EUROPEAN ACADEMIC RESEARCH
Vol. III, Issue 12/ March 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Scientific Approach on the Antimicrobial Potentials of Bioactive Phytochemicals of *Trema Orientalis* Leaves and Stalk

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Abstract:

The African countries are equipped with biodiversity rich plants in the world among which is trema orientalis. In this study, the active phytochemicals of trema orientalis plant (leaves and stalk) were investigated using standard methods. The extraction was done using hexane, ethyl acetate and methanol, successively. Bioactive compounds such as tannins, saponins, phenols, flavonoids, volatile oils, terpenoids, glycosides, steroids and alkaloids were found to be present. The antimicrobial activity of trema orientalis extracts were also studied using a well diffusion method. The extracts were tested against clinical isolates: Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus and Candida albican yeast extract. Results, based on concentration, showed that methanol extract of the stalk was active against all the micro-organisms than the corresponding ethyl acetate and hexane extracts of both plant parts investigated. The control drugs used yielded zones of inhibition higher than all the extracts.

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Key words: Bioactive phytochemicals, Leaves, Stalk, *Trema orientalis.*

1. INTRODUCTION

Africa countries, Nigeria inclusive, are rich with a biodiversity among the rich plants in the world, with wide varieties of plants used for therapeutic goals [1]. People rely on non-conventional medicinal sources, especially plants, for their primary healthcare [2]. Although several chemical investigations have been conducted to provide a scientific explanation for their use in traditional medicine [3,4], however, the inability to specify the mode of action, the targets biological ailment and the side effects of the molecules; has expose the populations to serious and sometimes irreversible damages from the use of traditional medicine. It is thus convenient to develop scientific approach on traditional medicine in order to locate the tolerant limits of its use.

It is the case of *Trema orientalis*, which is a plant, used much in an empirical way to treat pains, fevers, cough and hypertension as traditional medicine [5]. The stem bark decoctions are used as anti-dysentery [6]. The stems and twigs infusion are used to treat fever and toothache [7]. Both stem bark and leaf decoction of the *T. orientalis* are used to treat malaria, manage pain in muscles and aching bones as well as venereal disease [8,9]. There extracts are used as gargle, inhalation, drink, and vapour bath for relieve of toothache [10]. Unfortunately, even at preliminary level, there is no much scientific data on the toxicological and phytochemical study of this plant. Its virtues are also little known in scientific world. This study assesses the chemical composition of the leaves and stalk of *trema orientalis* with the objective of evaluating its bioactive compounds for the optimization of its use by modern medicine.

2. MATERIALS AND METHODS

Fresh samples of *T. orientalis* plant parts (leaves and stalk) were collected from a farmland in Iyara Ijumu, Kogi State and taxonomical identification was carried out at the herbarium of the Biological Science Department, University of Abuja. The samples were air-dried and then pulverized into fine powders which were subsequently used for chemical analysis.

Preparation of plant extract

The powdered samples (leaves and stalk) was successively extracted with solvents of varying polarities which includes n-hexane, ethyl acetate and methanol using soxhlet extractor in the order of their increasing polarities.

Phytochemical screening

Chemical tests were carried out on both plant parts using standard procedures to identify the plant constituents as described by various researchers [11,12,13]. The hexane, ethyl acetate and methanol extracts of *trema orientalis* samples were analyzed for the presence of alkaloids, flavonoids, tannins, steroids, saponins, glycosides, Balsams, triterpenoids, carbohydrates, phenols, and volatile oil, cardiac glycosides.

