TOXKTTY AND ANTIOXIDANT CHARACTERISTICS OF Vtfcex ***laid**** ROOT EXTRACT

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Abstract*

Vitet *doniana root extract from the wild in Keffi, Nasarawa State, Nigeria was screened for phytochemical, antimicrobial, toxicity and antioxidant characteristics. Tannin, saponin, carbohydrate, phenol, resin, terpenes and sterols were present. The crued extract had inhibitory activity on Bascillus subtilis at minimum inhibitory concentration ofl.Q mg/cm'; LC_M of 2.624 fig/cm* andpercent antioxidant of 96.84 which compared favour ably with ascorbic acid used as standard. TLC of extracts revealed four fractions while column chromatography revealed six fractions.*

Keywords: *Phytochemistry, antimicrobial, toxicity, antioxidant, chromatography*

INTRODUCTION

Vitex doniana (Verbanaceae) is called dinya or tinya (in Hausa); Tunci (in Fulani); mgwobe (in Tanzania) and muhomozi (in Uganda). The plant is a perennial shrub widely distributed in tropical West Africa extending eastwards to Uganda, Kenya and Tanzania. *V. doniana* is employed in the treatment of a variety of diseases. Hot aqueous extracts of leaves are used in the treatment of stomachache, rheumatic pains/ inflammatory disorders, diarrhea and dysentery (Etta, 1984) indicating that the plant's leaves possess anti-inflammatory and analgesic properties. The roots and leaves are used to treat nausea, colic and epilepsy in Nigeria (Iwu, 1993). The young leaves are used as vegetables for sauces and porridge, the extract of the stem-bark exhibited a marked dose related antihypertensive effect in both normotensive and hypertensive rats (Olusola *et al,* 1997). The stem-bark also demonstrated some of invitro rypanocidal activity against *Trypansoma brucei* (Atawodi, 2005). The aqueous methanol extract has also exhibited anti diarrhea activity. Iwueke *et al.* (2006) reported that the leaves of V. *'doniana* possessed antiinlammatory and analgesic properties.

Medicinal plants are the best sources of drugs. The increase in drug resistant strains of bacteria and recent appearances of bacteria strains with reduced susceptibility to antibiotics adds urgency for new infection fighting strategies. This research is aimed at search of medicinal plants which are bioactive are natural, biodegradable and environmentally friendly.

MATERIALS AND METHODS

Plant Material

Roots of *V. doniana* were collected in Keffi. The plant was identified at National Institute of Pharmaceutical Research and Development (NIPRD) in Idu, Abuja. The roots were cleaned, air died and pulverized. The roots were extracted with methanol using soxhlet extractor method described by Furniss *et al* (1978) for 6 hrs at a solutesolvent ratio of 1:10 w/w. The methanol extract was concentrated and subjected to phytochemical analysis using methods of Harbone (1992); Trease and Evans (1989). The antimicrobial activity of extract was evaluated using Bauer and Kirby (19%) disc diffusion method on standard strains of micro-organisms: *Escherichia coli, Bacillus subtillis, Pseudomonas aeriginosa, Candida albicans, Staphyllococus aureus, Aspergillos flavus* obtained from NIPRD laboratory. The minimum inhibitory concentration (MIC) was conducted using different concentrations 2.0,1.0, 0.5, 0.25, 0.125 mg/cm^s of the extract respectively.

Toxicity (LCjo) Test

The toxicity (LQo) test was carried out using the brine shrimp lethality bioassay (Meyer *et al,* 1982)

Antioxidant Analysis

Antioxidant free radical scavenging assay of roots extract at 250 ig/cm^3 was evaluated using the 1,1-dipheny 1-2-picryl hydrazyl pPPH) assay at 518nm, (Menser *et al,* 2001; Aderogba *et al,* 2004). It j was calculated using the formula: AA% = 100

$$\frac{\text{(Absorbance (Sample) -Absorbance (blank)}}{\text{Absorbance (Control)}}$$

AA%=Percentage ascorbic acid

UK results are expressed as mean + SEM (Standard *dot of* means)

Horn Layer and Column Chromatography
The TLC plates, preparation of the sorbent (Silica gel slurry) and development of the chromatogram were methods described y Hamilton and Hamilton (?987) and Touchstone and Dobbins (1978). The visualization of spots was achieved using daylight and iodine vapour. TLC was carried out using hexane: ethylacetate: methanol, 3:2:1. The extracts were fractionated by column chromatography using silica gel 60G (70-230 Mesh) packed in a glass column of 90 on long with 3.0 cm internal diameter.

