IN-VITRO ASSESSMENT OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *VITEX DONIANA* ON CLINICAL ISOLATE OF *SALMONELLA TYPHI*

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ABSTRACT

This research work aimed on the screening of Phytochemicals constituents and determination antimicrobial activities of the stem-bark and leaves extract (aqueous and methanol) of *Vitex doniana* using agar well diffusion to substantiate its use in the ethno-medicinal practices. The stem-bark and leaves of the plant was extracted and subjected to phytochemical and antibacterial screening. The phytochemical analysis carried out on the plant stem-bark extract revealed the presence of alkaloids, saponin, Anthraquinone, tannins, phenol, glycosides and flavonoids. On sensitivity test carried out on clinical isolate of *Salmonella typhi*, the result shows that both extracts were effective against the isolate with average zone of inhibition of 20.63mm. Statistic analysis of the result reveals that the aqueous extracts were more effective on the test isolate compared to methanolic extracts. The average activity index of the extracts was found to be 0.82 which showed that the plant extract can compete with standard antibiotics. The results obtained in this research were in line with the use of the plants under investigation as medicinal plants.

Keywords: *Vitex doniana; Salmonella typhi;* Phytochemicals; antimicrobial activity.

1.0 INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications (Baris *et al*., 2006). Medicinal plants are rich bio-resources of drugs (Hammer *et al*., 1999). A number of interesting outcomes have been found with the use of a mixture of natural products or plant extracts to treat diseases (Gibbons, 2003). The antimicrobial properties of plants have been investigated by a number of researchers worldwide though thorough biological evaluation of plants extracts is vital to ensure their efficacy and safety. These factors are of importance if plant extracts are to be accepted as valid medical agents for the treatment of infectious diseases (Tanaka *et al*., 2006) especially in light of the emergence of drug-resistant microorganisms. There is an increased interest in alternative therapies globally (WHO, 2003) and a consistent increase in the use of plant derived products as they are convenient alternatives or complementary to the use of orthodox or synthetic drugs. This

is due in part to adverse side effects of conventional drugs, the drift towards consuming 'natural' products as opposed to synthetics, as well as the increasing awareness of the beneficial effects of natural products and high inflation in the third world (Jacob *et al*., 2001). This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects, (Nair and Chanda, 2007).

Black – plum (*Vitex doniana*) of the family *Verbanaceae* is a tree crop that grows in open woodland and savannah regions of tropical Africa; it is the commonest of the *Vitex* species in West Africa (Dalziel *et al.*, 1964). It produces fruits which are plum–like, sweet and edible (Keay, *et al.*, 1964). The fruit is green when mature and changes to dark brown when fully ripe, with the pulp surrounding a hard stone containing 1 – 4 seeds. It is a savanna species and can therefore be found in Nigeria. It is known by the local names: Hausa – Dinya; Fulani – Galbihi; Yoruba - Ori nla; and Ibo – Ucha koro. The fruits are also referred to as black-plum or African olive (Glew *et al.*, 1997). It has been reported that syrup similar to honey was produced from the fruit and that physicochemical and sensory results showed that it can be substituted for other syrups as a nutritive sweetener (Egbekun, *et al.*, 1996). Earlier workers have reported the use of the fruits and leaves for medicinal purposes (Sofwora, 1993). In Nigeria, from information available from the indigenous traditional healers, a decoction of the chopped stem-bark part of *V. doniana* is prepared and taken orally for treatment of gastroenteritis. It is administered for ailments including diarrhea and dysentery. It is also taken to improve fertility and the juice may be squeezed into the eyes to treat eye troubles. The use of *V. doniana* suggests that it may possess antimicrobial activity. Various parts of the plant are used by traditional medicine practitioners in Nigeria in the management and treatment of several disorders which include rheumatism, hypertension, cancer, and inflammatory diseases (Sofowora, 1993). Kilani, (2006) assess the antibacterial effect of whole stem-bark of *Vitex doniana* against some Enterobacteriaceae, and supports the use of *Vitex doniana* by traditional medicine practitioners in the treatment of dysentery and gastroenteritis. He stressed further that, antimicrobial activity of the *Vitex doniana* extract could be attributed to the presence of phenolic compounds that have been liked with antimicrobial properties (Adejumo *et al*., 2013).

