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Phytochemical and Antimicrobial Studies on Vitex Chrysocarpa (Planch ex Benth.)

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Abstract

Ethanolic leaf, stem and root extracts of Vitex chrysocarpa were investigated for the presence and composition of these phytochemicals (alkaloid, flavonoid, phenol, tannin, sterol, hydrogen cyanide, anthraquinone, saponin and terpenoid) using standard techniques. Their antimicrobial activities at different concentration against some selected clinical pathogens (bacterial strains *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, Fungal strain *Aspergilus niger*) were also studied using agar diffusion method. Minimum Inhibitory Concentration against the organisms was also evaluated. Analysis of Variance (ANOVA) was employed in data analysis. Qualitative and percent quantitative phytochemical results showed that Ethanolic leaf, stem and root extracts contained these phytochemicals assayed but at varied quantities. Antimicrobial studies indicated that the ethanolic leaf, root and stem extracts of Vitex chrysocarpa inhibited the growth of the pathogens but at varied levels and the inhibition was extracts concentration dependent. However, the extracts showed higher inhibition against the fungal strain than the bacterial strains. Inhibitory effect of the leaf extract was significantly higher than those of the stem and root extracts with minimum inhibitory concentration of 50 mg/ml for S. aureus and *E. coli*, 25 mg/ml for *P. aeruginosa* and 12.50 mg/ml for *A. niger*. For the stem, the minimum inhibitory concentration is at 50 mg/ml for *S. aureus* and *P. aeruginosa*, 75 mg/ml for E. coli and 25 mg/ml for *A. niger*. For the root, minimum inhibitory concentration is at 75 mg/ml for *S. aureus* and *E. coli*, 25 mg/ml for *P. aeruginosa* and 50 mg/ml for *A.niger*.
Antibiotic had a better activity when compared to the extracts at the same concentration. The data obtained from this study showed that the plant possessed antimicrobial properties especially antifungal and could be used in the treatment of bacterial and fungal infections but more especially the latter.

INTRODUCTION

Plants and other substances of natural origin have been in used throughout the world for human and animal health care from time immemorial. This is especially in Africa where underdevelopment and poverty have made a large percentage of the people to rely almost entirely on traditional medical practices and folkloric use of plants [1, 2]. In recent times, interest in plant research has increased all over the world owing to its potential use in traditional medicine for the treatment of a range of diseases. Various medicinal plants have been identified and modern scientific approaches have been used to study their authenticity, safety and efficacy of their therapeutic use. Different substances have been identified in medicinal plants which are believed to be antimicrobial agents and these includes; different forms of alkaloids, diterpenes, saponins, flavonoids, tannins, sterols, phenols, different forms of other proteins as well as lipids [3]. Several plant species have been tested for phytochemical and anti-microbial properties but the vast majorities have not yet been adequately evaluated [1, 4-6].

Among the medicinal plants, locally used in our community for treatment and management of various ailments is *V. chrysocarpa*. The genus *Vitex* includes approximately 250 known species of trees and shrubs in tropical and sub-tropical regions, although a few species are also found in temperate zones [7]. In Nigeria, especially in south eastern region, many species of *Vitex* are found including *V. chrysocarpa*.

Vitex chrysocarpa (Planch ex Benth.) belongs to the family Verbenaceae. It is more or less shrub or small tree with short bole, up to 6-8 (-12) m high with rounded open crown. The various species of *Vitex* have been used to treat a range of human ailments, particularly related to insects, fungi, bacteria, snakes and poisonous spider and diseases associated with menstruation and gynaecological problems. In my community, *Vitex chrysocarpa* is among the medicinal plants local-

ly used for treatment and management of various ailments. However, no scientific study has been done on *V. Chrysocarpa*, hence the need for the present study. Accordingly, the problem and focus on this research is to ascertain the phytochemical constituents of the plant and its potentials to inhibit the growth of microorganism.

