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Phytochemicals and Antimicrobial Activity of *Vitexdoniana* (verbenaceae) on *Salmonella typhimurium*

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Abstract:
Phytochemicals from Vitexdoniana(Verbenaceae) leaves and stem bark were extracted with ethanol and distilled water as solvents using standard procedures. Aqueous solvent had 7 and 6 phytochemicals from leaves and stem bark as against the ethanolic with 5 and 3, respectively. All extracts irrespective of solvent and plant organs had flavonoids, tannins and phenols present, but devoid of carbohydrate. The antibacterial effect of the extracts was concentration dependent. Aqueous extract of stem-bark showed the highest inhibitory effect at the concentration of 36.0mg/ml. Cardiac glycosides was probable the most active principle in stem bark that provided relative efficacy over aqueous leaf extract which lacked it.

Keywords: *Vitexdoniana, phytochemical, Antimicrobial effect, MIC, ZI.*

1. Introduction

Infectious diseases account for a high proportion of health problems in Nigeria. Typhoid fever or enteric fever is one of such infectious diseases induced by bacteria of the salmonella group - *Salmonella typhi* and *Salmonella paratyphi* A, B and C. According to Ivanoff, *et al.* (1997), typhoid fever is rare in industrialized countries however; it remains a serious health threat in the developing world principally due to lack of potable drinking water, poor hygiene and poor feeding tendencies.Typhoid fever induced by *Salmonella typhior Salmonella paratyphi* is a major health problem with global incidence of 21 million cases and 200,000 (1-4% deaths worldwide) deaths per year (Crump *et al.,* 2004).

Salmonella has become a major threat to the society due to emergence of multi-drug resistance strains and its possible use as a potential candidate in bioterrorism (WHO, 2003).

The average incubation period of typhoid fever varies from 1 to 14 days, depending on the virulence of the organism as well as the species. However, when untreated, typhoid fever persists for three weeks to a month, death may occur in 10 to 30 percent of untreated cases. During the period of incubation, the infected patient may suffer from a variety of symptoms, such as altered bowel habit, fever with low pulse rate, headache,toxemia, enlargement of spleen, generalized weakness, and abdominal pains. Mortality rates associated with typhoid fever vary from region to region.Chicago typhoid fever mortality rate averaged 65 per 100,000 people a year from 1860 to 1900. The worst year was 1891, when the typhoid death rate was 174 per 100,000 persons (Brusch, 2006). Mortality rate associated with typhoid fever vary from region to region, with the highest reported from Indonesia, Nigeria and India (Miller *et al*., 1994).

Vitexdoniana is locally administered as a remedy for vitamins A and B deficienc.Vunchi*et al*. (2011) reported on the proximate vitamins and mineral composition of *Vitexdoniana* fruit pulp (protein, fat, carbonhydrate, vit. A, B_I, B_2, B_6, and C). Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent (Shibata *et al*., 2005; Betoni*etal*., 2006). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999; Lewis and Ausubel, 2006).

2. Materials and Methods

2.1. Plant Material

Fresh plants (leaves and bark) of *Vitexdoniana* were collected separately from fields in Kajuru local government area. The plant was taxonomically authenticated at the herbarium of the Biological Science Department,Ahmadu Bello University (A.B.U.),Zaria, Nigeria by comparing with the herbarium specimen and deposited with voucher specimen No1201.

2.2. Preparation of Extracts

2.2.1. Preparation of the Aqueous Extract

A 100g of the pulverized air-dried leaves and stems of the plant were separately soaked in 1000ml of distilled water in a clean covered extractor bottle at room temperature for 48 hours. The resulting mixture was filtered through a Muslin cloth and Whatman's No 4 filter paper. The extracts were concentrated at a temperature of 100^0C on a water bath (Harbone, 1998).

2.2.2. Preparation of the Ethanolic Extract

A 100g of the pulverized air-dried leaves and stems of the plant were soaked separately in 1000ml of ethanol in a clean covered extractor bottle at room temperature for 24 hours. The resulting extract was filtered through a Muslin cloth and Whatmans No 4 filter paper and thereafter concentrated under reduced temperature of 45^0C using a rotary evaporator (Harbone, 1998).The phytochemical screening of the various portions of the extract was conducted using standard procedures (Prashant*et al.*,2011).

2.2.3. Analysis of Phytochemicals

The phytochemical analysis of the plant components was carried out at the National Research Institute for Chemical Technology (NARICT) in Zaria and Department of Chemistry, Nigerian Defence Academy (NDA), Kaduna using the method described by Prashant*et al.* (2011). Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction process. Two extraction solvents (water and ethanol) were adopted and Standard procedures were followed.Test for alkaloids (Odebiyi and Sofowora,1978).Test for saponins (Sofowora and Odebiyi,1979).Test for tannins (Trease and Evans,1989).Test for terpenoids and cardiac glycosides (Harbone,1998).