This study was designed to screen the phytochemical constituents and potentiality of *Vitex doniana* stem back and leaves extract for antimicrobial effects against clinical isolate of *Salmonella typhi* recovered from diarrheic stool sample.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and Identification

The stem back and leaves of *Vitex doniana* were collected at about 7:30 am on 3rd October, 2016 at Ketawa village, Gezawa Local Government Area in Kano state, Nigeria. The identification and authentication of the plant materials was done at Herbarium in the department of Biological Science with the following voucher number DDC/BUK/HIF/0632. Samples were deposited there for future reference. The parts collected were washed thoroughly with distilled water and air-dried in a shade for two weeks, then cut into pieces and grinded into powder using a sterile pestle and mortar under laboratory condition. The powder was then kept in air tight container for future use.

2.2 Test organisms

Clinical isolates of *Salmonella typhi* was obtained from Department of pathology, Muhammad Abdullah Wase hospital for further experiment. Identification and characterization of the isolates was conducted there by using three procedures namely Gram staining, cultural characterization (using selective media) and biochemical characterization. The pure isolates of the test organism was inoculated in sterile slants containing Nutrient agar and transported to the department of Laboratory science Technology, School of Technology, Kano and preserved at 4℃ before use.

2.3 Extraction of plant material

Aqueous (water) and organic (methanol) solvents were used for extraction of the active components of the plant part. For aqueous extraction, water extraction method as described by Kilani (1986) was used. 25 g of each of the grounded leaf and stem back were extracted by successive soaking for 4 days using 250 ml of distilled water in a sterile conical flask. The extracts were filtered using Whatman filter paper and the filtrates concentrated in water bath at 70°C. The solid concentrated filtrate, now the extracts were then stored in universal bottles in the refrigerator at 4°C before use. For organic extraction, 25g of the powdered plant part was extracted in 250 ml of ethanol for 7 days mixture was filtered using Whatman No.1 filter paper and the extracts were evaporated to dryness using rotary evaporator. The solid residues obtained were reconstituted in 5% of Dimethylsulphuroxide (DMSO) at stock concentration, stored in the refrigerator at 4^0C until used.

2.4 Phytochemical Screening

This was done on different extract to ascertain the presence of bioactive component present in the leaves and stem back of *Vitex doniana.* Presence of Alkaloid, saponin, Glycoside, Tannin, flavonoid, phenol, reducing sugar, steroid, terpenoid, and Anthraquinone were determined using procedure described by Sofowora (1993).

2.5 Antimicrobial Assay

The agar well method was used to determine the antibacterial activity of the plant extracts. 0.1ml of the different standardized organism (0.5 Mc Farland) was inoculated on the surface of Mueller Hilton Agar in a sterile Petri dish and allowed to set and then labeled. A sterile cork borer 6mm was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 50mg/ml, 100 mg/ml, and 150mg/ml, 200mg/ml while the 5^{th} well contained 0.1ml of 100mg/ml Ciprofloxacin (Micro lab limited) which was used as control in this research. These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°c for 24 hours. After incubation, the diameter of the zones of inhibition around each well, were measured to the nearest millimeters (Kavanagh, 1977).

2.6 Evaluation of activity index of the extracts

The activity index (AI) of the crude extracts was calculated as described by Vedpriya *et al*. (2010).

$$\text{Activity index (AI)} = \frac{\text{Mean zone of inhibition of the extracts}}{\text{Zone of inhibition obtained from standard drug.}}$$

3.0 RESULTS

3.1 Phytochemical analysis

The phytochemical constituents of leaves and stem back extract of *V. doniana* is presented in table 1. The result showed that the both leaves and stem back extracts contain the following Phytochemicals alkaloid, saponin, tannin, Anthraquinone, Flavonoid, phenols, terpenoid and glycoside. On the other hand steroid and reducing sugar were absent.

Table 1: Phytochemical constituent of leaves and stem bark extracts

S/N	Phytochemical	Leaves extract	Stem extract
1	Alkaloid	++	++
2	Flavonoid	+	++
3	Glycoside	+	++
4	Reducing sugar	-	-
5	Saponin	++	+
6	Steroid	-	-
7	Tannin	+	+
8	Phenol	-	+
9	Terpenoid	+	+
10	Anthraquinone	+	+

++ = Abundance of phytochemical
+ = Presence of Phytochemical
- = Absent of Phytochemical

3.2 Antibacterial activity of the extract

The antibacterial activity of aqueous and methanolic extract of *Vitex doniana* leaves is presented in Table 2. The result showed that the aqueous extract demonstrated higher activity of 27mm at 200mg/ml. The zone of inhibition shown by the control (100mg/ml Ciprofloxacin) is found to be 13mm

Table 2: Antibacterial activity of aqueous and methanol leaves extracts *V. doniana* on *S. typhi*

