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EVALUATION ANTIBACTERIAL EFFECTS OF *GARCINIA KOLA* AND *VERNONIA AMYGDALINA* ON *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RESIDENTS OF ABUJA. NIGERIA.

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ABSTRACT

This study was carried out to ascertain the antibacterial effects of extracts of *Garcinia Kola* and *Vernonia amygdalina* on Staphylococcus isolated from residents of Abuja. Fresh Bitter Kola seeds and *Vernonia amygdalina* leaves were purchased from various markets in Abuja. Some of them were collected from domestic gardens. Extracts were prepared and tested upon isolates of *Staphylococcus aureus* obtained from various clinical smaples. The crude extract of Bitter Cola showed activity against *S. aureus*, with mean zones of inhibition (ZOI) of 6.36 ±0.36 mm (3.9 ±0.6 - 8.9±0.6); compared to mean ZOI of 5.96 ±0.62 mm **(**5.2 ±0.3 - 6.7±0.8) recorded in Bitter Leaf. Aqueous extracts of Bitter Cola exhibited higher antimicrobial activities, ZOI: 8.66 ±0.42mm (5.3 ±0.4 - 13.5 ±0.4), compared to mean ZOI of 5.96±0.62mm **(**5.2 ±0.3 - 6.7±0.8) recorded in *V. amygdalina*. On the other hand, aqueous extracts of Bitter Cola exhibited higher antimicrobial activities with a ZOI of 8.66 0.42 mm (5.3 ±0.4 - 13.5 ±0.4) compared to 6.36 ±0.36 mm (3.9 ±0.06 - 8.9 ±06 mm). Both crude and aqueous extracts of test plants exhibited significant ($P<0.05$) effects against *S. aureus*. The inhibitory activities of *G. kola* and *V. amygdalina* did not differ significantly ($p> 0.05$). The MIC of *Garcinia kola*

seed extract ranged from 0.045–0.049 mg/mL. While the MBC of the extract ranged from 0.1268–1.25mg/mL. Similarly, the MIC of Vernonia ranged from 0.0016–0.1486 mg/mL and the MBC ranged from 0.0611–2.45 mg/mL respectively. The MBC values were much higher than the MIC values. Thus, is suggestive of bacteriostatic action of *Garcinia* and *Vernonia* extracts on *S. aureus*.

KEYWORDS: Abuja, Staphylococcus, Vernonia, Garcinia, Antibacterial.

INTRODUCTION

Infection rate from *S. aureus* is high and recently; there has been a huge concern about the increased incidence of strains of the bacteria that fail to succumb to all but a few antibiotics (Boyanova and Mitov, 2013). This is a situation known as antibiotic resistance. Most experts think that this is due to the worldwide overuse of antibiotics (Akortha *et al.*, 2011).

In Nigeria, multidrug resistant *S. aureus* have been reported in hospital and non-hospital population (Chigbu and Ezeronye, 2003). Therefore, owing to the problem of resistance (Nwakaeze *et al.*, 2013) by *S. aureus* accounting for about 50% failure of circulating antibiotics; the local communities especially in Nigeria have relatively resorted to the use of herbs; which according to reports (Onwuliri *et al.*,2006), have been in existence hundreds of years before colonization. About 80% of the population depending on herbal medicine for its primary health care delivery (Elujoba *et al.*, 2005; Okigbo and Mmeka, 2008).

Garcinia kola is a medium sized tree of West and Central Africa origin, particularly popular in parts of Nigeria as a tree of the rain forests (Iwu, 1993). Its biological name is "*Garcinia kola*" and it belongs to the family of "Guittiferae". The Yorubas call it 'Orogbo', the Igbos calls it 'Agbilu, Adi' or 'Aki ilu' while the Hausas, know it as 'Namijin Gworo. *Garcinia kola* is highly valued for its edible nuts and traditionally used by African medicine men who believed that it had purgative, anti-parasitic, and antimicrobial properties (Adegboye *et al.*, 2008).