METHODS

Area of the Study
The experiments were carried at the Chemistry Laboratory at the National Root Crops Research Institute (NRCR) Umudike, Abia state.

Collection and Identification of Plant Materials

The leaves, stem and roots of *Vitex chrysocarpa* (Planch. ex Benth) were collected between April-June from a forest along Omambala/Ezu River bank in Aguleri, Anambra East Local Government Area of Anambra State and was identified by Prof. Okafor, J.C. a Consultant, Agro forester and Taxonomist in Enugu, Nigeria. The voucher specimens were deposited at the Department of Botany Herbarium, Nnamdi Azikiwe University, Awka.

Materials Used

The materials and instruments used for the study included plant specimen (*Vitex chrysocarpa*), blender (grinder), masking tape, mortar and pestle, moisture cans, crucibles, Whatman filter paper No 42, burettes, volumetric flasks, beakers, conical flasks, sample tubes, desiccators, spectrophotometer, muslim cloth, oven, measuring cylinder, spatula, electric scale, Bunsen burner(stove), funnels, aluminium foils, test tubes, syringes, pipettes, cotton wools, etc.

Chemical and Reagents Used

Ethanol (alcohols), concentrated acetic acid, sulphuric acid, diluted ammonia, water, ferric chloride, potassium ferrocyanide, ethyl acetate, hydrochloric acid, petroleum ether, sodium hydroxide, potassium hydroxide (potassium permanganate). Hydrogen peroxide, sodium chloride, copper sulphate, sodium picrate, methyl red, cresol green, folin-cio caltean reagent, folin-dennis reagent, Eriochrome black and solechrome dark blue.

Preparation of Plant Materials for Phytochemical Studies

Fresh leaves, stem and root of *V. chrysocarpa* were oven dried and blended with electric blender. 250 g of each of the ground samples were soaked in 200 ml of ethanol for 24 h. They were then filtered with Whatman filter paper No 42. The extracts (50% yields) were concentrated by means of rotary evaporator, and subjected to tests.

Phytochemical Screening

Qualitative phytochemical screening of the extracts was conducted to determine the presence of these phytochemicals: Hydrogen cyanide, Alkaloids, Anthraquinone, Flavonoids, Saponins, Sterols, Tannins, Phenols and Terpenoids. This was done using standard procedure as described by [8]. Quantitative phytochemical test of the extracts was conducted to determine the percent quantitative contents of above phytochemicals using standard procedure described by [8-10].

Antimicrobial Studies

Test Microorganisms

The following microorganisms: Bacterial strains (*Staphylococcus aureus* (NR 201), *Escherichia coli* (NR 202), *Pseudomonas aeruginosa* (NR 203), and Fungal strain: *Aspergillus niger* (NR 241), were collected based on their clinical and pharmacological importance.

Sources of Test Microorganisms

The pure cultures of the microorganisms were obtained from the pathology Department of National Root Crop Research Institute, Umudike, Abia State. The isolates were checked for purity and are maintained on nutrient broth at 4 ^{0}C in the refrigerator until when required.

Sample Preparation and Extraction Procedure

The fresh leaves, root and stem of *Vitex chrysocarpa* were oven dried at 65-70 ^{0}C and ground into fine powder using a mechanical grinder. Soxhlet extraction method was employed for the extraction of the plants active principles.

Ethanol Extraction

The ethanolic extracts of the plant was prepared by soaking the ground sample of the leaf, stem and root in 100 ml of ethanol. The concentration of each extract was determined by adding 100 g, 150 g, 200 g, and 250 g in 100 ml of ethanol. The experimental set-up was left for 24 h at room temperature and thereafter filtered using No. 1 Whatman filter paper. The extract was then concentrated to 50 ml of the original volume of the extract and stored in an air tight container in a refrigerator at 4 ^{0}C until when needed.

