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# PHYTOCHEMISTRY AND COMPARATIVE ANALYSIS OF THE ANTITUSSIVE POTENTIAL OF *Allium sativum* AND *Garcinia kola* AGAINST CLINICAL ISOLATES OF RESPIRATORY TRACT INFECTIONS.

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## ABSTRACT

Aqueous and ethanol extracts of *Allium sativum* and *Garcinia kola* were comparatively assessed for phytochemical compositions and antitussive activity on bacterial isolates from respiratory tract and associated clinical samples (nasal and throat swabs). Using standard phytochemical and analytical microbiological techniques, the results revealed the presence of alkaloids, flavonoids, cardiac glycosides and tannins, indicating that ethanol was a better solvent than water and of significantly ( $p < 0.05$ ) higher yields in *G. kola* than *A. sativum*. The antibacterial activity of *A. sativum* and *G. kola* were assessed by agar-disc diffusion technique against the isolated respiratory tract infection pathogens: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The results revealed broad-spectrum of antibacterial activity by ethanol extracts of both plants. The antibacterial activity was significantly ( $p < 0.05$ ) higher for the ethanol extracts of *G. kola* than those of *A. sativum*; while the aqueous extracts of both plants were inactive, except slight activity by *G. kola* against *S. aureus* and the streptococci. The bioactive plants extracts basically exhibited static activity independent of the concentrations and incubation periods used in the study.

## INTRODUCTION

Respiratory Tract Infection (RTI) is among the commonest infections, accounting for much consultation in general medical practices worldwide (Denyer *et al.*, 2007). The RTI pathogens are regularly transmitted via oral and nasal discharges from infected eyes, ears or both (Fuerst, 1978). Oral and nasal secretions inevitably contaminate the atmosphere during talking, laughing, sneezing, coughing and similar actions that produce droplets and droplet-nuclei. This is in addition to the aerial dissemination of saliva and mucus, the spread of oral, nasal and conjunctival secretions by hands, eating utensils, drinking glasses and improperly maintained swimming-pools, which may be contaminated by feces and urine as well as by respiratory secretions. Hence, the environment is constantly facing a formidable, unceasing and virtually ubiquitous onslaught of RTIs. Consequently, RTI and other associated airborne pathogens generally, are amongst the most difficult to control (Fuerst, 1978). Amongst the bacterial etiologic agents of RTI are *Staphylococcus aureus*, *Streptococcus* species, *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; *Mycobacterium tuberculosis*, *Haemophilus influenzae*; *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Chlamydia psittacosis*, *Bordetella pertussis*, as well as anaerobes such as *Bacteriodes* species (Duerden *et al.*, 1990; Laurence *et al.*, 1997; and Denyer *et al.*, 2007). The pathogenesis of these bacteria often result in the production of secretions, exudates, transudates or extraneous materials from the respiratory tract by the irritation of the airways and lungs mostly the bronchi, resulting in cough (Rang and Dale, 1994; Laurence *et al.*, 1997). Cough is a protective reflex mechanism, the sole purpose of which is to remove foreign materials and secretions from the bronchi and bronchioles of the

Lower Respiratory Tract (LRT), (Rang and Dale, 1994). Cough is caused by the irritation of airways: the bronchi and trachea. Accordingly, it may be inappropriately stimulated by conditions not associated with either excess secretions or foreign material, such as inflammation or neoplasia. In this case, antitussives (cough-suppressants) may be used, even though they merely suppress the symptoms without influencing the underlying conditions (Rang and Dale, 1994).