Bitter Leaf, *Vernonia amygdalina* belongs to the plant family Compositae. In Nigeria, the Edo calls it Oriwo, Hausa, Chusar doki (a horse tonic food containing the leaves), Fatefate/mayemaye (a food prepared from the leaves). The Ibibio calls it Atidot, the Igbos, Onugbu; Tiv, Ityuna and Yoruba, Ewuro (Uzoigwe and Agwa, 2011). *Vernonia amygdalina*, a member of the Asteraceae family; is a small shrub in tropical Africa. *V. amygdalina* is

commonly called Bitter Leaf because of its bitter taste. The plants are used in traditional medicine (Iwalokun *et al.*, 2006). *Vernonia amygdalina,* Bitter Leaf, a shrub; 10 meters tall; is much branched and densely pubescent. Leaves are alternate, blade ovate-elliptical to lanceolate, cuneate or rounded at base, terminal Inflorescence Flowers; style hairy, brown to black, crowned by the much longer pappus bristles.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF STUDY PLANT MATERIALS

The two plants used in this study: Bitter cola (*Garcinia kola)* and *Vernonia amygdalina* (Bitter Leaf); were collected from home gardens, NIPRD garden and purchased from markets in Abuja and identified using standard methods (Christinah and Roland, 2012). The identification was authenticated by a plant taxonomist in the Department of Medicinal Plant Research and Traditional Medicine; National Institute for Pharmaceutical Research and Development (NIPRD), Abuja Nigeria.

PREPARATION OF *GARCINIA KOLA* SEED SAMPLES

The outer testa of each *Garcinia kola* seeds were removed washed and air-dried for about 24 hours. Each seed was then cut into small bits pellets with a kitchen knife. The resulting pellets were subsequently dried in electric oven for 12 hours at 40^0C. The dried seed pellets were blended into fine powder, using a manual grinder and then sieved with 10 micrometer sieve and kept in air-tight container for further use. Portions of the resulting powder were used for extraction and phytochemical analysis and the remained reconstituted with normal saline to obtain suspensions of appropriate concentration for oral administration.

PREPARATION OF BITTER LEAF SAMPLE

Leaves of *V. amygdalina* were batched into three parts of 300g each. The weighed samples were macerated in 2.3 liters of each of the solvents and covered with cellophane. The mixture was allowed to stay for 24 hours, after which it was filtered using Muslim cloth and vacuum filtration. The filtrates were concentrated using a rotary evaporator. The concentrated aqueous extracts were dried slowly in water bath while methanol and ethanol extracts were freeze dried (Momoh *et al*., 2010).

PREPARATION OF EXTRACTS OF *VERNONIA AMYGDALINA* LEAVES AND OF *GARCINIA KOLA* SEEDS

Three hundred grammes (300g) of the milled *G. kola* seed powder was added into solvents: distilled water, ethanol and methanol respectively. This was mixed for ten minutes, left to stand for 24 hours and filtered through a giant funnel with a collector below, according to Momoh, 2010. The extracts of the seeds were prepared in accordance with the method of Basri and Fan (Nwaokorie *et al.*, 2010).

DETERMINATION OF *STAPHYLOCOCCUS AUREUS* SUSCEPTIBILITY TO EXTRACTS OF *GARCINIA KOLA* AND *VERNONIA AMYGDALINA*

Staphylococcus aureus isolates were cultured on Nutrient Agar following the method described by (Naima *et al.*, 2013). A 4mm cork-borer was used to make the appropriate number of holes inside the solid sterile nutrient agar in a sterile Petri dish containing about 0.5 McFarland *S. aureus*. Plates were swabbed with cotton wool impregnated with the organisms prepared. Five holes were bored in one plate. Each of the holes was filled with different concentration of prepared crude extract solutions. The first three wells were filled with solution of the extract at concentrations of 200 mg, 100 mg and 50 mg/mL. The other two wells were filled with a positive control Ciprotab (Ciprofloxacillin) antibiotic (1.25 mg/mL) and Sterile water (negative control). The plates were then allowed to stand for 20 min to allow proper diffusion of the solution into the medium before incubation. The test plate cultures were incubated in an incubator at 37°C for 24 hours. The zones of inhibition of *G. kola* / *V. amygdalina* extracts were observed and measured. Antimicrobial activity was evaluated by measuring the zones of inhibition against the test organisms. The experiment was replicated two times and zones of inhibition reported as mean ±SD.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) TEST PLANT EXTRACTS ON *S. AUREUS* ISOLATES