Antimicrobial sensitivity test

The agar-well diffusion method as described by Saliu et al[14], was used to determine the growth inhibition abilities of the test organisms by the *trema orientalis* extracts. Cystine Lactose Electrolyte Deficiency (CLED) agar was prepared and 25ml each was poured into sterile Petri dishes. The agar was put in a laminar flow machine and allowed to solidify. A loopful of cultured organisms (*Staphylococcus Aureus*, *Escherichia Coli*, *Klebsiella Pneumonia* and *Pseudomonas aeruginosa* with fungi candida *albican)* were inoculated uniformly into separate agar

and then carpet stripped. With the aid of a sterile cork-borer, four wells were bored on the agar sufficiently separated and kept at least 15 mm from the edge of the plate and 25 mm from well to well to prevent overlapping of zone. With the aid of a syringe, 0.1 ml of a known concentration of the leaf extracts at different concentration of 600 mg/ml, 500 mg/ml, 400 mg/ml, 300 mg/ml, 200 mg/ml and 100 mg/ml were introduced into separate well labeled (holes). This was done in duplicates and the inoculated plates were incubated at 37 ^{0}C for 24 hours. With the aid of a meter rule, the zone diameter of inhibition of growths were measured and recorded to the nearest millimeter. Standard antibiotic drugs, *Cotrimoxazole* (*septrin*), *Gentamycin* and tetracycline, were used at concentration ranging from 40 mg/ml to 100 mg/ml as positive control to provide and evaluate the degree of inhibition of the extracts due to its broad spectrum. While the solvents served as negative control.

3. RESULTS AND DISCUSSION

The results of the phytochemical screening and antimicrobial sensitivity of *Trema orientalis leaves* are as represented below:

Table 1: Phytochemical screening of the leaves and stalk of *Trema orientalis* extract

	Leaves			Stalk		
Phytochemicals	**Hexane**	**Ethyl acetate**	**Methanol**	**Hexane**	**Ethyl acetate**	**Methanol**
Tannins	+	+	+	-	-	+
Saponins	-	+	-	+	+	-
Phenols	-	+	-	-	+	-
Flavonoid	+	-	-	-	-	-
Volatile oils	+	+	-	+	+	-
Terpenoids	+	+	-	-	+	-
Glycoside	+	+	-	+	+	-
Steroids	-	-	+	+	+	-
Alkaloids	+	+	-	+	+	+

Keys: + = present, - =absent

Table 2: Zone of inhibition (mm) of the antimicrobial activities of *trema orientalis* extracts against test isolates

		Leaves			Stalk		
Isolates	**Concentration (mg/ml)**	**Hexane**	**Ethyl acetate**	**Methanol**	**Hexane**	**Ethyl acetate**	**Methanol**
S. aureus	600	NI	NI	NI	NI	NI	6
	500	NI	NI	NI	NI	NI	4
	400	NI	NI	NI	NI	NI	NI
	300	NI	NI	NI	NI	NI	NI
	200	NI	NI	NI	NI	NI	NI
	100	NI	NI	NI	NI	NI	NI
E.coli	600	8	8	NI	NI	28	28
	500	4	4	NI	NI	23	25
	400	4	4	NI	NI	22	18
	300	3	3	NI	NI	18	18
	200	3	3	NI	NI	15	10
	100	3	3	NI	NI	10	8
K. pneumonia	600	NI	NI	NI	NI	NI	29
	500	NI	NI	NI	NI	NI	27
	400	NI	NI	NI	NI	NI	23
	300	NI	NI	NI	NI	NI	21
	200	NI	NI	NI	NI	NI	20
	100	NI	NI	NI	NI	NI	NI
P. aeruginosa	600	35	15	6	36	NI	20
	500	32	15	5	28	NI	15
	400	31	15	5	27	NI	9
	300	24	7	5	20	NI	9
	200	22	4	4	17	NI	5
	100	20	NI	4	10	NI	3
C. albican	600	NI	NI	NI	NI	NI	17
	500	NI	NI	NI	NI	NI	15
	400	NI	NI	NI	NI	NI	14
	300	NI	NI	NI	NI	NI	18
	200	NI	NI	NI	NI	NI	NI
	100	NI	NI	NI	NI	NI	NI

Keys:
E.coli- Escherichia coli S. aureus - Stapylococcus aureus K. pneumonia – Klebsiella pneumonia P. aeruginosa - Pseudomonas aeruginosa C. albican – Candida albican
NI = No inhibition

Table 3: Zone of inhibition (mm) of the antimicrobial activities of the control drugs against test isolates

