IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 11, Issue 6 Ver. III (June. 2017), PP 16-19
www.iosrjournals.org

Antibacterial Activity of Ethanol and Aqueous Extracts of *Garcinia Kola* and *Allium Sativum* against Some Respiratory Pathogens: Alternative Antimicrobials?

Martin E. Ohanu[1]; Jerome U. Nduka[1]; Otuu, C. Fred[2]; Elvis N, Shu[3]Ikechukwu S. Nkechinyere[4]

[1]*Department of Medical Microbiology, College of Medicine, University of Nigeria, Enugu Campus*
[2]*Department of Pharmaceutics, University of Nigeria, Nsukka*
[3]*Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Enugu Campus*
[4] *Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka*

Abstract: *The evolution of resistant respiratory pathogens against conventional antibiotics is of public health concern. The development of plant-based antimicrobials will stem the tide of resistant microbial proliferation. This study brought forth the case of microbial activity of Garcinia kola and alium sativa ethanol and aqueous extracts. These plant products are chewed in Africa and beyond, against mainly respiratory infections. The respiratory organisms involved in this study were (Staphlococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Escherichia coli, klebsiella pneumoniae and Pseudomonas aeruginosa).*

Methods *Punch hole agar diffusion method was used to study the antibacterial activity of ethanol and water extracts of G. kola and A. sativum after obtaining pure cultures of the bacterial pathogens.*

Results *The combination of A.sativum and G.kola ethanol extracts showed statistically significant ($p < 0.0001$) antibacterial activity to all the organisms different from that of G.kola and A.sativum extracts individually (using student t test). There was statistically significant effect ($p<0.0001$ for each) against S. pneumoniae and S pyogenes in particular with 43mm, and 40mm, mean inhibitory zones respectively, and against S. aureus, K. pneumoniae, E. coli and P aeruginosa with 24mm, 20mm, 18mm and 16mm mean inhibitory diameters respectively. The minimum inhibitory concentration for the combined extracts was statistically significant when compared to single extracts for all the organisms except E coli, S pyogenes and K. pneumoniea.*

Conclusion: *The combinations of G. kola and A. sativum have strong antimicrobial effect and were effective against susceptible organisms causing upper respiratory tract infections.*

Keywords*: Antibacterial, Garcinia kola, Allium sativum, Respiratory pathogens.*

I. Introduction

Worldwide, infectious diseases have become a worrying cause of death [1]. Contributing to these infectious diseases include new and known respiratory bacterial pathogens with their increasing resistance to antibiotics [1, 2]. The development of new treatments would entail the development of new antimicrobials [1, 2], such as plant antimicrobials (phytomedicines) [3, 4]. The anti infective properties of some plant extracts are not in doubt [5, 6]. Quinine from the bark of cinchona tree is useful in the treatment of malaria. The development of phytomedicines usually starts as botanicals used in their crude form [7]. These then undergo scientific processing to identify the active molecules down to clinical trials to establish efficacy and safety [2]. *Garcinia kola*, also known as bitter kola is cultivated and distributed throughout West Africa. The seeds are used in the treatment of bronchitis, throat infections [8] and malaria [9]. It contains biflavonoids, xanthones and benzophenones [10] but the antimicrobial properties of this plant are attributed to the benzohenone, and flavanones [11]. *Allium sativum* commonly called Garlic, a rhizome whose anti bacterial activity is attributed to diallyl sulfide and diallyl disulfide [12] that act on arylamine N-acetyl Transferase of the organisms [13,14]. Although numerous works on their individual antimicrobial effects have been done [15, - 17], we are not aware of any recent work on their combined antibacterial effect. Since both plants (*G. kola* and *A.sativum*) are chewed slowly in the oropharyx before being swallowed, the long transit time gives an enhanced opportunity to affect upper respiratory tract pathogens. Our aim is to investigate the effects of their extracts singly and combined on some known respiratory pathogens in our environment.

II. Materials And Methods

Test organisms

Pure cultures of *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *E. coli*, *k.pneumoniae* and *P. aeruginosa* were isolated from the clinical specimens of respiratory tract (throat swab and sputum) from the Department of

Medical Microbiology in University of Nigeria Teaching Hospital , Enugu. Specimens arriving at the chest unit of the Microbiology laboratory were cultured on blood, chocolate and McConkey agars. Suspect colonies were then isolated and identified according to standard laboratory protocol [18]. The cultures were maintained on nutrient agar and chocolate blood agar slants at 37^{o} C during the period of the study.