Previously, the most powerful antitussives were the narcotic agents, but a few non-narcotic agents have been reported to prove powerful enough to have gained extensive popularity (Asperheim and Eisenhauer, 1981). Presently, many medicinal plants based on folkloric claims and traditional (herbal) medical practices could possess antitussive and expectorants potentials, amongst which are *Allium sativum* and *Garcinia kola*. *Allium sativum* (Garlic) is a species in the onion family *Alliaceae*, generally distinguished by their characteristics pungent, hot flavor (Iwu, 1984;1999; Okigbo and Igwe, 2004; Sofowora, 2005; Eja *et al.*, 2007). Amongst the claimed folkloric pharmacological usefulness of *Allium sativum* are its use as an antimicrobial agent against many infections and diseases notably the RTI by preventing and treating common-cold and cough. This assertion has the backing of the long tradition in herbal medicine which garlic is used for coarseness and cough due to irritation from pharyngitis, bronchitis, bronchopneumonia. It is also an effective cold-remedy as well as an expectorant for coughs (Iwu, 1984; 1999, Ashur, 1986; Sofowora, 1993; 2008, 1996; Eja *et al.*, Iwu, 1999; Burkill, 2002; Bushmann *et al.*, 2006; Okoli *et al.*, 2007; Joshi, 2008 and Focho *et al.*, 2009).

Similarly, *Garcinia kola* (Bitter-kola) belongs to the family *Guttiferae*, and is abundantly found and distributed in the moist forests of West and Central Africa, growing as a medium size tree of 10 – 18m high. Amongst the many folkloric claimed pharmacological and medicinal uses of its seeds, roots, leaves and stem-bark, is the use of the seeds as an antimicrobial agent in the treatment of many infections and diseases, including RTI (Sofowora, 1993; Hostettmann *et al.*, 1996; Lewis and Elvin-Lewis, 1997; Iwu, 1999; Akaochere *et al.*, 2002; Burkill, 2002; Okigbo and Igwe, 2004; Bushmann *et al.*, 2006; Okoli *et al.*, 2007; Joshi, 2008; Sofowora, 2008; Focho *et al.*, 2009).

Generally, there is a dearth and paucity of information on the empirical antimicrobial effects of *Allium sativum* and *Garcinia kola* as antitussives against respiratory tract infections induced cough. Thus, this paper examines and reports the efficacy of folkloric claim of the potency of these plants against RTI- pathogens, as potential non-narcotic antitussives and expectorants.

## **MATERIALS AND METHOD**

### **Plants Collections, Identification and Preparation**

*Allium sativum* (Garlic) bulbs and *Garcinia kola* (Bitter-kola) seeds were obtained from Araria market in Aba, Abia State, Nigeria and identified by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo Nigeria. The garlic bulbs and bitter kola seeds were respectively washed with clean water, to reduce the microbial load and air-dried. Thereafter, the outer covering of both garlic and bitter-kola were peeled-off and the materials sliced into cutlets, air-dried and subsequently pulverized. The samples were weighed and stored in air-tight polyethene bags at room temperature.

### **Extraction and Phytochemical Screening**

Cold extraction of the respective pulverized plants parts were carried out by macerating 400 g of the samples in 100 ml distilled water and ethanol, with intermittent stirring for 24 h (aqueous) and 72 h (ethanol). The samples were filtered using cotton-wool and Whatman No.1 filter paper. The filtrate were concentrated to dryness *in vacuo* at 40 °C, and stored at 4 °C for subsequent assays. Phytochemical screening of the respective plants extracts was carried-out according to the standard phytochemical procedures (Harbone, 1983; Iwu, 1984; Trease and Evans 2002; Pearson, 2005; Sofowora 2008).

### **Isolation, Characterization and Identification of Clinical Bacterial Isolates**

Clinical samples comprising sputum, throat and nasal swabs were respectively and aseptically collected from patients at the University of Uyo Health Centre, into samples-stoppered Bijou bottles containing 10 ml of microbiological saline and were immediately taken to the laboratory for cultivation. Isolation of RTI-bacteria was aseptically carried-out by inoculating 1.0 ml aliquots of ten-fold serially diluted samples in both general-purpose and selective/differential media such as Nutrient Agar (NA), Mannitol Salt Agar (MSA), Blood Agar (BA), MacConkey Agar (MCA) and Eosin Methylene Blue agar (EMB), by the standard pour-plate technique and incubated at 37 °C for 48 h (Collins and Lyne, 1979). The isolated bacterial cultures were aseptically purified by repeated sub-culturing twice by streaking on the freshly prepared nutrient agar plates and maintained at 4 °C in agar slants. The clinical bacterial isolates were characterized and identified based on standard cultural, morphological and biochemical properties.

