotic sensitivity test reveals the presence of multiple antibiotic resistant bacteria. The agar well diffusion method was used to assay the antibiotic activities of the extract. The extracts were prepared from *T. orientalis* using Methanol and petroleum ether as extraction solvents. The antibacterial assay shows the efficacy of the methanol and petroleum ether extracts except on *S. aureus* ATCC 43300 and *Shigella dysenteriae*. The highest zones of inhibition by methanol and petroleum ether extracts were recorded on *Salmonella typhi* of 22.003 ± 0.003 mm

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and  $32.002 \pm 0.002$  mm, respectively. The fungal isolates were susceptible to the extracts with the zones  $11.67 \pm 0.33$  mm and  $13.67 \pm 0.33$  mm. The results of the sensitivity test compared well with that of the antimicrobial test. The Minimum Inhibitory Concentration ranged between 3.125- 25.00 mg/mL. *T. Orientalis* contains bioactive compounds that has antimicrobial effects. The leaf of *Trema orientalis* has broad - spectrum activity, hence *T. Orientalis* methanol and Petroleum ether extracts is recommended for exploration as source of antimicrobial agents.

**Keywords:** Antibacterial; antifungal; clinical isolates; trema orientalis; resistance.

## 1. INTRODUCTION

Medicinal plants contain active components, which is being exploited in traditional medical practice for the treatment of various illnesses over the years [1]. These active components which usually include terpenoids, alkaloids saponins etc are potentially useful for the development of therapeutic agents.

Medicinal plants have a therapeutic ability on infectious diseases and mostly generate little side effects [2]. In pharmaceutical field, medicinal plants are mostly used for the wide range of substances present in plants have been used to cure chronic as well as infectious diseases [3]. Medicinal plants are increasingly moving into the pharmaceuticals, cosmetics, and nutraceuticals [4] estimated conservatively that, between 60 - 90% of the population of the non-industrialized countries rely on medicinal plants as source of their health care needs, either totally or partially. In pharmaceutical field, medicinal plants are mostly used for the wide range of substances present in plants have been used to cure chronic as well as infectious diseases [4].

*Trema orientalis* is a medicinal shrub or tree belonging to the family Ulmaceae. Locally, it is known as a charcoal tree or gunpowder tree [5]. The Ulmaceae family consists of 15 genera, and 200 species are distributed over tropical and temperate regions of the northern hemisphere [6]. Locally, it is known as a charcoal tree or gunpowder tree. It is named for Chikan or Jibon in Bengali, Nalita in English and Gio in Hindi. The leaves and fruit are reported to be eaten in the Democratic Republic of Congo [7]. The leaves and the bark are used to treat cough, sore throats, asthma, bronchitis, gonorrhoeas, yellow fever, toothache, and as an antidote to general poisoning [8]. *Trema orientalis* is also used in traditional medicine to treat and manage many diseases such as hypertension, malaria, diarrhea, female sterility. It's also used as an inhalant, anti-helminthic, antidote to general

poisoning, febrifuge, anti-dysentery, anti-convulsion, anti-diabetic, analgesic, anti-sickling [7, 9]. These activities are due to the presence of biologically active compounds such as polyphenols, saponins, flavanoids, triterpenoid which have been confirmed in this plant [10]. Pathogenic microorganisms, such as bacteria, viruses, parasites and fungi cause infectious diseases, which are considered a significant threat to human health especially with the increase in antibiotic resistance. This study assays the antifungal and antibacterial activity of the Methanol and Petroleum ether extracts of *T. orientalis*.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification Test Organisms

Pure culture of clinical Isolates was obtained from Donbosco hospital, Akure, Ondo State. Typed microorganisms includes: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E.coli*, *Salmonella typhi*, while clinical isolates includes: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Streptococcus faecalis*, *Shigella dysenteriae*, *Proteus mirabilis*, *Haemolytic Streptococcus viridian*, Fungi isolates includes: *Aspergillus niger*, *Candida albicans*, *Aspergillus flavus*. The isolates were confirmed using conventional techniques [11].