Plants

A.sativum bulbs and *G. kola* fruits were used after identification by a Botanical Taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka

Extraction

Aqueous extracts

These were obtained by cold maceration, following the method of Chantarasriwong et al [8]. Weighed100g dry powdered plants were macerated in cold distilled water for 24hrs to obtain water extraction. The extracts were then filtered using Whatman No1 filter paper and the filtrate was evaporated in vacuo and dried using a rotary evaporator at 60^0C [18]. The final dried material was kept in Labeled sterile bottles and stored at -20^0C.

Ethanol extracts:

Aliquot (100g) of each dry powdered plant was macerated in 95% ethanol for 24hours to ensure complete extraction of ethanol soluble active ingredients [8]. The extracts were filtered using Whatman filter paper No1, and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 60^0C. Dried extracts were stored in labeled sterile screw capped bottles at – 20^0C. 4g of the resulting concentrated extracts were each dissolved in 10ml of 3% tween 80 (to give a concentration of 400mg/ml). Dilutions of the extracts were prepared as required.

Screening for antibiotic activity

The punch-hole Method was used [18]. The holes at 8mm diameter and 4mm depth were made in the plates of nutrient agar and chocolate blood agar, using a sterile Cork borer. The plates were streaked with loop-full of overnight cultures of the test organisms. The holes were then filled with 0.3ml (containing 120mg/ml) of extract. For the combination of *A.sativum* and *G. kola*, 0.3ml of each was added into the holes. Controls, (where extracts were replaced with 3% sterile nutrient broth) were similarly performed. The plates were incubated at 37˚C for 24 hours. The inhibition zones sizes were measured on the underside at the plates using calipers. A zone of 18mm (including diameter of ditch) was taken as indicator of active inhibition. The tests were repeated seven times with the extracts for each organism and the mean diameter results calculated.

Determination of Minimum Inhibitory Concentration (MIC).

This was carried out by the standard broth dilution Method using Muellar Hinton Broth (MH)[18]. The broth cultures of the test organisms were diluted to correspond to tube 0.5 at Mcfarland tube and incubated under 10% $C0_2$. 0.3 ml quantities of the diluted cultures ($3x10^5$) were introduced into tubes of MH broth containing graded concentrations of the investigated plant extracts (0.3 mg/ml-100mg/ml). After 24 hour incubation at 37^0C, the tubes were examined for growth as determined by turbidity when compared with controls (without plant extracts).This was repeated seven times for each extract and the averages calculated. The MIC was taken as the minimum concentration of extract that inhibited growth.

Data Analysis

Statistical analysis was done using Graph Pad Prism 6. Student t test was used to compare the mean diameters of each extract versus the combination and p values lower than 0.05 (95% confidence interval) were considered statistically significant.

III. Results

The results of testing the antibacterial activities of *G. kola* and *A.sativum* by the punch hole method are presented in Table 1, while the minimum inhibitory concentrations are in Table 2. From the antibiogram, *S. pneumoniae* and *S. pyogenes* were both susceptible to *A.sativum* and *G.kola* individual ethanolic extracts being 24mm or more. In the combination of *A.sativum* and *G.kola* ethanolic extract, there was strong activity, against *S. pneumoniae* and *S. pyogenes* showing inhibitory diameters of 43 mm and 40 mm respectively. This was statistically significant when compared to the individual extracts ($p < 0.0001$ for each). *S. aureus*, *K. pneumoniae* , *E. coli* and *P. aeruginosa* have 24mm, 20mm, 18mm and 16mm inhibitory diameters respectively, The controls showed no inhibition. There was less antibacterial activity by the water extracts hence they were disregarded. A similar pattern was seen in the Minimum inhibitory concentration, being 10 and 15mg/ml for *S. pneumoniae* and *S. pyogenes* respectively in the combination of *G. cola* and *A. sativum* .

IV. Discussion

The antibacterial effect of the mixture of the extracts was significantly different from that of *G.kola* and *A.sativum* extracts individually for each of the organisms ($p<0.0001$). The antibacterial effects of the individual extracts were significantly different in just four of the organisms, ($p<0.05$) but for *P.auroginosa* and *S. pyogenes*