The Minimum Inhibitory Concentration (MIC) was determined using the agar dilution method as described and modified by Adejare *et al.* (2013**)**. Each of 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml concentrations of Garcinia *kola* aqueous and ethanolic extracts into 100ml of Mueller Hinton Agar (MHA), were mixed vigorously to obtain a homogenous mixture. The inoculated, serially diluted extract was incubated at an appropriate temperature with the test organism for about 18 hours. After incubation, the culture was observed for

microbial growth (presence of turbidity). One untreated culture was used as control to compare with the MIC of ideal antimicrobial agent.

For Bitter leaf (*Vernonia amygdalina*), each of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml concentrations were prepared for both the aqueous and alcoholic extracts. Twenty grammes (20)g, 10g, 5g and 2.5g of each of the extract was introduced into a 100 ml of MHA. This was mixed vigorously to achieve homogeneity. Comparative control experiment was simultaneously carried out using sterile distilled water (negative control) and Ciprotab (positive control).

The concentration at which there was no visually detectable bacterial growth was taken as the MIC (Nwaokorie *et al.*, 2010).

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The MBC was determined using the method of Vila *et al*. (2010) with small modifications. Approximately, 2µL of the sample from Minimum Inhibitory Concentration assay was spread onto freshly prepared MHA plates, incubated at 37°C for 24 hours and monitored for the presence of bacterial growth. The MBC were taken as the lowest concentration that did not allow bacterial growth on the surface of the agar plates. The concentration at which there was no bacterial growth after inoculation in Mueller Hinton agar was taken as MBC.

RESULTS

Table 1: Antimicrobial activities of *Garcinia kola* seed and *Vernonia amygdalina* leaf extracts.

Test plant	***S. aureus* isolates**	**Zone of Inhibition (mm)**	
		Crude extract	**Aqueous extract**
Bitter Cola	*S. aureus*$_{CISN-1}$	8.9 ±0.6	13.5 ±0.4
	S. aureus$_{CISN-2}$	5.7 ±1.4	6.9 ±0.3
	Isolate - 011	8.7 ±0.8	5.3 ±0.4
	Isolate - 017	3.9 ±0.6	7.3 ±0.5
	Isolate – 026	4.6 ±0.7	10.3 ±0.5
	Mean ±SD	**6.36 ±0.36**	**8.66 ±0.42**
Bitter Leaf	*S. aureus*$_{CISN-1}$	5.7 ±3.4	4.7 ±0.6
	S. aureus$_{CISN-2}$	5.2 ±0.3	5.0 ±0.4
	Isolate - 011	6.4 ±0.7	4.6 ±1.0
	Isolate - 017	5.8 ±0.5	12.2 ±0.6
	Isolate – 026	6.7 ±0.8	8.8 ±0.3
	Mean ±SD	**5.96 ±0.62**	**7.06 ±0.58**

Zones of inhibition = Mean ±SD values of duplicate challenge of *S. aureus* on cultured plates, ± = Standard deviation.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration of ethanol extracts of *G. kola* and extracts on *S. aureus.*

Organism		
MIC/MBC	***S. aureus***	***S. typhimurium* (control)**
MIC values of Ciproftab (mg/mL)	0.0049	0.0178
MIC values of *G. kola* (mg/mL)	0.045	0.84
MBC values of Ciproftab (mg/mL)	0.1268	0.1565
MBC values of *G. kola* (mg/mL)	0.25	1.25

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol extracts of *V. amygdalina* leaf on *S. aureus.*

