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Phytochemical and In-vitro Antibacterial Investigation of *Vitex doniana* Leaves, Stem Bark and Root bark Extracts.

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Abstract: The leaves, stem bark and root bark of *Vitex doniana* were gradient extracted and subjected to phytochemical and bacterial screening. The presence of various phytochemicals in the extracts was observed. The sensitivity test carried out on Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli gave zones of inhibition ranging from 4-20mm in most of the extracts. The Minimum Inhibition Concentration(MIC) was 1000 µg/cm^3 while maximum MIC was 2000µg/cm^3 for the bacteria used. Analysis of the bark extract revealed the presence of α-terpineol

Key words: *Vitex doniana*, phytochemicals, MIC, In-vitro, *α*-terpineol

INTRODUCTION

People world-wide have applied poultices and imbibed infusions of thousands of indigenous plants dating to prehistory. Human disease management in Nigerian history also provides evidence of the relationship between plants and medicine (Bannerman *et al*., 1986; Hammer, 1999; Raghavendhra *et al*., 2006; Ayandele and Adebiyi, 2007).

Research has shown that collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products known as secondary metabolites (Fatope *et al.*, 2001).

Medicinal plants are therefore, plants that contain these substances in one or more organs (root, stem, bark and flower) which can be used for therapeutic purposes and treatment of ailments. Plant chemicals useful in medicine are utilized mainly by incorporating them into medicines.

Secondary metabolites are also used as precursors for the manufacture of new or synthetic drugs and to help in the elucidation of the physiological mechanisms in drug development or testing. For example, medicinal plants with anti-inflammatory activity are widely employed in the traditional treatment of several disorders. The inflammatory response involves a complex array of enzyme activation, mediator release, cell migration, tissue breakdown and repair (Vane and Bolting, 1995)

Some important groups of these phytochemicals include: alkaloids, glycosides, steroids, flavonoids, fats, phenols, resins, saponins, tannins and terpenes. (Finar, 1975).

Medicinal plants represent a rich source from which antimicrobial agents may be obtained. These plants are generally of local origin and herbalists use them without sufficient scientific knowledge, often relying on past experiences and observations orally passed on, or in recent times, written (Bennerman *et al.*, 1986). They have been used successively as laxatives, anti-malarial, analgesics, anti-inflammatory drugs etc.

One of the earliest records of herbal medicine is the use of Chanlonoogra oil from species of *Hydrocapus guartin***,** which was known to be effective for the treatment of leprosy in China between 2730 and 300BC (Le Strange, 1977).

The use of plant and animal parts in medicine has since been widely documented in the records of ancient China, India and Egypt. This practice was based on series of "trial and error", and not substantiated by scientific means then. Over the years however, these methods have produced results of proven efficacies alongside conventional modern medicine (Chopra *et al*., 1956). In recent times, herbal medicines have become an integral part of the Primary Health Care system of many nations (Fajimi and Taiwo, 2000).

Vitex doniana sweet, (family *Verbanaceae*) is a perennial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania; and high rainfall areas. It is found in the middle belt of Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano states (Etta, 1984). It is variously called *dinya* (Hausa), *dinchi* (Gbagyi) and *oriri* (Yoruba) *ejiji* (Igala) and *olih* (Etsako).

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It is a deciduous evergreen tree, usually 4-8m high, occasionally up to 15m with a dense rounded crown. Its bark is light grey with numerous vertical fissures. Branchlets are not hairy and leaves are long stalked with 5-7 leaflets. Flowers are numerous, white tinged purple, usually borne in short, stout axilliary cymes on a long stalk. Calyx and pedicels are densely hairy. The fruit, which ripens between May and August, is a drupe consisting of a thin exocarp, the edible mesocarp (pulp) and a thick woody endocarp (Irvine, 1961; Etta, 1984; Bouquet *et al*., 1971; Iwu, 1993) and is used for jams and beverages (Egbekun *et al* 1996; http//www.worldagroforestry.org/sea/Products/…/AF/…/SpeciesInfo.asp 20-11-2010).