the difference were not significant (p>0.05). Comparing the effects of the mixture of the extracts on the micro-organism, they were extremely sensitive (p<0.0001). In the case of *S. pneumoniae, S .pyogenes, K.pnuemoniae* and *E. coli* they were slightly different when comparing the mixture with *A.sativum* (p=0.01). For the MIC, the differences in effect on *E.coli* to *S. pyogenes* and to *K. pnuemoniae* were not significantly different (p>0.05).
In assessing the strength of the antibiotic effects of the mixture on the organisms, it is obvious that *S. pneumoniae* is the most susceptible, then *S. pyogenes, K. pneumoniae, E. coli,*and *S. aureus,* while *P. aeroginosa* is the least susceptible. Emerging infectious diseases and increasing rate of resistance to antibiotics by known pathogens is worrisome. Recent reports show that *G. kola* has antibacterial effects against a variety of organisms [19, 20]. Allicin from *A.sativum* in its pure form or coupled with thiosulfinate [21] has been found to exhibit, antibacterial activity against a wide range of Gram- negative and Gram-positive bacteria, antifungal activity particularly against *Candida albicans*, antiparasitic activity against the protozoans *Entamoeba histolytica* and *Gardia lamblia*, antiviral activity[22] and anti cancer activity[23]. From the results of our study, extracts from the two plants showed strong antibacterial activity on some respiratory pathogens. *S. pneumoniae* and *S. pyogenes* are known respiratory pathogens. The latter is known to have serious post infection consequences [24]. The plants are also used as anti mycotic and anti-cancer agents [25, 26] . The ultimate aim will be the purification and concentration of the active ingredients of both plants as drugs against susceptible organisms. *G. Kola* is a good source of carbohydrate, dietary fiber and protein. [27]. It also has a high level of calcium, potassium, sodium in addition to anti-inflammatory and antioxidative properties [27]. It is good to note that the biological and chemical stability of garlic derived allicin can be affected by the solvent agent used for its extraction [28] *G. kola* and *A. sativum* also have activity against the vaginal *Candida species* (*C albicans, C. tropicalis, C. glabrata* and *C. parapsillosis* [29].

There are growing concerns about herbal medicinal products now becoming increasingly popular as they are often purported to be without side-effects. There could be drug-drug and herb-drug interactions that could be of public health importance [30] Temperature affects the effect and quality of the herbs like black garlic (obtained by heating raw garlic at high temperature with controlled humidity).[31] The use of herbal medicines has been on the increase in many developing and industrialized countries. A study in South East Nigeria showed that the prevalence of herbal medicine use was high and most of the determinants observed are modifiable [32], thus there is need to institute appropriate control measures by relevant authorities to tackle this problem. Conceivably, plant extract/herbs certainly provide an option for people to treat oral infections based on scientific proof. However, this is an in-vitro study that did not explain blood concentrations of active ingredients of the extracts for possible systemic use which offers consideration for herbal medicine as alternative antimicrobials.

V. Conclusion

The extracts have antimicrobial activities against the test organisms with significant synergistic interactions when compared with the activities of the individual extracts.

Acknowledgements

The authors are grateful to the technical staff of Pharmacology and Therapeutics Department of University of Nigeria, for their assistance.

Footnote.

Author contributorship: OM contributed to the concept, design of the study, literature review and statistics, while NJ.OF and SE contributed immensely to the design and laboratory work. All authors revised the manuscript for important content and approved it for submission.

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Table 1: Antibacterial spectrum of ethanolic extracts of *A.sativum* and G.kola. (Mean Inhibitory Zone in mm).

INHIBITORY ZONE DIAMETER

Organisms	GK+G		Garcinia		p Value	Garlic		p Value
	Mean	SEM	Mean	SEM	ʧ	Mean	SEM	*
S. pneumoniae	43	0.5281	28	0.3689	< **0.0001**	24	0.5533	< **0.0001**
P.aeruginosa	16	0.4364	10	0.9476	< **0.0001**	12	0.6494	< **0.0001**
S. pyogenes	40	0.5948	24	0.2974	< **0.0001**	24	0.6851	< **0.0001**
S. aureus	24	0.4286	18	0.3086	< **0.0001**	12	0.1844	< **0.0001**
E. coli	18	0.2182	10	0.6851	**0.0055**	16	0.8081	< **0.0001**
K.pnuemoniae	20	0.4809	12	0.1429	< **0.0001**	14	0.5281	< **0.0001**

GK: Garcinia kola
G: Garlic
ʧ : t test of GK+G &GK
***: t test of GK+G & G**
SEM: Standard Error of Mean

Table 2: Minimum inhibitory concentration (MIC) mg/ml of *A.sativum* and *G. kola*

MINIMUM INHIBITORY CONCENTRATION

Organisms	GK+G		Garcinia		p Value	Garlic		p Value
	Mean	SEM	Mean	SEM	ʧ	Mean	SEM	*
S.pneumoniae	10	0.2857	20	0.2857	< **0.0001**	25	0.3595	< **0.0001**
P.aeruginosa	25	0.1429	30	0.1429	< **0.0001**	35	0.202	< **0.0001**
S.pyogenes	15	0.4286	35	0	< **0.0001**	30	0.1429	< **0.0001**
S. aureus	20	0.2857	25	0	< **0.0001**	45	0	< **0.0001**
E. coli	16	0	14	0.2608	< **0.0001**	18	0.2974	< **0.0001**
K. pneumoniae	17	0.3689	15	0.2857	**0.0169**	20	0.1844	< **0.0001**