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ANTIMICROBIAL EFFECTS OF THREE TROPICAL PLANT EXTRACTS ON *STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI AND CANDIDA ALBICANS*

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Abstract

Antimicrobial activities of the leaf extracts of *Cymbopogon citatrus* (lemongrass) and *Vernonia amygdalina*(bitter leaf) and the seed extracts of *Garcinia kola* (bitter kola) were carried out. *G. kola* had effect only on *Staphyococcus aureus* and *Escherıchıa coli* with no inhibition on *Candida albican*s. Ethanol, cold water and hot water extracts of *Vernonia amygdalina* and *Cymbopogon citratus* showed inhibition on the three organism but *G. kola* ethanol, cold water and hot water extracts only inhibited *S. aureus* and *E. coli* with no inhibition on *Candida albicans.* The organism's susceptibility varied with more inhibition to *S. aureus* and least to *Candida albicans*.

Key words: Antimicrobials, , plant extracts, G*arcina, Vernonia, Cymbopogon,*

Introduction

In Africa phytomedicine has been in existence for hundreds of years ago ever before the colonial administration and is still in use today with about 80% of the population depending on herbal medicine for its primary health care delivery (Elujoba et al, 2005; Okigbo and Mmeka, 2006, Anon 2007a). Infectious disease accounts for one half of all deaths in the tropical countries irrespective of efforts made in controlling the incidence of epidemic (Iwu,1993; Okigbo and Ajalie, 2005).

Three plants were selected for this work based on ethnobotanical evidence of the plant in the community. *Garcinia kola,* bitter kola (Guttiferae) – A plant of west and central Africa origin (Iwu, 1993). *G. kola* is used as a masticatory (stimulate the saliva) substance and a good preventive agent against dysentery while the fruit pulp is used for the treatment of jaundice or high fever, the bark is used in medicinal preparations to help heal various ailments (Adebisi, 2007; Anon, 2007b). *C. citratus*, lemon grass (Poaceae/Graminae) is a native to India but found growing naturally in tropical grass land (Anon, 2007d). It used in Ayurvedic medicine to help bring down fevers and threat infectious diseases (Anon, 2007c). Traditionally, it is believed to cleanse the body so used as an antioxidant. *Vernonia amygdalina*, bitter leaf (Compositae) is a plant whose origin is in Nigeria (Anon, 2000). They are used in traditional medicine as a tonic and remedy against constipation, fever, high blood pressure and many infectious diseases (Iwalokun et al., 2006). The leaves are used instead of hops in beer production in Nigeria (Anon, 2000). The aim of this research is to determine the antimicrobial and antifungal properties of their extracts.

Materials and Methods

The medicinal plants used *V. amygdalina* (BOT/NAU/1314), *C. citratus* (BOT/NAU/2015) and *G. kola* (BOT/NAU/1518) seeds were collected in December, 2006 from Alor and Ojoto in Anambra State, Nigeria. The plants were identified by Prof. C.U. Okeke of Botany Department, Nnamdi Azikiwe University, Awka.

Aqueous Extraction (Cold Water): The method of Al-Magboul et al. (1997) as modıfıed by Okıgbo and Omodamıro (2006) was used. It was filtered with sterile filter paper (labline filter paper) inserted in a funnel and the

filtrate evaporated in a water bath at 100°C to dryness. The standard extracts obtained were stored in a refrigerator at 4°C until required for use. *G. kola* was filtered using muslin cloth.

Aqueous Extraction (Hot Water): 12g of the weighed plant material was soaked in 100ml of hot water boiled for thirty minutes into a conical flask for 24hrs. It was filtered using filter paper and evaporated.

Organic Solvent Extraction Using Ethanol: 12g of the plant was soaked in 100ml of 99.9/100% ethanol for 24hrs at room temperature with occasional stirring. The content was filtered and evaporated to dryness in a water bath at 78°C. The extract was collected and stored in the refrigerator at 4°C until required for use.

Table 1: Antimicrobial activities of the three plant extracts on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*

	Vernonia amygdalina			*Garcinia kola*			*Cymbopogon citratus*		
Pathogen	Ethanol	Cold aqu.	Hot Aqu	Ethanol	Cold	Hot aqu	Ethanol	Cold aqu.	Hot Aqu
S. aureus	+	+	+	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+	+	+	+
Candida albicans	+	+	+	-	-	-	+	+	+

+ = inhibition ; -=no inhibition

Table 2: Mean zone of inhibition (mm) of plant extracts of *Vernonia amygdalina*, *Garcinia kola* and *Cymbopogon citratus*

	Diameter zone of inhibition (mm)								
	Vernonia amygdalina			*Garcinia kola*			*Cymbopogon citratus*		
Pathogen	Ethanol	Cold H20	Hot Aqu	Ethanol	Cold Aqu	Hot Aqu	Ethanol	Cold Auu	Hot Aqu
S aureus	15±2	11±0.58	9±1	26±1	10±0	7±0	16±1.752	10±0.58	8±0
Escherichia coli	10 ±0	8±0.58	7±1	20±0.67	10±1.48	8±0	12±0	11±1.15	8±0
Candida albicans	7±0	7±1	6±0	-	-	-	9±01.73	10±00.58	7±0.63

These values are means of three measurements at different point of the zones on the cultured plates (Mean ± S.D.). Sterile water used as negative control did not show any effect on the test organism.