V. doniana is employed in the treatment of a variety of diseases. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea and dysentery (Irvine, 1961; Etta, 1984) indicating that the plant's leaves may possess anti-inflammatory and analgesic properties among others. The roots and leaves are used for nausea, colic and epilepsy ((Bouquet *et al*., 1971; Iwu, 1993). In eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meals. The anti-hypertensive effect of extract of the stem bark of *Vitex doniana* has been reported (Olusola *et al*., 1997). The extract exhibited a marked dose-related hypotensive effect in both normotensive and hypertensive rats (Olusola *et al*., 1997). Extracts of stem bark of *V. doniana* have also demonstrated some level of *in vitro* trypanocidal activity against *Trypanosoma brucei brucei* (Atawodi, 2005). The aqueous methanol extract has also exhibited anti-diarrheal activity (Agunu *et al*., 2005).

Most medicinal plants presently employed by local herbalists are used without scientific investigation. It is therefore important to access and document the ethno-medical claims of medicinal plants. In view of the various trado-medical applications of *V. doniana,* scientific investigation could help in the search for new drugs especially those which can be used in treatment and management of several ailments.

MATERIALS AND METHODS

Sampling and Identification:

Samples of *Vitex doniana* stem bark, root bark and leaves were collected in transparent polythene bags from Maikonkele, Bosso Local Government Area of Niger State and identified at the Biological Sciences Department of the Federal University of Technology, Minna.

Sample Treatment:

The samples were sun dried, crushed using pestle and mortar and micronized with a laboratory blender before storage in plastic bags prior to solvent extraction.

Extraction Process:

Each of the powdered sample was subjected to gradient extraction with Petroleum Ether (60-80), Chloroform and Methanol successively, with a soxhlet extractor. The sample was extracted for 48 hours with each solvent. The extract was dried on a water bath (60^{o}C) and the residue cooled and stored in a sterile container till required.

The Test Organisms:

Pure isolates of *Escherichia coli, Shigella dysentariae*, *Salmonella typhi* and *Staphylococcus aureus* were obtained from the Microbiology Department of the Federal University of Technology, Minna.

Phytochemical Screening:

Phytochemical screening of the extracts was done using standard procedures by Harborne (1973), Trease and Evans, (1983) and Sofowora. , (1984)

Microbial Screening of the Extracts:

The agar diffusion method was used as described by Oyeleke, *et al*., (2008) and Jimoh *et al*., (2010). Sterile nutrient agar was prepared and placed in labeled Petri dishes and allowed to gel. Wells were bored into the nutrient agar using a 4 mm sterile cork borer. Each crude extracts was reconstituted by adding 2cm3 of its mother solvent. 0.2cm^{3} of the reconstituted extract was dispensed into each well and allowed to diffuse for 30minutes. The test organisms were inoculated onto the labeled Petri dishes with a swab stick before incubating at 37^{o}C for 24 hours.

Determination of the Minimum Inhibitory Concentration (MIC):

In the determination of the MIC, the agar dilution method was used. 0.2, 0.1, 0.05 and 0.025g of the extracts were dissolved in 5cm^3 of sterile distilled water. 1cm^3 of each of the extracts was added to 19cm^3 of sterile molten nutrient agar, mixed thoroughly and then poured into sterile Petri dishes to make a final concentration of 2000, 1000, 500 and 250 µg/ cm^3 respectively. A loop full of the standardized bacteria culture was used to inoculate the plates which were then incubated at 37^{o}C for 24hrs. Minimum concentration which showed no turbidity was taken as the MIC.

Chromatography:

Thin layer chromatography (TLC) on the crude fractions using several solvent mixtures on AR60 silica gel established the best solvent mixtures. Preparatory TLC was then conducted and the different fractions were subjected to phytochemical and microbial screening. Finally, column chromatography was carried out on the ethanolic extract and the pale yellowish band observed was eluted and analysed by GC/MS.

RESULTS AND DISCUSSION

Table 1: Result of the phytochemical screening of crude extracts of *Vitex doniana* extracts.