Antimicrobial Activity

The agar diffusion method as described by [11, 12] was adopted for the study. Standardised Nutrient broth culture of the test isolate containing approximately 10^7 cells/ml organisms was used. 0.1 ml of the broth culture was introduced into sterile Petri dishes and 15 ml of motten nutrient agar poured into the Petri dishes. The contents were thoroughly mixed and allowed to solidify. Three holes each measuring 5.0 mm in diameter were made in each of the solid agar plates using a sterile cork borer. 0.04 ml of the different concentration of plant extracts were transferred into the holes using a Pasteur pipette. Two Petri dishes containing a particular bacterium were used for each concentration of the extracts. The plants were thereafter allowed to stand for 1 hour for pre-diffusion of the extracts [11] and were subsequently incubated at 37°C for 24 hours. After incubation, the plates were collected and the zones of growth inhibition were measured. The extent of inhibition was expressed in terms of the diameter of the inhibition zone as mea-

sured with a transparent metre rule. The effects of the extracts on bacteria and fungi pathogens were compared with those of the standard antibiotic ampicillin fungabacter for bacteria and fungi as standard control respectively.

Statistical Analysis

The results were analyzed using ANOVA. The Duncan's multiple range test significance was used to test the difference among treatments. All analyses were carried out at 5% level of significance.

RESULTS

Results are presented in Tables 1, 2, 3, 4, 5, and 6 and Figure s 1a and 1b.

Result revealed that the ethanolic leaf, stem and root extracts of *Vitex chrysocarpa* contained all the phytochemicals assayed (alkaloid, flavonoid, tannin, sterol, phenol, hydrogen cyanide, anthraquinone, saponin, terpenoid) but in varied quantities (Table 1). The leaf contained significantly the highest composition of alkaloid, tannin, sterol, phenol, HCN, saponin (2.77 ± 0.01, 1.06 ± 0.00, 0.24 ± 0.01, 0.13 ± 0.01, 4.71 ± 0.01 and 1.82 ± 0.05 respectively (Table 1). The stem contained significantly the highest composition of flavonoid and terpenoid (0.78 ± 0.00 and 2.78 ± 0.03 respectively (Table 1). The root contained significantly the highest composition of anthraquinone (2.88 ± 0.03) (Table 1). These phytochemicals were reported to be responsible for many antimicrobial activities of different plant species [13, 14]. Pharmaceutical and therapeutic values of plants and their products lie on the presence of these phytochemicals in them [15, 16]. Flavonoids have been reported to be synthesized by plants in response to microbial infections and are good antibacterial agents. Tannins have been demonstrated to have antibacterial activities [17]. Alkaloids are known to have effects on the central nervous system and act as antipyretic such as morphine, a painkiller. Similarly, saponins which are a special class of glycosides have been found to possess antifungal activity [18].

The results in (Tables 2, 3, 4, 5) showed that *V. chrysocarpa* extracts at 100, 150, 200 and 250 mg/100 ml all had inhibitory effects on tested pathogens but at varied levels. This indicated that the plant possesses antimicrobial properties. The inhibition of bacterial strains (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*) suggests that the plant possesses broad spectrum of antibacterial properties which could be used in the treatment of skin diseases and food poisoning of which the pathogens are commonly implicated. The inhibition of the fungal strain (*Aspergillus niger*) suggests also that the plant possesses antifungal property and could be used in the treatment of skin fungal infections. The antibacterial and antifungal activities of the Vitex c*hrysocarpa* were due to the presence of various secondary metabolites such as tannins, phenols, saponins, terpenoids, alkaloids, flavonoids, glycosides etc, which have been found *in vitro* to have antimicrobial properties [19, 20]. From the study, the leaf showed significantly higher inhibitory effects against the tested pathogens when compared to root and stem extracts. According to [21] this could be attributed to presence of higher bioactive compounds in the leaf extract than in the stem and root extracts. The result revealed inhibitory effect of the extracts against the pathogens to be in direct proportion to the concentration of the extracts (i.e. as the concentration increases the sensitivity and susceptibility of the test organism increases).