2.2.4. Preparation of the Bacteria Inoculum

Pure culture of *Salmonella typhimurium* was obtained from the Bacteriology Laboratory of Federal College of Veterinary and Medical Laboratory Technology (FCVMLT) Vom, Jos Plateau State, Nigeria. This was sub-cultured and re-identified to ensure its purity. Cultures were grown and maintained on Nutrient Agar slope at 37^0C for 48hours.Bacterial isolate of *Salmonella typhimurium* was cultured into Eosin Methylene Blue (EMB) agar plates and incubated for 24 hours at 37^oC.A single colony was then cultured into 5ml Mueller Hinton Broth for 4 hours at 37^oC.The density of the bacterial culture for antibacterial sensitivity test was adjusted to 0.5 McFarland standard (1.0×10^8 CFU/ml) using the McFarland standard (Cheesbrough, 2005).

2.2.5. Preparation of Plant Extract Sensitivity Discs

The plant extracts (aqueous and ethanolic) were diluted serially in two fold dilution. Initial doses of 0.5g/10ml, 1.0g/10ml, and 2.5g/10ml were prepared by dissolving 50mg, 100mg, and 500mg of the extract each in 1ml of normal saline, respectively. For standard control; the same concentration of the extract was replaced with ciprofloxacin. Twenty micro liter (20µl) from each of the wells was then used to impregnate the blank sterilized discs (Oxoid,UK). The impregnated discs were dried in an incubator at 37^0C for 24 hours and immediately used for the sensitivity test.

2.2.6. Disc Diffusion Method

Disc diffusion method for antimicrobial susceptibility test was carried out according to Kirby Bauer's method (Bauer *et al.*, 1966) to assess the antibacterial activities of the plant extracts. A bacterium culture (which had been adjusted to 0.5 McFarland standards), was used to lawn Mueller Hinton agar plates evenly using a sterile cotton swab. The plates were dried for 15minutes and then used for the sensitivity test. The discs which had been impregnated with the different concentrations of plant extract were placed on the Mueller-Hinton agar surface.Each test plate was made of six discs which had one positive control, which is a standard commercial antibiotic disc, one non-chemical control, and four treated discs. Each plate had four treated discs placed at equal distances to each other besides the controls. The plates were then incubated at 37^0C for 24 hours and then examined for zones of inhibition that were measured using calipers and recorded in millimeter (mm). Test was conducted in triplicates and diameter of means of zones of inhibition determined.

2.2.7. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibition concentration (MIC) was determined using inhibitory concentration in diffusion (ICD) method (Guerin-faublee*et al.*, 1996). It was done by carrying out the diffusion test with twelve discs of different concentrations of the plant extracts similar to the concentration used in the sensitivity test against the bacterial isolate. The lowest concentration that inhibited the growth of *S.typhimurium* was noted and considered as its MIC value.

3. Results

The result of the phytochemical screening of the plants components is presented in Table 1. This shows that in the aqueous extract, flavonoids, tannins, saponins, terpenoids and phenols are present in both leaves and stem bark of the plant. Also, alkaloids and glycosides are only present in the leaves of the aqueous and ethanolic extracts, while cardiac glycosides was present in the aqueous extract of the stem bark of the plant. For the ethanol extract, flavanoids and tannins were present in both leave and stem bark of the plant. Saponins, terpenoids and cardiac glycosides are absent in ethanolic extract of both leaves and stem bark of the plant.

The aqueous leaf extract had seven of the nine phytochemicals screened for while the stem bark had six. Carbohydrate and cardiac glycosides were absent in the aqueous leaves extract whereas the stembark lacked alkaloids, carbohydrate and terpenoids. The

ethanolic leaf extract had five of the nine phytochemicals but tested negative to saponins, carbonhydrate, cardiac glycosides and terpenoids; whereas ethanolic stem-bark extract had three of the nine phytochemicals, but was deficient in saponins, alkaloids, carbohydrates, cardiac glycosides, glycosides and terpenoids. Hence carbohydrate was consistently absent in the leaves and stembark extracts of the two solvents extracts as showed in Table 1.

Phytochemical constituents	Aqueous Extract		Ethanolic Extract	
	Leaves	Stem-bark	Leaves	Stem-bark
Flavonoid	++	+++	++	++
Tannins	+++	+++	+++	+++
Saponins	+++	++	– –	– –
Alkaloids	++	– –	++	– –
Carbohydrate	– –	– –	– –	– –
Cardiac glycosides	– –	++	– –	– –
Glycosides	++	– –	++	– –
Terpeniods	++	++	– –	– –
Phenols	++	++	++	++
Total no of phytochemicals extracted	7	6	5	3

Table 1: Qualitative Phytochemical Composition of Leaves and Stembark Extracts of Vitexdoniana.

Key: - - =Absent
+ = slightly present
++ = Moderately present
+++ =Highly present

Antimicrobial result of leaf and stem bark extract of *Vitexdoniana* is presented in Table 2. The result showed that aqueous extract of the leaf and stem bark at concentrations of 50,100 and 500mg/ml had zones of inhibition of 6.70, 7.30, 10.00, and 6.60, 30.30, 36.00(mm) respectively. The ethanolic extract of the leaf at concentrations of 50,100 and 500mg/ml had zones of inhibition of 0.00, 13.30 and 28.00(mm) respectively while the ethanolic extract of the stem bark at concentrations of 50,100 and 500mg/ml had a zone of inhibition of 10.00,16.60 and 20.00 (mm), respectively.