Inocula of the isolated clinical bacteria were standardized to 0.5 MacFarland Nephelometer turbidity, with cells density approximately  $1.5 \times 10^8$  cfu/ml following the method of Tilton and Howard (1987); Baron and Finegold (1990); with some modifications (Ekong *et al.*, 2004). Cultures of Gram-positive bacteria were diluted to factor 3; while those of Gram-negative bacteria were diluted to factor 5 (Ekong *et al.*, 2004).

### **Antibacterial Activity of Garlic Bulb and Bitter-Kola Seed Extracts**

The antibacterial activity of the garlic bulbs and bitter kola seeds against the clinical bacterial isolates was evaluated using the standard spread-plating technique and agar-disc diffusion method (Collins and Lyne, 1979). A loopful of 0.1ml standardized bacterial cultures was respectively spread-plated on NA on which the extracts impregnated paper discs of 6mm diameter were then placed. The set up was incubated at 37 °C for 24 h (Collins and Lyne, 1979). Paper-discs of equal diameter impregnated with cefuroxime (CFX) and cotrimoxazole (CTZ) served as the positive controls.

The sizes of Inhibition Zone Diameters (IZD) were measured and correspondingly deducted from the diameter of the filter-paper discs. Thus, IZD less than or equal to the diameter of the paper discs indicated either inactivity or resistance; while zones greater than the paper discs diameter indicated activity or sensitivity. Potency of the extracts was evaluated by activity-index, calculated as the ratio of extracts activity to that of the controls (Udofia *et al.*, 2012; Ekong and Udoh, 2015a). The higher the activity index, the more potent the extracts.

### **Determination of Minimum Inhibitory Concentration (MIC) of the Plants Extracts**

The MIC of garlic bulb and bitter-kola seeds extracts were respectively determined by the macrobroth-dilution method (Tilton and Howard, 1987; Baron and Finegold, 1990), with some modifications (Ekong *et al.*, 2004). Tubes of two-fold serially diluted extracts with nutrient broth (NB), were inoculated with 0.1 ml aliquots of the respective standardized broths-cultures and incubated at 37 °C for 24 h. Uninoculated NB served as negative control. The MIC of the extracts were taken as the least concentrations of the extracts that inhibited the growth of the test bacteria with respect to turbidity of the control.

## **RESULTS AND DISCUSSION**

The yields of *Allium sativum* and *Garcinia kola* extracts and their phytochemical compositions are presented in Table 1.

The yield varied with the plants and the solvents used for extraction. In both plants, ethanol extracts recorded significant ( $P < 0.05$ ) higher yields in the descending order *Garcinia kola* (78.0 %) > *Allium sativum* (60.0 %), compared with the yields of the aqueous extracts of both plants which *Garcinia kola* (60.0 %) still recorded significant ( $P < 0.05$ ) higher yield to that of *Allium sativum* (56.0 %). In this study, cold maceration irrespective of its associated low yield was preferred because of its low or room temperature operational requirement. As such, the

technique does not involve the use of excessive heat, as the soxhlets apparatus, thereby providing protection to thermolabile bioactive phyto-constituents. Similar, reports of low extractable yields by cold maceration have been reported (Ibrahim *et al.*, 2010; Prajapati and Patch, 2010; Udofia *et al.*, 2012). Nevertheless, the relative higher yields obtained with ethanol extracts, may possibly suggest the superior extractable properties of ethanol. This could be attributed to the major function of the solubility of the phyto-constituents in ethanol, a more polar solvent than water. Hence accountable for the low yields obtained with the aqueous extracts.