Table 1: Percent Quantitative Phytochemical Compositions of the Stem, Leaf and Root of Vitex chrysocarpa (%)

Phytochemicals	Plant Parts			
	Stem	Leaf	Root	P value
Alkaloid	1.850 ± 0.000^{a}	2.770 ± 0.014c	1.370 ± 0.014b	**
Flavonoid	0.780 ± 0.000^{c}	0.690 ± 0.000b	0.450 ± 0.000a	**
Tannin	0.835 ± 0.021^{b}	1.060 ± 0.000c	0.630 ± 0.000a	**
Sterol	0.190 ± 0.000^{b}	0.240 ± 0.014c	0.150 ± 0.014a	**
Phenol	0.130 ± 0.014^{a}	0.131 ± 0.014a	0.160 ± 0.014b	**
HCN	3.845 ± 0.007^{b}	4.710 ± 0.014c	0.910 ± 0.014a	**
Anthraquinone	2.290 ± 0.014^{b}	1.910 ± 0.014a	2.880 ± 0.028c	**
Saponin	1.435 ± 0.021^{b}	1.815 ± 0.050c	1.180 ± 0.028a	**
Terpenoid	2.780 ± 0.028^{c}	1.640 ± 0.014a	1.765 ± 0.050b	**

Results are in mean ± standard deviation. Rows followed by the same letter are not significantly different
** There is significant different (P < 0.05)

Table 2: Inhibitory Activity of the Leaf, Stem and Root Extracts of *Vitex Chrysocarpa* at 100 mg/100 ml of Ethanol

Plant Part	*P. aeruginosa*	*S. aureus*	*A. niger*	*E. Coli*
Control	12.78 ± 0.04^{d}	9.41 ± 0.01^{d}	13.55 ± 0.07^{d}	8.73 ± 0.18^{d}
Leaf	3.45 ± 0.00^{c}	2.78 ± 0.03^{c}	3.84 ± 0.00^{c}	2.41 ± 0.01^{c}
Root	2.71 ± 0.01^{a}	1.92 ± 0.00^{b}	2.61 ± 0.01^{a}	1.65 ± 0.00^{a}
Stem	2.84 ± 0.02^{b}	1.85 ± 0.00^{a}	2.75 ± 0.00^{b}	1.73 ± 0.01^{b}
P value	**	**	**	**

Control = Penicillin
** P < 0.05, column followed by the same letter are not significantly different

Table 3: Inhibitory Activity of the Leaf, Stem and Root Extracts of *Vitex Chrysocarpa* at 150 mg/100 ml of Ethanol

Plant part	*P. aeruginosa*	*S. aureus*	*A. niger*	*E. coli*
Control	15.76 ± 0.00^{d}	14.00 ± 0.00^{d}	17.50 ± 0.00^{d}	13.73 ± 0.04^{d}
Leaf	4.78 ± 0.04^{c}	3.23 ± 0.04^{c}	4.89 ± 0.05^{c}	2.73 ± 0.04^{c}
Root	3.85 ± 0.01^{a}	2.81 ± 0.01^{b}	3.76 ± 0.08^{a}	1.85 ± 0.00^{a}
Stem	4.23 ± 0.04^{b}	2.68 ± 0.04^{a}	4.33 ± 0.04^{b}	2.45 ± 0.00^{b}
P value	**	**	**	**

** P < 0.05, column followed by the same letter are not significantly different

Table 4: Inhibitory Activity of the Leaf, Stem and Root Extracts of *Vitex Chrysocarpa* at 200 mg/100 ml of Ethanol