Plant components solvents / conventional drugs	Conc. of plant extracts and conventional drugs (mg/ml)	Zones of inhibition (mm)
Aqueous leaf	50	6.70
Aqueous leaf	100	7.30
Aqueous leaf	500	10.00
Aqueous stem bark	50	6.60
Aqueous stem bark	100	30.00
Aqueous stem bark	500	36.00
Ethanolic leaf	50	-
Ethanolic leaf	100	13.30
Ethanolic leaf	500	28.00
Ethanolic stem bark	50	10.00
Ethanolic stem bark	100	16.60
Ethanolic stem bark	500	20.00
Kenflox 20%	500	40.70
Ciprofloxacin	500	26.00
Distilled water	00	-

Table 2: Antimicrobial Activity of Aqueous and Ethanolic Extracts of Vitexdoniana against Salmonella typhimurium

KEY:(-)=No zone of inhibition, Conc=Concentration
The minimum inhibitory concentration result of plant extract against the bacteria is presented in Table 3. The result showed light growth in the aqueous extract of both leaf and stem bark at 50mg/ml, no growth was observed in the ethanolic extract of the leaf and stem bark at 50mg/ml. Concentrations of 25mg/ml produced high growth in the aqueous extract of the leave and stem bark. Concentrations of 12.5mg/ml of aqueous extract of the leaf and stem-bark showed light growth while concentrations of 6.25mg/ml of both aqueous and ethanolic extracts of the leaf and stem bark was observed to be the MIC.

Plant extract	Concentrations of plant extract (mg/ml)	Reaction on *Salmonella typhimurium*
Aqueous leaf	50	+
Aqueous leaf	25	+++
Aqueous leaf	12.5	+
Aqueous leaf	6.25	0*
Aqueous stem bark	50	+
Aqueous stem bark	25	+++
Aqueous stem bark	12.5	+
Aqueous stem bark	6.25	0*
Ethanolic leaf	50	-
Ethanolic leaf	25	+++
Ethanolic leaf	12.5	+
Ethanolic leaf	6.25	0*
Ethanolic stem bark	50	-
Ethanolic stem bark	25	+++
Ethanolic stem bark	12.5	+
Ethanolic stem bark	6.25	0*

Table 3: Minimum Inhibitory Concentrations (MIC) Of Plant Extracts Against Salmonella typhimurium.
KEY: 0= MIC, -=No growth, ++= Slight growth, +=Moderate growth, +++= High growth*

4. Discussion

In terms of the solvent of extraction and plurality of phytochemicals registered, aqueous leaf and stem bark extracts with 7 and 6 items had a relative advantage over ethanolic extracts with 5 and 3, respectively(Table 1).The presence of biologically active compounds (flavonoids, tannins, saponins, phenols, terpenoids) in the leaf and stem-bark extract of *V. doniana* has made the plant to become a potential antimicrobial reference point against pathogenic organisms. Tannins for example have been reported to interfere with bacterial cell protein synthesis and is important in the treatment of ulceration or inflamed tissue and also in the treatment of intestinal disorders (Igbinosa*et al.,* 2009). Alkaloids have been reported to be pain killers while saponins have managing effect against inflammation (Igbinosa*et al*., 2009; Hussain*et al.*, 2009). Flavonoids are also important against inflammation and antimicrobial as well. Furthermore, the presence of terpenoids and saponins in the aqueous extract of both leaf and stem-bark of the plant but absent in the ethanol extract (Table2) is in line with the findings of Malu (2008), who reported that type of solvent and extraction methods used affect the content of the extract.

The result of the antimicrobial activity of the leaf and stem-bark of the medicinal plant *Vitexdoniana* presented in Table 2 shows that among the tested plant parts, the aqueous extract of the stem-bark produced the highest activity against *Salmonella typhimurium* with a zone of inhibition of 36.0mg/ml. The result also showed that the effect of the extract appears to be effective at higher concentration of 500mg/ml since activity was observed to be very low (6.7mm and 6.6mm) at lower concentrations of 50mg/ml. However, the active principle in the plant extract that might have contributed to the relative efficacy of the stem-bark over the leaf extract could be speculated to be the cardiac glycoside that was present in only stem-bark extract, but absent in aqueous leaf extracts (Table 1).

The result of the inhibitory action of the extract against *S. typhimurium* revealed minimum inhibitory concentration value of 6.25mg/ml (Table 3) implying that at a concentration of 6.25mg/ml the growth of the bacteria was inhibited.

5. Conclusion and Recommendation

The result obtained from this study has shown that the crude extract of *Vitexdoniana* is effective against *S.typhimurium*.

Further studies should be carried out to isolate the specific bioactive constituents responsible for inhibiting *Salmonellatyphimurium* and development of therapeutic drugs for treatment of salmonellosis and enteric fever. Such will even go a long way in administering lower doses of the plant extract than the current 500mg/ml that turned out as the best dose in this research.

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