Table 1: Extractable Yield and Phytochemical Composition of *Allium sativum* and *Garcinia kola*

(a) Yield		
Plant samples	Extractable Yield (mg)	
	Aqueous extract	Ethanol extract
<i>Allium sativum</i> (bulb)	2.85 ± 0.05 (56.0%)	3.08 ± 0.08 (60.0%)
<i>Garcinia kola</i> (seed)	3.03 ± 0.06 (60.0%)	3.95 ± 0.05 (78.0%)
(b) Phytochemistry		
Phytoconstituents	Inference	
	<i>Allium sativum</i>	<i>Garcinia kola</i>
<i>Alkaloids</i>		
Alkaloids	++	+
Saponins	-	-
Tannins	-	+++
Phlobotannins	-	-
Anthraquinones	-	-
Flavonoids	+++	++
Terpenes	-	-
Cardiac glycosides	+	++
i) Salkowski	+	++
ii) Keller-killiani	++	++
iii) Libermann	+	++

\* = significant (P<0.05); - = absent; + = slightly present; ++ = moderately present; +++ = abundantly present

The phytochemical composition in both plants indicated the presence of alkaloids, flavonoids and cardiac-glycosides; while tannis was only present in *Garcinia kola*. The presence of these and other phytochemicals in the plants may be responsible for the antibacterial activities. This assertion is in line with previous reports that the presence of these and other phytochemicals are responsible for the wide-array of biological and pharmacological, including antimicrobial activities of medicinal plants (Iwu, 1984; Sofowora, 1993; Trease and Evans, 2002; Pearson, 2005, NNMDA, 2006; Sofowora, 2008).

The bacterial species isolated from the respiratory tracts and other associated clinical samples were: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Salmonella typhi* (Table 2). These clinical isolates are known sensitive and resistant Gram-positive and Gram-negative pathogenic bacteria, the etiologic agents for the many mild-opportunistic and fatal superficial-systemic human infections. Equally, some of these clinical bacterial isolates-*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, are the aucthocthonous respiratory tract microflora, where the samples were obtained (Fuerst, 1978; Rosenberg and Cohen, 1983; Volks and Wheeler, 1984; Konemann *et al.*1994). However, under certain body physiological imbalances, these bacteria's respiratory tract microflora, are liable and become the underlying etiologic agents of the various RTIs, which are the commonest of infection frequently reported in hospitals attendance worldwide (Denyer *et al.*, 2007). Equally, the RTI by these ubiquitous etiologic agents cause irritation or inflammation of the airways, which frequently results in cough (Fuerst, 1978; Duerden *et al.*, 1990; Laurence *et al.*, 1997; Denyer *et al.*, 2007).

**Table 2:** Cultural /biochemical characterization and identification of RTI and associated clinical bacterial isolates

Media/Colony Description	Colony morphology	Sugar Fermentation															Probable organism
		Gram reaction	Motility	Indole	MR	VP	Catalase	Coagulase	Citrate	Urease	Oxides	Mal	Suc	Man	Lac	Glu	
NA – milky colonies BA – yellow colonies with β-haemolysis MSA – yellow colonies	cocci in clusters	+	-	-	-	-	+	+	-	-	-	A	A	AG	A	AG	<i>Staphylococcus aureus</i>
NA – milky/white colonies BA – white colonies with β-haemolysis	cocci in chains	+	-	+	-	-	-	-	-	-	-	AG	AG	A	A	A	<i>Streptococcus pyogenes</i>
NA - milky colonies BA - white/unpigmented mucoid colonies surrounded with zone of incomplete α-haemolysis	Cocci in short chains	+	-	-	-	-	-	-	-	-	-	AG	AG	A	A	AG	<i>Streptococcus pneumoniae</i>
MCA - mucoid/Pink colonies	short rods	-	-	-	+	+	+	-	+	+	-	AG	AG	AG	AG	AG	<i>Klebsiella pneumoniae</i>
MCA - red non-mucoid colonies EMB - metallic-sheen colonies CLED- yellow colonies	short rods	-	+	+	+	+	-	-	-	-	-	A	AG	A	AG	A	<i>Escherichia coli</i>
NA – blue green spreading colonies EMB- light purple colonies	short rods	-	+	+	+	+	+	-	+	+	-	-	A	A	-	A	<i>Pseudomonae aeruginosa</i>
MCA - light purple colonies SS - black colonies TSI - black colonies	straight rods	-	+	-	+	+	+	-	+	-	-	A	-	A	-	A	<i>Salmonella typhi</i>
MCA - light purple colonies SSA – black colonies TSI – black colonies	long/straight rods	-	-	-	+	+	-	-	+	-	-	AG	A	A	-	A	<i>Shigella dysenteriae</i>