### 2.2 Antibiotics Susceptibility Profile

Antibiotic susceptibility testing was performed using conventional antibiotic sensitivity disc using the Kirby Bauer disk diffusion method [12] and interpretation, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. The antibiotics used include: Gentamycin (CN) 10 µg, Streptomycin (S) 30 µg, Pefloxacin (PEF) 10 µg, Tarivid (OFX) 10 µg, Septrin (SXT) 30 µg, Chloramphenicol (CH) 30µg, Sparfloxacin (SP) 10 µg, Ciprofloxacin (CPX) 10 µg,

Amoxicillin (AM) 30 µg, Augumentin (AU) 30 µg, Ampiclox (APX) 30 µg, Erythromycin (E) 10 µg, Zinacef (Z) 20 µg, and Rocephin (R) 25 µg.

### 2.3 Collection of Plant

The fresh leaves of *Trema orientalis* was harvested from the forest at Kajola Layade via Ile-Ife, Osun State and at Federal University of Technology Akure (FUTA) campus where they were growing naturally in their habitat and authenticated in the Department of Crop, Science and Pest Management, at The Federal University of Technology, Akure. The leaves were air-dried for two weeks and ground into a fine powder with an electric blender (Phillips Model blender), after which it was stored in an air tight container before use. The crude extracts were obtained by soaking 400 grams of dried plant leaves and stem powder in 1000mL Methanol and Petroleum ether separately, stirring intermittently, for 72 hrs, sieved with a muslin cloth and filtered with No 1 Whatman filter paper. Extract was further concentrated at  $27 \pm 2^\circ\text{C}$ , the weights of the dried extracts were measured and transferred into a clean container and kept in the refrigerator before use.

#### 2.3.1 Phytochemical analysis of plants

Qualitative and quantitative phytochemical analyses were carried out on the extracts using standard chemical methods, as described by [14].

#### 2.3.2 Reconstitution of Extracts

The extract was reconstituted by dissolving varying of the plant extracts separately in 1mL of already prepared Tween 20(20%) to get a concentration of each extract 0.50 mg/mL 50 mg/mL [15].

### 2.4 Standardization of Inoculum

The freshly prepared nutrient broth was inoculated with test organisms and incubated for 24 h at  $37^\circ\text{C}$ . A 0.2 mL aliquot from the cultured broth was aseptically dispensed into 20 mL of freshly prepared nutrient broth and incubated for 2 to 3 h at  $37^\circ\text{C}$  to standardize to 0.5 McFarland standard of Barium sulfate solution which is equivalent to  $1 \times 10^6$  CFU [16].

### 2.5 Antimicrobial Assay of Leaf Extract

The methanol and petroleum ether extract of the leaves of *Trema orientalis* was evaluated for antimicrobial activity using the agar well diffusion method [17]. Standardized inoculum of each test microorganisms was spread onto sterile Mueller Hinton agar plate for bacterial isolates and potato Dextrose Agar for fungal isolates. The plates were allowed to gel, and a sterile cork borer of diameter 8 mm was used to bore wells in the agar plates. The extracts were reconstituted in 20% Tween 20. A 0.5 mL of 50 mg/mL of the extract was aseptically dispensed into the wells; the plates were allowed to stand for 20 mins for proper diffusion to take place and then incubated at  $37^\circ\text{C}$  for 24 h and  $25^\circ\text{C}$  48 h for bacterial and fungal isolates respectively. Zones of inhibition were recorded in millimeters (mm). The standard commercial antibiotic disc was used as the positive control. The zones of inhibition were measured and recorded.

### 2.6 Minimum Inhibitory Concentration of the Extracts

A 5mL of the extract was dispensed into different concentrations ranging from 50 – 3.125 mg/mL. Plates were seeded with bacterial suspension ( $1 \times 10^6$  cfu/mL), wells were bored on the agar plates, and varying concentrations of the extract were dispensed into each hole. The plates were incubated for 24 h at  $37^\circ\text{C}$ , and Zones of inhibition were measured in millimeters [18].