EXTRACT	Concentration (mg/ml)/ zone of inhibition (mm)				
	50	**100**	**150**	**200**	**Control**
ALE	17	20	27	25	25
MLE	19	23	24	24	25

ALE = Aqueous leaves extract, MLE = Methanol leaves extract,
Control = 100mg/ml Ciprofloxacin

The antibacterial activity of aqueous and methanolic extract of *Vitex doniana* stem back is presented in Table 3. The result showed that the aqueous extract demonstrated higher activity of 24mm at 200mg/ml. The zone of inhibition shown by the control (100mg/ml Ciprofloxacin) is found to be 13mm

Table 3: Antibacterial activity of aqueous and methanol stem bark extracts *V. doniana* on *S. typhi*

EXTRACT	Concentration (mg/ml)/ zone of inhibition (mm)				
	50	**100**	**150**	**200**	**Control**
ASE	18	19	20	24	25
MSE	13	17	20	20	25

ALE = Aqueous stem bark extract, MLE = Methanol stem bark extract,
Control = 100mg/ml Ciprofloxacin

3.3 Activity index of the extract

The activity indices of the extracts are presented in Table 4. The result showed that MLE has the highest activity index of 0.90, followed by ALE 0.89, then ASE 0.81 and the lowest activity is shown by MSE 0.70.

Table 4: Activity index of the extracts

Extracts	Total ZOI	Average ZOI	Activity index
ALE	89	22.25	0.89
MLE	90	22.50	0.90
ASE	81	20.25	0.81
MLE	70	17.50	0.70
Average	**82.5**	**20.63**	**0.82**

ALE = Aqueous leaves extract, MLE = Methanol leaves extract, ALE = Aqueous stem back extract, MLE = Methanol stem back extract, ZOI = Zone of inhibition

4.0 DISCUSSION

The phytochemical characteristics in the plant leaves and stem back showed that alkaloid, tannins, saponin, flavonoid, Anthraquinone, terpenoid, phenol and glycoside are present in almost all solvent extracts while glycosides and reducing sugar are absent in the extract. The phytochemical characteristics possessed by *V. doniana* may be attributed to their antimicrobial properties. The finding of this study agrees with that conducted by Emmanuel *et al*. (2015) on Phytochemical and Antimicrobial Screening of the Stem-Bark extracts of *Vitex doniana*, the results revealed that the preliminary phytochemical screening of the stem back extract contain alkaloids, saponins, tannins, glycosides and flavonoids.

The antibacterial activity of aqueous and methanolic extract of *Vitex doniana* leaves that the aqueous extract demonstrated higher activity of 27mm at 200mg/ml. The antibacterial activity of aqueous and methanolic extract of *Vitex doniana* stem back showed that the aqueous extract demonstrated higher activity of 24mm at 200mg/ml. The zone of

inhibition shown by the control (100mg/ml Ciprofloxacin) is found to be 13mm. The result of this finding demonstrated that the plant is effective against the test isolate. This is in line with the study conducted by Kilani, (2006) to assess antibacterial activity of whole stem back methanolic extract of *Vitex doniana* against some members of Enterobacteriaceae including *Salmonella typhi, Shigella* and *Escherichia coli*. The result of his study shows that the stem back extracts were able to inhibit the growth pattern of the tested microorganisms. According to him, the results suggest that *V. doniana* may be valuable in the management of dysentery and gastroenteritis infections. The antimicrobial activity of Alkaloids and Flavonoids of Vitex doniana seed extracts was studied by Okoye, (2015) in which the result of the study shows that the aqueous extracts of Alkaloids and Flavonoid of the plant *inhibited the* growth of the following bacteria successfully, *P. aeruginosa, B. subtilis, S. aureus, E. coli and S. typhi* and this is inconformity with the present study.

The activity indices of the extracts (Table 4) showed that MLE has the highest activity index of 0.90, followed by ALE 0.89, then ASE 0.81 and the lowest activity is shown by MSE 0.70. The average activity index of the extract is found to be 0.82 which indicated that the extract can compete to the standard antibiotic used.

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results of phytochemical analysis revealed the presence of alkaloid, saponin, tannin, anthraquinone, Flavonoid, phenols, terpenoid and glycoside which are the active components of the plant responsible for the medicinal values of the plant. The extracts exerted antibacterial activity against *S. typhi.* Each of the extracts used showed a large zone of inhibition against the organisms used confirming the use of the stem-bark in the treatment of diseases like typhoid fever, diarrhoea and dysentery. The plant could therefore serve as source of bioactive agents for production of new drugs.

5.2 Recommendation

It is recommended that further studies or investigations on toxicity test on the bioactive ingredients should be carried

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