NA – Nutrient agar; BA= blood agar; MSA = mannitol salt agar; MCA= MacConkey agar; EMB = Eosin methylene blue agar; CLED = Cystine lactose electrolyte deficient agar; TSI – Triple sugar iron agar; MR = Methyl red; VP = Voges Proskauer; Mal = maltose; Suc= sucrose, Man= mannose, Lac=lactose; Glu = glucose; + = present/positive reaction or growth; - = absent/negative reaction or no growth; A= Acid production; AG = Acid and Gas production.

Antibacterial activity and potency of both plants against the clinical bacterial isolates indicated a broad-spectrum of activity. Ethanol extracts of both plants recorded activity, which was significantly ( $P < 0.05$ ) higher for *Garcinia kola* than *Allium sativum*; while only the aqueous extract of *G. kola* was bioactive, compared with the standard antibiotics (Table 3). The relatively low antibacterial activity by ethanol extracts of *A. sativum*, even though, recorded the most abundant presence of majority of the phytoconstituents screened (such as alkaloids and flavonoids) could be attributed to either the nature and types of these phytoconstituents and /or the stronger presence of both cardiac-glycosides and tannins in *Garcinia kola*.

Irrespective of the assumed phytochemical disparity and the corresponding activity differential between the plants, the degree of antimicrobial and other biological activities of medicinal-plants is universally acknowledged as the functions of the phytochemicals present. Thus, the presence of the aforementioned phytochemicals could be responsible for the antibacterial activities; hence, the antitussive properties of the plants under study (Bhat *et al.*, 2009; Joshi, 2008; Kar, 2007). Evidently, alkaloids and flavonoids in most medicinal plants have been reported to possess antitussive and expectorant properties (Bhat *et al.*, 2009; Yadava and Jain, 2009). Also, specifically, garlic given its characteristic pungency and after an overnight eating or as warm beverage decoction has been reported to be sensitive to cold and constantly rattling mucous in bronchi, with high expectorant property. Similar biological activities had been reported on prolonged consumption of bitter kola (Kar, 2007; Joshi, 2008).

Table 3: Antibacterial activity and potency of *Allium sativum* and *Garcinia kola* extracts against the RTI clinical bacterial isolates

RTI-clinical bacterial isolate	Antibacterial activity /inhibition zone diameter, IZD (mm)					
	<i>Allium sativum</i>		<i>Garcinia kola</i>		Controls	
	AO	ETH	AO	ETH	CFX	CTZ
<i>Staphylococcus aureus</i>	-	7.0 a=1.4 b=0.77	8.0 a = 1.6 b = 0.89	10.0 a = 2.0 b = 21.1	5.0	9.0
<i>Streptococcus pyogens</i>	-	9.0 a = 0.69 b = 0.9	10.0 a= 0.77 b = 1.00	13.0 a = 1.00 b = 1.30	13.0	10.0
<i>Streptococcus pneumoniae</i>	-	10.0	10.0 a = 0.77 b = 1.00	12.0 a = b =	14.0	12.0
<i>Klebsiella Pnuemoniae</i>	-	8.0 a= 0.5 b = 1.0	-	8.0 a = 0.50 b = 1.00	16.0	8.0
<i>Pseudomonas aeruginosa</i>	-	7.0 a = 0.88 b = 1.2	-	9.0 a = 1.1 b = 1.5	8.0	6.0