Isolates	Tetracycline (40mg/ml)	Gentamycine (20mg/ml)	Septrin (100mg/ml)
S. aureus	10	14	0
E.coli	30	25	18
K. pneumonia	35	20	14
P. aeruginosa	30	27	15
C. albican	0	0	0

All extracts showed no inhibition against the negative control

The phytochemical analysis carried out on the leaves of *trema orientalis* plant parts (leaves and stalk) show the presence of tannins, saponins, phenols, flavonoids, volatile oils, terpenoid, glycoside, steroids and alkaloids in at least one of the extracts for both plant parts under investigation. Results from similar analysis carried out on the seed of *trema orientalis* by Akin-Osanaiye *et al.* (2014)[15] also shows the presence of tannin, saponin, alkaloid, terpenoid, balsam, volatile oil and cardiac glycoside. These compounds are known to be biologically active and therefore will aid the antimicrobial activities of *trema orientalis*. These secondary metabolites exert antimicrobial activities through different mechanisms.

Tannins play a role as an antimicrobial and antidiarrhea agent. Review on the biolological activities of tannins showed that it has anticancer activity and can be used in cancer prevention [16], thus suggesting that *trema orientalis* has potential as a source of important bioactive molecules in suppressing the growth of cancer tumor. Another compound observed is the flavonoids, which was present in only the hexane extract of the leaves. According to N'Guessan and Zhi, flavonoids are metabolites which express a good antioxidant activity [17,18]. Steroids, also should be noted, are of importance and interest in pharmacy due to the relationship with such compounds as sex hormones, therefore it can be used as vegetables for expectant mothers or breast feeding mothers to ensure balance, since steroidal structure could serve as a potent

starting materials of this hormones. Glycoside has been used for over 2 centuries as stimulants in cases of heart failure. This perhaps justifies the already locally establish function of *trema orientalis* plant in the treatment and management of hypertension. The samples may exhibit cytotoxic effect and growth inhibition against variety of cell making due to the presence of saponins. However, at moderate amount, they may not be deleterious to the user.

The antimicrobial activities of each of the 3 extracts against 4 clinical isolates: *Escherichia coli, staphylocollus aureus, pseudomonas aeruginosa and klebsiella pneumonia* and a yeast isolate, candida albican as shown in table 2, indicates the assessment of the potency of the leave and stalk extracts for the observation for inhibition zones that occurred on the culture plates. Tetracycline, Gentamycine, and septrin were used as positive controls because of their broad sprectrum (table 3). Among all the extracts used, the methanol extract of the stalk is seen to be more active against the tested bacterial strains when compared with other solvent extracts for both leaves and stalk plant parts. Similar result has been reported on the seed of *trema orientalis* where hexane and ethanol extracts showed activity against *staphylocollus aureus, escherichia coli* and *salmonella paratyphi*[15]. The observed variation in sensitivity pattern of the tested clinical isolates used may be related to the genetic diversity among the organisms. Although it might be possible to experience a better inhibitory effect if whole plant extract instead of individual plant parts have been used.

Most of the extract for both plant parts has MIC value at 100 mg/ml on the inhibited organisms except in few cases where higher concentration inhibitory effect was observed. From all these results therefore, it can be inferred that the activities of the extracts are concentration dependent. The control used yielded zones of inhibition higher than all the extracts. Though it is very possible that at much high

concentration and observable time limit, there could be bactericidal effect on more of the organisms. However, results from this study proved that the plant parts can be used in the treatment of bacteria diseases.

4. CONCLUSION

The results of this study revealed that the leaves and stalks of *trema orientalis* contain some major bioactive compounds that can inhibit the growth of microorganism there by proven very effective as alternative source of antibiotics. It is shown from the results obtained that *Esherichia coli* and Pseudomonas aeruginosa are more susceptible to the plant extracts compared to *Klepsiella pneumonia* and *staphylocollus aureus.* However, the extracts of *trema orientalis* should be further subjected to trials after isolation and characterization of the active components as well as elucidation of structure of the components. Sooner than expected, we may be able to develop a broad spectrum drug from *trema orientalis* plant which will be able to cure humans of various ailments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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