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ANTIBACTERIAL ACTIVITY AND BRINE SHRIMP LETHALITY OF *VITEX DONIANA* SWEET, STEM BARK EXTRACTS

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ABSTRACT
The stem bark of Vitex doniana was extracted with n-hexane, chloroform, ethylacetate, acetone, ethanol and water sequentially using cold extraction method. The fractions were tested for antibacterial activity using disc agar diffusion technique and brine shrimp lethality followed by screening for secondary metabolites. All the fractions showed a remarkable activity against some respiratory tract pathogenic bacteria particularly the n-hexane and ethanol soluble fractions at 100 µg/disc, 300 µg/disc and 500 µg/disc concentrations. The Brine shrimp test showed highest toxicity in n-hexane and ethanol soluble fractions with LC_{50} values of 6.7674µg/ml and 5.3421µg/ml respectively. Phytochemical analysis of the fractions revealed the presence of alkaloids, steroids, cardiac glycosides, tannins, reducing sugars and flavonoids.

Keywords: Vitex doniana, Brine Shrimp Lethality, Antibacterial properties, Respiratory tract pathogens, Phytochemicals.

INTRODUCTION

Bacterial resistance to antibiotics in community acquired respiratory tract infections is a serious problem increasing at an alarming rate all over the world (Kohno *et al*., 2008). *Streptococcus pneumoniae*, one of the main organisms implicated in respiratory tract infections has developed multiple resistance mechanisms to combat the effects of most commonly used antibiotics, particularly the beta lactams and macrolides (Brown, 1993). Therefore, continued search for effective antibiotics through screening of bioactive plants is very essential and one of the intensive area of natural product research today.

Vitex doniana SWEET (Verbenacaea) is a deciduous tree with a pale brown to grey white bark. It has palmate fingered leaves and white or yellowish white with blue or red centered flowers. The fruits are green, glabrous with small white dots and later turn yellowish brown then black when matured (Hans-Jurgen, 1990). In the northern part of Nigeria, the chewed leaves are put to improve healing. A decoction of the bark with the root of *Fiscus sycomorus* serve as remedy for cough, throat infections and chest pain, while the powdered mixture serves as anti dote against snakebites (Irvine, 1961). Iwueke *et al*., (2006) studied the anti-inflammatory and analgesic properties mediated by prostaglandin synthesis inhibition of leaves of *V. doniana*. The anti-hypertensive effect of extract of the stem bark of *V doniana* has been reported (Olusola *et al*., 1997). The aqueous methanol extract was shown to exhibit anti-diarrheal activity (Agunu *et al*., 2005)

During the recent years biological, chemical and physical properties as well as nutritional values of *V doniana* were reported (Abu, 2002; Egbekum *et al*., 1996; Ladeji and Okoye, 1993). But a number of terpenoids, flavanoids and phenolic compounds were isolated and reported from other *Vitex* species (Ono *et al*., 2000; Suksamram *et al*, 1999; Achri *et al*., 1984).

This study screened the various extracts obtained from stem bark of *Vitex doniana* against some selected bacteria causing respiratory tract infections and determined their brine shrimps lethality.

MATERIAL AND METHODS

The stem bark of *Vitex doniana* was collected around Bayero University, Kano, Nigeria, air dried under shade and ground into fine powder.

Extraction

The powdered stem bark of *Vitex doniana* (200g) was soaked in n-hexane (500 cm^3), chloroform (500 cm^3), ethylacetate (500 cm^3), acetone (500 cm^3), ethanol (500 cm^3) and water (500 cm^3) for three weeks sequentially using cold extraction method. Thereafter, the fractions in each case were filtered with whatman's No 1 filter paper. The extracts were concentrated at 40 ^{o}C using rotary evaporator. The fractions obtained were weighed and labeled VD1 (n-hexane soluble fraction), VD2 (chloroform soluble fraction), VD3 (ethylacetate soluble fraction), VD4 (acetone soluble fraction), VD5 (ethanol soluble fraction), and VD6 (water soluble fraction). Each fraction was screened for phytochemicals and antimicrobial activity.