Plant extracts	Alkaloids	Steroids	Flavonoids	Tannins	Glycosides	Saponins
LPet	+	+	-	+	-	-
BPet	-	+	+	+	-	-
RPet	-	-	+	-	+	+
LCh	-	-	-	+	+	-
BCh	-	+	+	-	+	+
RCh	+	-	-	-	+	-
Let	+	-	+	+	+	+
Bet	-	+	+	+	+	-
Ret	-	+	+	+	+	+

Key: L= Leaves; B=Bark; R=Root;
Pet= Petroleum ether; Ch= Chloroform; Et= Ethanol;.
+ = Present; - = Absent.

Table 2: Results of antimicrobial screening of crude extract of *Vitex doniana* extracts.

	Zone of inhibition (mm)			
Extracts	*S.aureus*	*S.typhi*	*P.aeruginosa*	*E.coli*
LPet	10	20	10	4
LCh	10	14	10	10
Let	10	10	10	10
BPet	10	10	4	8
BCh	14	4	8	10
Bet	-	10	10	4
RPet	10	4	10	8
RCh	10	-	4	4
Ret	-	-	10	4

Key: L= Leaves; B=Bark; R=Root;
Pet= Petroleum ether; Ch= Chloroform; Et= Ethanol;.

Table 3: Minimum Inhibitory Concentration (MIC) of *Vitex doniana* extracts, (µg/cm^3).

Extracts	*S.aureus*	*S.typhi*	*P.aeruginosa*	*E.coli*
LPet	-	-	-	-
LCh	2000	-	-	-
Let	1000	2000	-	2000
BPet	-	2000	-	-
BCh	-	-	-	2000
Bet	1000	1000	1000	1000
RPet	-	-	-	-
RCh	-	-	-	-
Ret	1000	1000	1000	1000

Key: L= Leaves; B=Bark; R=Root;
Pet= Petroleum ether; Ch= Chloroform; Et= Ethanol;

Table 4: Result of antimicrobial screening of TLC fractions of *Vitex doniana* extracts.

TLC Fractions (Solvent Mixture)	Extracts	*S.aureus*	*S.typhi*	*P.aeruginosa*	*E.coli*
R_f 0.47 (Ch/MeOH;2:1)	LCh	2	2	-	6
R_f 0.55 (Ch/MeOH;4:2)	LEt	-	1	-	-
R_f 0.45 (Pet/MeOH;1:2)	BCh	6	-	4	4
R_f 0.64 (Pet/Ch;1:1)	BEt	6	1	4	4
R_f 0.47 (Pet/Ch;2:1)	RPet	4	-	-	-
R_f 0.43 (MeOH)	REt	1	2	2	2

Key: L= Leaves; B=Bark; R=Root;
Pet= Petroleum ether; Ch=Chloroform; Et =Ethanol

Table 5: Phytochemical screening of TLC fractions of *Vitex doniana* with significant antimicrobial activity.

Extracts	TLC fraction (Solvent mixture)	Alkaloids	Steroids	Flavonoids	Tannins	Glycosides	Saponins
LEt	R_f 0.55 (Ch/MeOH; 4:2)	-	-	-	+	+	-
BCh	R_f 0.45 (Pet/MeOH 1:2)	-	+	-	-	+	-
BEt	R_f 0.64 (Pet/Ch 1:1)	-	-	-	+	+	-
REt	R_f 0.43 (MeOH)	-	-	-	+	+	-

Key: L= Leaves; B=Bark; R=Root;
Pet= Petroleum ether; Ch= Chloroform; Et= Ethanol;
+ = Present; - = Absent

Result of the phytochemical screening of *Vitex doniana* is presented in Table 1. It shows that most of the phytochemicals were absent in the petroleum ether and chloroform extracts of the plant while the ethanolic extracts contained almost all. Only saponins and tannins were present in the chloroform extract of the leaves while the chloroform extract of the root contained only alkaloids and steroids. Alkaloids were absent in the ethanolic extracts of the root and alkaloids and saponins in the bark of the same extract. This result agrees with that of Iwueke *et al.* (2006) and Kubmarawa *et al.* (2007) who reported the presence of alkaloids, tannins, glycosides, steroids and flavonoids in the ethanolic extracts of this plant.