### 2.7 Statistical Analysis

Data obtained from the study were subjected to a one-way analysis of variance. Treatment means were compared using Duncan's New Multiple Range Test (DNMRT) at a 5% level of significance with the aid of SPSS version 20.

## 3. RESULTS

The antibiotic sensitivity patterns of the isolates are presented in Table 1a and 1b for Gram positive isolates and Gram negative isolates respectively. The isolates showed multiple antibiotic resistance. The percentage yield of extract per extraction solvent is presented in Table 2. Methanol rated best for *Trema orientalis* in the study. The qualitative phytochemical properties of the extracts are presented in Table 3. Figs. 1 and 2 shows the antimicrobial activity of extracts against fungi and bacteria

**Table 1a. Antibiotics sensitivity test for positive control Gram-negative organisms**

Isolates	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
<i>Escherichia coli</i>	0.000±0.000	0.000±0.000	0.000±0.000	18.010±0.006	0.000±0.000	0.000±0.000	0.000±0.000	16.507±0.003	0.000±0.000	0.000±0.000
<i>Salmonella typhi</i>	0.000±0.000	0.000±0.000	7.010±0.006	9.507±0.003	0.000±0.000	0.000±0.000	13.503±0.003	15.007±0.003	15.017±0.009	0.000±0.000
<i>Pseudomonas aeruginosa</i>	0.000±0.000	0.000±0.000	0.000±0.000	1.007±0.003	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000
<i>Proteus mirabilis</i>	0.000±0.000	0.000±0.000	10.007±0.007	16.010±0.002	0.000±0.000	0.000±0.000	0.000±0.000	13.503±0.003	9.010±0.006	0.000±0.000

Gram-negative: SXT –Septrin, CH-Chloramphenicol, SP- Sparfloxacin, AM Amoxicillin, AU AUgmentin, CN- Gentamycin, PEF- Pefloxacin, OFX-Tarivid, S-Streptomycin

**Table 1b. Antibiotics sensitivity test for positive control Gram-positive organisms**

Isolates	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
<i>Staphylococcus aureus</i>	15.010±0.006	9.507±0.003	0.000±0.000	0.000±0.000	7.010±0.006	0.000±0.000	6.020±0.012	0.000±0.000	0.000±0.000	0.000±0.000
<i>Streptococcus</i>	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	11.010±0.006	11.020±0.000	16.510±0.006	16.003±0.003

Key; Gram-positive PEF- Pefloxacin CN- Gentamycin, APX- Ampiclox, Z- Zinnacrf, AM Amoxicillin, R-Rocephin, CPX- Ciprofloxacin, S- Streptomycin, SXT- Septrin, E- Erythromycin

**Table 2. Percentage yield of the leaf by solvents of extraction**

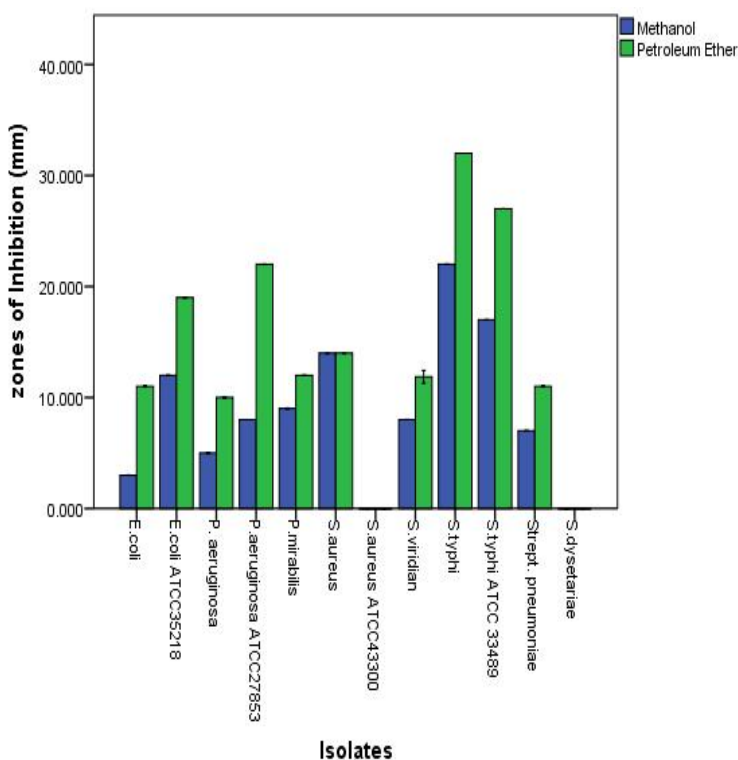
Plant	Solvents	Powdered sample (g)	Extract recovered (g)	Percentage yield (%)
<i>Trema orientalis</i>	Methanol	400	29.04	75
	Petroleum ether	400	8.17	6