Phytochemical Analysis of the Fractions

Phytochemical analysis for qualitative detection of secondary metabolites (tannins, steroids, cardiac glycosides, flavonoides, saponins and alkaloids) were performed on each fraction as described by Harbone, 1975, Evans and Trease, 1995, Brain and Tunner, 1975, El-olemy *et al*., 1994, and Sofowora , 1984.

Sources of Microorganisms

Pure cultures of *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, E. coli, Salmonella typi and Pseudomonas aeruginosa* were obtained from Microbiology Laboratory, Department of Biological Sciences, Bayero University, Kano. These bacterial cultures were maintained in nutrient agar slant at 4^{o}C before use.

Preparation of Inocula

The inoculum was prepared from the stock cultures which were maintained on nutrient agar slant at 37^{o}C overnight and subcultured in nutrient broth using a sterilized wire loop and incubated at 37^{o}C for 24 hours. The density of suspension to be inoculated was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (1% v/v).

Preparation of Sensitivity Discs

Discs of about 6mm diameter were punched from whatman's No.1 filter paper using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 110^{o}C for 24hours .The stock solution of 50mg/ml of the plant extract was prepared by dissolving 0.1g of each fraction in 2ml Dimethylsulphoxide (DMSO). Concentration of 30mg/ml and 10mg/ml were prepared by dissolving 0.6ml and 0.2ml of the stock solution into 0.4ml and 0.8ml of DMSO respectively. One milliliter (1ml) of the extract from 50mg/ml, 30mg/ml and 10mg/ml concentrations were each transferred into separate bottles containing 100 discs. Since each disc can absorb 0.01 ml, the three bottles yielded discs of 500µg/disc, 300µg/disc and 100µg/disc respectively (British Pharmacopoeia, 1998).

Antibacterial Susceptibility Test

Disc agar diffusion technique described by Bauer and Kirby (1966) was employed for antibacterial assay. Three concentrations for each fraction of the plant extract were prepared such as 500µg/disc, 300µg/disc and 100µg/disc. These concentrations of the plant extract were subjected to antimicrobial susceptibility test against the selected organisms. Sterile wire loop loaded with the standard culture was used in streaking agar plates evenly and aseptically (in an inoculation chamber). The prepared discs, and disc containing only DMSO (as negative control) were aseptically pressed firmly using sterile forceps unto the inoculated plates. The set up was incubated at 37^{o}C for 18 hours. The zone diameter of inhibition was measured to the nearest whole number using meter rule.

Brine Shrimp Lethality Test (BST)

Brine shrimp lethality bioassay was carried out using brine shrimp larvae (*Artemia salina*) to test the cytotoxicity of the plant extract. Each test material (20mg) was weighed and dissolved in absolute methanol (2ml). From this solution, 500, 50, 5µl corresponding to 1000, 100, 10µg/ml of the material were transferred into three separate vials, using a micro-syringes (each dosage was tested in triplicate) and 500µl of solvent was also added to a control vial. The solutions in the control plus the 9 vials were allowed to evaporate overnight at room temperature. After 48 hours, 3.5ml of sea water and 10 shrimps were introduced into each vial and the volume in each vial was made up to 5ml with sea water. After 24 hours, the number of surviving shrimps in each vial, including the control were counted and recorded. LC_{50} values were determined at 95% confidence interval from the total mortality by analyzing the data using Finney Probit software programme (Meyer *et al.*, 1982; and Mitscher *et al.*, 1972).

RESULTS

The powdered stem bark of *V doniana* extracts was found to contain some secondary metabolites (Table1). The extracts from *V doniana* particularly n-hexane and ethanol fractions showed greater level of toxicity against both brine shrimps and some selected microorganisms as shown on Tables 2 and 3 respectively.

Table 1 Phytochemical Constituents of *Vitex doniana* Extracts

Secondary metabolite group	VD1	VD2	VD3	VD4	VD5	VD6
Saponins	+	-	+	-	-	-
Alkaloids	-	-	-	+	-	-
Phlobatannins	-	-	-	-	-	-
Cardiac glycosides	-	+	+	+	+	+
Flavonoids	-	-	-	-	-	-
Steroids	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-
Tannins	+	+	+	+	+	+
Resins	-	-	-	-	-	-
Flavonosides	-	-	+	+	-	+
Reducing sugar	-	+	+	+	+	+