Plant part	*P. aeruginosa*	*S. aureus*	*A. niger*	*E. Coli*
Control	16.53 ± 0.11^{d}	20.38 ± 0.11^{d}	18.00 ± 0.00^{d}	15.80 ± 0.00^{d}
Leaf	5.75 ± 0.00^{c}	7.20 ± 0.00^{c}	6.41 ± 0.01^{c}	4.50 ± 0.00^{c}
Root	4.50 ± 0.00^{a}	5.43 ± 0.25^{a}	4.81 ± 0.01^{a}	3.81 ± 0.01^{a}
Stem	4.80 ± 0.00^{b}	6.30 ± 0.00^{b}	5.75 ± 0.04^{b}	4.17 ± 0.02^{b}
P value	**	**	**	**

** P < 0.05, column followed by the same letter are not significantly different

Table 5: Inhibitory Activity of the Leaf, Stem and Root Extracts of *Vitex Chrysocarpa* at 250 mg/100 ml of Ethanol

Plant part	*P. aeruginosa*	*S. aureus*	*A. niger*	*E. coli*
Control	20.75 ± 0.35^{d}	19.25 ± 0.35^{d}	21.38 ± 0.04^{d}	23.00 ± 0.00^{d}
Leaf	10.27 ± 0.02^{c}	8.16 ± 0.00^{c}	11.33 ± 0.04^{c}	7.50 ± 0.00^{c}
Root	9.20 ± 0.00^{b}	6.43 ± 0.04^{a}	8.75 ± 0.00^{a}	6.50 ± 0.00^{b}
Stem	8.73 ± 0.04^{a}	6.52 ± 0.03^{b}	9.30 ± 0.00^{b}	6.28 ± 0.04^{a}
P value	**	**	**	**

** P < 0.05, column followed by the same letter are not significantly different

Table 6: Minimum Inhibitory Concentration (MIC) of the Extracts (mg/ml)

S/No	Organisms	Leaf	Stem	Root	Control
1	*S. aureus*	50	50	75	25.0
2	*E. coli*	50	75	75	25.0
3	*P. aeruginosa*	25	50	25	12.50
4	*A. niger*	12.50	25	50	12.50

Figure 1: a, Vitex chrysocarpa plant and b, Twig of Vitex chrysocarpa.
Source: self collection from wild

The result also revealed the minimum inhibitory concentration at which the leaf extracts will show inhibitory activity against the organism to be at 50 mg/ml for *S. aureus* and *E. coli*, 25 mg/ml for *P. aeruginosa* and 12.50 mg/ml for *A. niger*. For the stem, the minimum inhibitory concentration is at 50 mg/ml for *S. aureus* and *P. aeruginosa*, 75 mg/ml for *E. coli* and 25 mg/ml for *A. niger*. For the root, minimum inhibitory concentration is at 75 mg/ml for *S. aureus* and *E. coli*, 25 mg/ml for *P. aeruginosa* and 50 mg/ml for *A. niger*. This means that any concentration below these MICs (minimum inhibitory concentrations) the extracts will not show inhibition (Table 6).

DISCUSSION

This study revealed that the plant extracts possessed bioactive compounds that have antibacterial and antifungal activities against some human pathogens, which justified their use locally for treatment of infectious diseases.

This study has showed that the leaf of *Vitex chrysocarpa* showed significantly higher composition of all the phytochemicals assayed except for HCN, Anthraquinone and Terpenoid and therefore serves as a better source of this phytochemicals for medicinal purposes than the stem and root. The *V. chrysocarpa* extracts both showed antibacterial and antifungal activities however, the leaf extract showed better inhibition than the stem and root extracts indicating that it is a better antimicrobial agent than the stem and root. The data obtained from the study indicated that the plant possessed antimicrobial potentials. This study thus provides scientific insight to further determine bioactive natural agents and investigate other pharmacological properties of this great medicinal plant. Furthermore, before use in human being isolation of pure compound, toxicological study, and pharmacological activity should be carried out thereafter.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author CVI designed the study, CVI and JCO wrote the protocol and interpreted the data. Author JCO gathered the initial data and performed preliminary data analysis. Authors CVI and JCO managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

Authors have declared that no competing interests exist.

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