isolates. The methanol and petroleum extracts of *Trema orientalis* exerted an inhibitory action on all the clinical pathogenic organisms used except for *S.aureus ATCC43300* and *Shigella dysenteriae*. The zones of inhibition of the extracts on the isolates range from 5.002 ± 0.001mm to 22.003 ± 0.003 mm and for petroleum ether extract between 5.002 ± 0.001mm to 32.002 ± 0.002 mm. It also reveals

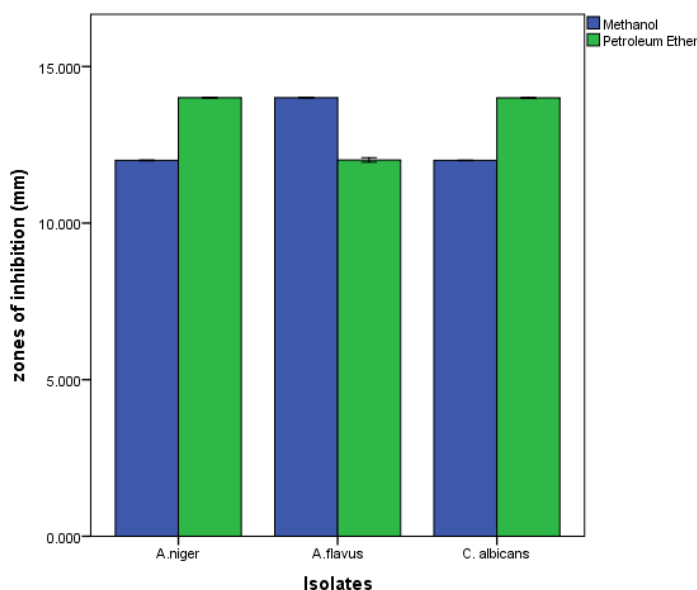
that the two extracts, methanol, and petroleum ether, exercised the highest inhibitory actions on *Salmonella typhi* which is usually a causative agent of typhoid fever, with zones of inhibition 22.003 ± 0.003mm and 32.002 ± 0.002mm respectively. Table 4 shows the minimum inhibitory concentration of the extracts against the clinical Isolates.

**Table 3. Qualitative phytochemical properties of the extracts of *Trema orientalis***

Phytochemicals	Methanol	Petroleum ether
Saponin	+	-
Tannin	+	+
Phlobatannin	-	-
Flavonoid	+	+
Steroid	-	-
Terpenoid	+	+
Alkaloid	-	-
Antraquinone	-	-
CARDIAC GLYCOSIDE		
Legal test	+	+
Keller kiliani test	+	+
Salkowski test	+	+
Lieberman test	-	-



**Fig. 1. Zones of inhibition (mm) of crude extract of methanol and petroleum ether of *Trema orientalis* at 50 mg/mL against selected isolates**