Table 2 shows the results for the antimicrobial screening of the crude extracts of *Vitex doniana* showing the petroleum ether extract of the leaves having the highest activity with a zone of inhibition of 20mm against *Salmonella typhi*. Other extracts had zones of inhibition ranging from 4mm-14mm against almost all the organisms. However, *Staphylococcus aureus* was resistant to the ethanolic extract of both the stem bark and root while *Salmonella typhi* showed resistance to only the ethanolic extract of the root.

The MIC result for *Vitex doniana* is shown in Table 3. Results obtained showed that different concentrations of the extracts were able to inhibit the growth of some of the organisms. The growth of the organisms was inhibited at concentrations between 1000µg/cm^3 and 2000µg/ cm^3 All the organisms were resistant to the petroleum ether extract of the leave and the chloroform extract of the roots at all concentrations which ranged from 250µg/ cm^3 -2000µg/ cm^3. These results however give an indication of the effectiveness of the plant extracts as chemotherapeutic agents.

In the TLC analysis, first spot of the ethanolic extract of the bark of *Vitex doniana* had an R_f value of 0.64 with a mixture of petroleum ether/chloroform 1:1 as solvent. Other TLC fractions showed antimicrobial activity against some of the test organisms as shown on Table 4. Fractions of the ethanolic extracts of the leave and bark of the plant were active against all the test organisms with zones of inhibition ranging from 1mm-6mm. Only *Pseudomonas aeruginosa* showed resistance to the chloroform fraction of the leave while *Salmonella typhi* was resistant to the chloroform fraction of the bark.

Phytochemical screening of these fractions as shown in Table 5 revealed the absence of alkaloids, saponins and flavonoids. However, all the fractions contained glycosides while tannins were present in only the spot obtained from the ethanolic extracts of the stem and root barks. Tannins and glycosides are both present in these fractions because both of them usually have a glucose ring in them. The steroid found present in the chloroform extract of the leaves may be a glycosidic steroid.

The presence of glycosides in the extracts of *Vitex doniana* and their antimicrobial efficacy against these organisms agrees with the findings of Ebana *et al.* (1991) which says cardiac glycosides inhibit pathogenic bacteria. Also Nguji (1998) reported that tannins are important in herbal medicine and are applied to the healing of wounds and arrests bleeding. This also agrees with the findings of Agunu *et al.* (2005) who reported that the leaves have anti-diarrhoeal activity. Most of the organisms were resistant to fractions of the other extracts which indicate that these extracts possess more than one active component. Harborne (1973) reported that the activity of plant extracts can sometimes change after fractionation and a pure compound eventually isolated may lack the activity of the original extract.

Column chromatography on the ethanolic fraction of the bark of *Vitex doniana* (R_f 0.64 petroleum ether/chloroform 1:1) gave a gummy light yellow compound, with no absorption in the UV above 200nm. The infrared (IR) spectrum of which showed a broad (-OH) band at 3250 – 3510cm^{-1} and a weak (C=C) peak at 1635cm^{-1}. In the MS analysis there was a peak (M – 1) at m/z 135. Other peaks were observed at m/z 121, m/z 93, m/z 81 and m/z 59, identifying the compound as α-terpineol. Trace amounts of β-terpineol was also detected.

Conclusion:

The result revealed the antibacterial effects of some extracts of *Vitex doniana* on S. *aureus*, S. *typhi*, P. *aeruginosa* and E. *coli.* It also showed the presence of an α-terpineol. This compound is found in *Ravensara aromatic, Canavas sativa, Camellia sinensis and Coffea arabica.* (http//:www.book.google.com.ng/book, 2010). It is known for its exceptional antiviral, immune-stimulant, anti-infectious, antibacterial, neurotonic and psychic stimulant. Other properties are muscle relaxant, antalgic, expectorant and anti-catarrh, (Baudoux, 2010).

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