Key: + = present , - = Absent, VD1= n-hexane soluble fraction, VD2= chloroform soluble fraction, VD3= ethylacetate soluble fraction, VD4= acetone soluble fraction, VD5= ethanol soluble fraction and VD6= water soluble fraction

Table 2 Brine shrimp lethality Assay of *Vitex doniana* Extracts

Fractions solvent	Fractions code	LC_{50} value (µg/ml)
n-Hexane	VD1	6.7674
Chloroform	VD2	27.2112
Ethyl acetate	VD3	139.7600
Acetone	VD4	27.3830
Ethanol	VD5	5.3421
Water	VD6	49.2631

Key: VD1= n-hexane soluble fraction, VD2= chloroform soluble fraction, VD3= ethylacetate soluble fraction, VD4= acetone soluble fraction, VD5= ethanol soluble fraction and VD6= water soluble fraction

Table 3 Antibacterial susceptibility of clinical isolates to *Vitex doniana* Extracts

Fraction	Concentration (µg/disc)	Test organisms with Zone of inhibition in (mm) K.P	S.P	S.A	E.C	S.T	P.A
VD1	100	10	13	10	7	11	11
	300	19	21	15	10	10	10
	500	22	24	17	12	12	13
VD2	100	06	07	06	09	07	10
	300	06	00	06	06	20	22
	500	06	08	06	11	23	20
VD3	100	11	07	06	06	16	06
	300	06	07	06	06	26	10
	500	06	06	06	12	29	11
VD4	100	27	06	06	06	09	06
	300	06	06	14	06	11	07
	500	17	07	06	06	10	06
VD5	100	14	13	12	08	11	11
	300	19	21	15	10	12	12
	500	22	24	17	12	12	15
VD6	100	11	07	06	06	16	6
	300	06	07	06	06	26	10
	500	06	06	06	12	29	11

Note: Zone of inhibition for disc = 6mm.
Key: K.P = *Klebsiella pneumoniae*, S.P = *Streptococcus pneumoniae*, S.A = *Staphylococcus aureus*, E.C = *Escherichia coli*, S.T = *Salmonella typi*, P.A = *Pseudomonas aeruginosa*.
VD1= n-hexane soluble fraction, VD2= chloroform soluble fraction, VD3= ethylacetate soluble fraction, VD4= acetone soluble fraction, VD5= ethanol soluble fraction and VD6= water soluble fraction

DISCUSSION

Result of the phytochemical analysis revealed the presence of some secondary metabolites in the plant extracts including tannins, steroids, cardiac glycosides, flavonoides, saponins and alkaloids. However, steroids, tannins and reducing sugars were found to be present in all the fractions while anthraquinone, resins and phlobatannins were absent in all the fractions. Some of these metabolites particularlythe flavonoids were reported to be responsible for antimicrobial activity associated with some ethnomedicinal plants (Yusha'u *et al.*, 2008). In addition to alkaloids and tannins that are well documented for antimicrobial activity (Sign and Bhat, 2003). The result of the brine shrimp lethality test (BST) showed good activity by all the fractions with highest toxicity observed in n- hexane (VD1) and ethanol (VD5) soluble fractions with LC_{50} value 6.7674µg/ml and 5.3421µg/ml respectively. The lower LC_{50} (in µg/ml) indicate the potential and efficacy of the extracts as drug. Antibacterial susceptibility test result indicated that all the fractions showed good degree of activity against the test organisms. However, VD1 and VD5 soluble fractions were found to be more active against all the respiratory tract pathogenic bacteria while chloroform (VD2) soluble fraction was found to be inactive against *Klebsilla pneumonia, Staphylococcus aureus and Streptococcus pneumoniae* at all the three concentrations tested (100 µg/disc, 300 µg/disc, and 500 µg/disc). Results of antimicrobial sensitivity obtained agree with the reports of Arokiyaraj et al., (2009) and that of Nwachukwu and Uzoeta (2010) in a similar study of *V doniana* (leaves) acetone, methanol as well as ethanol extracts.

Conclusion and Recommendation

The promising result of antibacterial bioassay and brine shrimp lethality test obtained in this study justified the efficacy of the plant in traditional medicine. Therefore, activity guided isolation and characterization to uncover the active agent(s) is recommended.

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