The packing of the column was as described by Furniss *et al.* (1978) and Popoola *et al.* (1994). The fractions were eluted using hexane: ethylacetate: ethanol in the ratio 3:2:1, respectively. TLC of the eluted fractions was carried out using hexane: ethylacetate, 4:1

Melting Point and pH Measurements
The melting point of the crude and fractions of extract obtained through column chromatogfapl\y are carried out by method described (Furniss *et al*, 1978) while pH was carried out using pH meter

RESULTS AND DISCUSSION
Fhytochemical Test
Phytochemicals present in the root extract were carbohydrates, tannin, saponin, phenol, resin, terpenes, sterols.

Antimicrobial Activity
The methanol extract of the roots inhibit the growth of *Bascillus subtilis* at minimum inhibitory concentration (MIC) of 1.0 mg/cm^3.

Toxicity(LC_{50})Test
The lethal concentration dose (LC^) 2.624 ug/cm^3 at 95% confidence interval.

Antioxidant Analysis
The antioxidant analysis of the extract revealed 96.84% at concentration of 250 ig/cm^3
Thin layer and column chromatography

The phytochemical components (secondary constituents) present in the roots extract of *V. doniana* have been reported to be responsible for bioactivity of the plant (Okwu, 2004; Zhao, 2007). The extracts inhibit the growth of *Basdllus subtilis* at MIC of 1.0 mg/cm^3. The microbe is the causative agent for most disease, which the plant is used traditionally to remedy. Tannin, saponin and phenols are major phenolic compounds associated with antioxidant activity of medicinal plants. It prevents oxidative cell damage, lowers the risk of heart disease (Okwu and Okwu, 2004), it protects against allergies, inflammations, microbes, viruses and tumours (Okwu and Omodamiro, 2005; Okunade, 2002). Tannins are antiseptic, have astringent properties and hasten the healing of wounds (Felix, 1982). Saponins have characteristics foaming properties and also lowers the amount of cholesterol in blood. Therefore, the use of *V. doniana* roots extract for curative purposes traditionally is

Table 1: Result of thin layer chromatography of crude *Vstex doniana* root extracts

Extract	Rf	Colour of spots
Crude *V. doniana* root	0.95	Light Yellow
	0.90	Brown
	0.55	Brown
	0.50	Brown

Table 2: Result of column chromatography of crude root extract of *V. doniana*

Fractions *Vitex doniana* roots	R_f	Colour of spots	Melting Point ^oC	pH
1-3	0.92	Light yellow	30-33	8.37
4	0.90	Light yellow	35-37	8.32
5	0.55	Light yellow	40-42	8.14
6-7	0.03	Brown	45-48	8.28
8-9	0.06	Dark brown	60-63	8.04
10-11	No separation	Dark brown	45-47	7.58

justified. The LC_{50} 2.624 $\mu g/cm^3$ of the roots extract signifies that extract could be very effective in controlling and inhibiting the growth of micro-organisms as it is capable of inhibiting vital metabolic processes thereby causing disorders in micro-organisms resulting in their perversion of behaviour or death (Fatope, 1995). The antioxidant activity, 96.84% is quite very high and compare favourably with a standard antioxidant, ascorbic acid with AA 93.7%, which was used as a control in this research. This signifies that the extract contained natural antioxidant namely saponin, tannin, phenol which can stimulate the human system and induce secretion of protective enzymes from the liver into the system and also block the damage of free radicals to genetic materials (Okwu, 2004). The other phytochemicals present in the extract also aid in stimulating antioxidant repairing mechanisms which subsequently increase the antioxidant scavenging mechanism for radicals in the system (Quettier-Dellen *et al.*, 2000). The thin layer chromatography and column chromatography revealed four and six components fractions respectively with different R_f values and corresponding characteristics colour of spots. The spots were not identified as no standard R_f values table was available as of the time of compiling this report. The melting point indicated that fractions obtained are pure as melting point indicated the level of purity of substance while the pH values showed that the extracts could be weakly basic in nature

CONCLUSION

The result of this research showed that crude root extract of *V. doniana* is effective in inhibiting growth of microorganisms and could be used as a source of natural antioxidant. Therefore, the traditional use of this plant for treatment of some diseases conditions is justified.

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