Table 3: Antibiotics disc sensitivity zone of inhibition (mm)

Antibiotics	*Staphylococcus aureus*	*E. coli*	*Candida albians*
Clotrimazole	-	-	++(35mm)
Amoxyclillin	++	+ (14mm)	-
Cephaloxin	-	+ (16mm)	-
Clexacillin	-	++	-
Erythromycin	++	++	-
Agumentin	++	++	-
Nitrofurantin	-	++	-
Ofloxacin	++	++	-
Ciproxin	++	++	-
Cefuroxime	++	-	-
Gentamicin	++	-	-
Naladixic acid	++	-	-

++ =>30mm (highly sensitive); + = < 20mm (sensitive); - = no inhibition

Tests for Potency of Bacteria and Yeast Pathogen

The clinical Isolates of the microorganisms were obtained from the Medical Microbiology Laboratory of Nnamdi Azikiwe University Teaching Hospital, Nnewi, (NAUTH). *E. coli* and *Staphylococcus aureus* were sub-cultured in Nutrient Agar and *Candida albicans* was sub-cultured in sabourand Dextrose Agar (SDA). An already made gram positive and gram negative (Asodisks Atlas Diagnostics, Enugu, Nigeria) antibiotic sensitivity disc was bought from the market (Table 3).

Plant Extracts Disc Preparation

The plant extract disc was prepared from labline filter paper by punching with a cork borer of 6mm diameter. The disc was autoclaved at 121^{o}C for 15mins. O.2gm of each plant extract was diluted with 10ml of water to give a concentration of 20mglml and this was added to discs. For the ethanol extracts, 0.2gm was first dissolved in 1ml of 99.9/100% ethanol due to its inability to dissolve in water initially and oily nature. Then, further dilution in 10ml of water to give 20mg/ml. The plant extract disc was dried in an oven and stored in refrigerator until required for use.

Culturing and Sensitivity Testing

The test organism (*S. aureus and E. coli)* were cultured on Nutrient Agar plates prepared by dissolving 28g of Nutrient Agar in one liter of water. *C. albicans* was cultured on Sabourand Dextrose Agar prepared by dissolving 64g in one liter of water. The media was autoclaved at 121^{o}C for 15mins. 9ml of this media was poured in plates and left to gel. The plant extract discs were placed in the triplicate cultured plates (disc-diffusion method) using a sterile forceps. The discs were placed far from each other to avoid overlap of zone of inhibition. The culture was incubated in an incubator for 24hrs at 37^{o}C. After 24hrs, the zone of inhibition of plant extracts was observed and measured. Sterile water disc was used as negative control and antibiotic disc used as positive control.

Statistical Analysis

Test for significance in the zone of inhibition was done using a 3-factor Nested classification in Randomized Complete Block Design and with analysis of variance (ANOVA) to know the effectiveness of each plant extract and the susceptibility of the test organism.

Result and Discussion

The plant extracts have antimicrobial properties (Table 1). *V. amygdalina* ethanol and aqueous extracts had inhibition on *S. arureus* (gram +ve) and *E. coli* (gram –ve) and against *C. albicans*. This is similar to Al-Magboul et al's (1997) research but contrary to Ashebir and Ashenafi (2007) observation of the plants' ability to inhibit *E. coli*. *G. kola* has a good antimicrobial properties (Ezeifeka et al., 2004; Madubunyi et al., 1995; Iwu, 1993). The ethanol extract of *G. Kola* had a good bactericidal properties on *S. aureus* (26mm zone of inhibition) and on *E. coli* (20mm zone of inhibition) with no inhibition on *C. albicans* (Table 2). This is due to low concentration of plant extract used. The aqueous extract inhibited *S. aureus* and *E. coli* only in line with their reports. The ethanol and aqueous extracts of *C. citratus* inhibited *S. aureus, E. coli* and *C. albicans.* Anon (2007c) reported that *C. citratus* possessed bactericidal and fungicidal properties but this disagreed with Kramer (2002) report of the plant's inability to inhibit *C. albicans*, *Pseudomonas aeruginosa* (gram negative) and *Bacillus cereus* (gram positive) bacteria. The difference in antimicrobial properties of a plant extract is attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), contamination by field microbes, adulteration and substitution of plants, incorrect preparation and dosage (Calixto, 2000; Okigbo and Omodamiro, 2006; Okigbo and Igwe, 2007).

The plant extracts were more susceptible to *S. aureus* (gram +ve) followed by *E. coli* (gram –ve) and then *C. albicans* (Table 2). Plant extracts show stronger retardation effect on the gram positive test strains than on the gram-negative ones (Desta, 1993; Okigbo et al., 2005; Ashebir and Ashenafi, 2007). It can also be seen in standard antibiotics used in which Amoxycillin and Cephaloxin had more effect on *S. aureus* than on *E. coli* (Table 3). This study highlighted the inhibitory properties of the three plants on the test organisms, the minimum inhibitory concentration of each extract of the plant and the best solvent for extraction. Furthermore the study revealed that *C.citratus, V. amygdalina* and *G. kola* plants used possessed the ability to inhibit the three pathogens.

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