**Fig. 2. Zones of inhibition (mm) of crude extract of methanol and petroleum ether of *Trema orientalis* at 50 mg/mL against fungal isolates**

**Table 4. Minimum inhibitory concentration of both extracts**

Isolates	Methanol extract mg/mL	Petroleum extract mg/mL
<i>Escherichia coli</i>	25	6.24
<i>E. coli typed</i>	6.24	6.24
<i>Pseudomonas aeruginosa</i>	25	3.125
<i>P.aeruginosa typed</i>	6.24	6.24
<i>Proteus mirabilis</i>	6.24	3.125
<i>Staphylococcus aureus</i>	25	3.125
<i>S. aureus typed</i>	NZ	NZ
<i>Streptococcus viridian</i>	3.125	6.24
<i>S. pneumonia</i>	6.24	3.125
<i>Salmonella typhi</i>	3.125	25
<i>S. typhi typed</i>	12.5	6.24
<i>Aspergillus niger</i>	6.2	3.125
<i>A. flavus</i>	12.5	6.24
<i>Candida albicans</i>	3.125	3.125

KEY: NZ- No Zone

#### 4. DISCUSSION

Antibiotic-resistant infections are on the increase, they pose threat to human health as well as an economic burden on the country's healthcare system, patients and families [19]. The isolates used in this study showed multiple antibiotic resistance. The high rate of resistance towards these antibiotics may be due to their overuse because they are readily available over the counter, substitution of dose without finishing the

initial dose [20]. Findings from this research shows variation in the quantity of the percentage recovery of the extracts; rates petroleum ether as the best solvents in the extraction of higher-end yield and this result agrees with the previous study that alcohol especially methanol is a better solvent for extraction of active substances against microbes from medicinal plant. The phytochemicals present in the extracts are tannin, flavonoid, saponin, terpenoid, cardiac glycoside. These phytochemical compounds are

known to play roles in the bioactivity of medicinal plants and elicit inhibitory effect on microorganisms [21].

The activity of the methanol extract of *T. orientalis* leaves is due to the presence of bioactive compounds in *T. orientalis* leaves. The extracts showed broad-spectrum activity as they were able to inhibit Gram-positive, Gram-negative bacteria and fungal isolates. [19] reported that extracts can have a broad-spectrum activity, which is usually as a result of the various polar and non-polar bioactive constituents present in the methanol extract of *Trema orientalis* leaves. [5,19,21-22] recorded that antimicrobial activities shown by crude extracts of many natural resources, including plants, is usually as a result of the secondary metabolites present in them. These metabolites may be able to penetrate the outer phospholipidic membrane of Gram-negative bacteria and peptidoglycan layer of Gram-positive bacteria to inhibit or kill them. The bacterial isolates were found to be more susceptible to the extracts than fungal isolates and, this may be attributed to different factors exhibited by fungi to resist antimicrobial agents such as point mutation, dosages too low or treatment courses that are not long enough [23]. Generally, *Salmonella typhi* was more susceptible to both extracts. Low MICs in the study indicates that *Trema orientalis* have credible bacteriostatic effects on the bacteria compared to the antibiotics [24], and can be useful for the control of infections caused by the multiple antibiotic resistant clinical isolates in this study. The effects of the standard antibiotics, and the leaf extracts against the bacterial and fungal isolates varied per organism.

## 5. CONCLUSION

The leaf of *T. orientalis* has broad-spectrum activities; it has antibiotic and antifungal properties on clinical isolates. *T. orientalis* can serve as special tool for strengthening pharmaceutical industries to fight infections caused by fungi and multiple antibiotic resistant bacteria. More also, methanol and petroleum ether extracts of *T. orientalis* possess a significant inhibitory effect against tested pathogens. Petroleum ether extract of *T. orientalis* rated best in inhibiting the multiple resistant bacteria isolates compared with the methanol extract. The demonstration of their antimicrobial activity against the test isolates is a pointer to their possibility of looking for

alternative antibiotic substances in this plant for the development of newer antibacterial agents.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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