Aq = Aqueous extract; Eth = Ethanol extract; CFX = Cefuroxime; CTZ = Cotrimoxazole;  
- = No activity; a and b = potency of extracts with respect to CFX and CTZ respectively

The absence of and low antibacterial activities of the aqueous extracts of *A. sativum* and *G. kola* respectively, could be linked to the low extractable and solubility strength of water compared to ethanol as solvents in the phytochemical extraction. This is an indicator of the nature and type of the phytochemicals which may likely be sparingly soluble in water-a least polar solvent compared to ethanol, a more polar solvent. Thus, in many cases, the excellent extractable and solubility properties of ethanol as vehicle and the corresponding excellent antimicrobial activities of its extracts is well documented. This excellent extractable and antimicrobial activity informed why locals, usually prepare the concoction/infusions of bitter kola ethanol (akai-kai, local gin). Alternatively, they copiously drink the local gin after chewing bitter kola for its synergistic or potentiating effect for either prophylactic or therapeutic options for cough prevention or treatments. To this, end by inference from the

study, the decoctions, infusions and concoctions of these plants as herbal remedies for cough, cold and other RTI, should be prepared with ethanol, but not with water for enhanced and optimal performances.

Table 4: Minimum Inhibitory Concentration (MIC) of *Allium sativum* and *Garcinia kola* against RTI Clinical bacterial isolates

	MIC of extracts (mg/ml)			
	<i>Allium sativum</i>		<i>Garcinia kola</i>	
	AQ	ETH	AQ	ETH.
<i>Staphylococcus aureus</i>	-	500.0	-	500.0
<i>Streptococcus pyogens</i>	500.0	250.0	500.0	250.0
<i>Streptococcus pneumoniae</i>	500.0	250.0	500.0	250.0
<i>Klebsiella Pnuemoniae</i>	1000.0	250.0	500.0	500.0
<i>Pseudomonas aeruginosa</i>	-	500.0	-	500.00

- = turbidity/growth

The MIC of the plants extracts against the clinical bacterial isolates (Table 4) showed remarkable predominant high MIC values ranging between (250.0 – 1000.0 mg/ml), with a corresponding lower static activity which was highest for the aqueous extract of *G. kola* (500.00 – 1000.0 mg/ml) and comparatively lower for ethanol extracts of both plants (250.0 – 500.0 mg/ml). The absence and /or high MIC values of the aqueous extracts against the isolates, compared to those of the ethanol extracts further supported and confirmed the superior antibacterial and antitussive/expectorant properties of the ethanol extracts. Also, this finding clearly indicates the direction for their use in the preparations of decoctions, infusions and concoctions as herbal remedies for cough, cold and associated RTIs. The bioactive ethanol extracts elicited basically, static activity irrespective of the concentrations used and incubation period. This may be an indication that the plants extracts should be frequently taken or administered for optimal performance and potency, as antibacterial agents and antitussive-expectorants in RTIs.

### CONCLUSION

This study has revealed the broad-spectrum bacteriostatic activity and antitussive potentials of the *Allium sativum* and *Garcinia kola* extracts. The predominantly bacteriostatic activities were comparatively higher in ethanol extracts of *G. kola* than *A. sativum* and absent in aqueous extracts of *A. sativum*. However, both plants contained alkaloids, flavonoids and cardiac-glycosides; while only *G. kola* has tannins. The roles of these bioactive agents in respiratory and cardiovascular infections chemotherapy are well documented. Following these positive findings, it is recommended that further extraction with other polar solvents and subsequent fractionation with both polar and non-polar solvents should be carried out, as means of purification and isolation of the active-principles(s).

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