Organism		
MIC/MBC	***S. aureus***	***S. typhimurium* (Control)**
MIC values of Ciproftab (mg/mL)	0.0016	0.01486
MIC values of *V. amygdalina* (mg/mL)	0.06	0.0145
MBC values of Ciproftab (mg/mL)	0.127	0.0611
MBC values of *V. amygdalina* (mg/mL)	2.45	1.25

DISCUSSION

The aqueous extract of *G. kola* exhibited higher antimicrobial activities than *Vernonia amygdalina* using the same solvent of extraction. It has also been reported by several workers that solvents actually extract the different antimicrobial substances (Ahmad *et al.*, 1998; Ibrahim *et al.*, 2009; Vaghasiya *et al.*, 2011). Antimicrobial activities of crude extract of *G. kola* seed extract gave inhibitory activities of as high as 8.66mm against test *S. aureus* isolates. *G. kola* has been reported with good antimicrobial properties (Ezeifeka *et al.*, 2004; Nwaokorie *et al.*, 2010). The ethanol extract of *G. kola* had good bactericidal properties on *S. aureus.* This agrees with Al-Magboul *et al.*, (1997). Our result is in tandem with Ahmad and Beg (2001) who reported zones of inhibition of less than 10 mm. The minimum inhibitory concentration and proximate composition, justifies the result of the antimicrobial activities. Phenolic content has been confirmed a key factor in most isolates were known resistant isolates, which could be responsible for the low zones of inhibition. The difference in antimicrobial properties of a plant extract has been attributed to not only plant materials, but prevailing physical factors (temperature, light water), field microbes (Okigbo and Omodamiro, 2006; Okigbo and Igwe, 2007; Atangwho *et al.*, 2009). In addition, *S. aureus*

bacteria are prokaryotes with thin cell wall and relatively simple genetic system, which enhance easy penetration of bioactive substances, leading to impact on the bacteria genetic system as a result of bioactive interruption (Prescott *et al.*, 1999; Wang *et al.*, 2014).

The antibacterial activities of crude extracts of *G. kola* agrees with the findings of Sibanda *et al.*, (2008) on *in vitro* antibacterial regimes of crude aqueous and acetone *G. kola* seed extracts. This is also consistent with the inhibition of growth of *S. aureus* reported by Sibanda and Okoh, (2008) and Christinah and Roland, (2012) on the antimicrobial effects of *G. kola* seeds extracts. Many studies have shown that saponins, tannins, flavonoids and phenolic compounds contain antimicrobial properties (Subrahmanyam *et al.*, 2001; Osman *et al.*, 2003; Tawaha *et al.*, 2007). Antibacterial activity of *V. amygdalina* against some Gram-negative and Gram-positive bacteria has also been reported, with suggestions that Bitter Leaf could be effective on drug resistant microorganisms, and in wounds dressing (Iwalokun *et al.*, 2003; Tula *et al.*, 2011; Uzoigwe and Agwa, 2011). *G. kola* on the other hand has been attributed with good antimicrobial and antiviral properties (Iwu, 1993). The seeds are used in the treatment of bronchitis and throat infections. Similarly in a recent study, crude extract of *G. kola* exhibited *in vitro* antimicrobial activities against both Gram-positive and Gram-negative organisms compared to streptomycin and tetracycline (Adegboye *et al.*, 2008).

Susceptibility of *S. aureus* to Vernonia and Garcinia are indicative of the efficiency of ethanol as an efficient solvent (Seanego and Ndip, 2012; Vaghasiya *et al*., 2011); while the poor activity of water extract is also in line with previous findings (Nwaokorie *et al.*, 2010; Jayalakshmi *et al*., 2011); who noted that water is not not a good solvent. This was linked to possible insolubility of important bioactive compounds of Garcinia and Vernonia extracts (Essawi *et al.*, 2010); Seanego and Ndip, 2012). The results obtained from this study justify the use of this plant in traditional medicine and provide leads which could be further exploited for the development of new and potent antimicrobials.

The higher MBC values than the MIC suggest a possible bacteriostatic effect of the plants even at at low concentration.

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