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ELIXIR FORMULATIONS OF METHANOLIC EXTRACT CONCENTRATES OF *GARCINIA KOLA*, *KOLA ACUMINATA* AND *KOLA NITIDA* SEEDS AND THEIR ANTIMICROBIAL ACTIVITY.

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Abstract

The crude methanolic extracts of *Garcinia Kola* (Guttferae), *Kola acuminata* and *Kola nitida* (Sterculiaceae) seed powders were screened against some bacterial isolates consisting of six different Genera namely: *Streptococcus, Staphylococcus, Escherichia, Proteus, Klebsiella* and *Pseudomonas*, most of which were isolated from the respiratory tract and the rest from other sources like wounds, vagina and urethra. The extracts were found to be active on all the Gram-positive bacteria and some of the Gram-negative bacteria including *Pseudomonas aeruginosa*. The extracts were then formulated into stable Elixir formulations at a concentration of 15mg/ml and screened against the same bacterial isolates. They were found to be active against all the selected Gram-positive bacteria used in this study while some of the Gram-negative bacteria tested were also susceptible to the three elixir formulations.

Key words: Elixir formulations, *Garcinia Kola, Kola acuminata, Kola nitida*, antimicrobial activity.

Introduction

For thousands of years, human beings based on their peculiar socio-cultural and religious ideologies and beliefs, have adopted different therapeutic strategies of using the various natural materials for treating various illness among them. Bioactive compounds extracted from medicinal plants can be tested for their pharmacological activity, which may facilitate the synthesis of more potent drugs with reduced toxicity (Ebena *et al*, 1991; Williams, 1996; Pamplona-Roger, 1999; Manna & Abalaka, 2000). Furthermore, the active components of herbal remedies have the advantage of being in combination with many other substances that appear to be inactive within the plant. However, these complimentary components give the plant as a whole, a safety and efficiency much more superior to that of its isolated and pure active components, which often present higher levels of toxicity (Sheriff, 2001).

Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious disease including opportunistic infections in AIDS. Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and *Garcinia* biflavonones used in traditional African system of medicine, have been found to be active against a wide variety of microorganisms (Iwu *et al.*, 1999).

Traditional medicine is currently gaining prominence in Nigeria and over 80% of the world's population still depend on medical plants (WHO, 1991).

The seeds of *Garcinia kola* are used in the treatment of bronchitis and throat infections. They are also used to prevent and relieve colic, cure chest colds and relieve cough. Also the plant is used for the treatment of liver disorders and as chewing stick (Iwu, 1993). The seeds are used in folk medicine and in many herbal preparations for the treatment of ailments such as laryngitis, liver disorders and others (Iwu, 1982). In 1999, the British Broadcasting Corporation (BBC) reported that *Garcinia* fruits halted the deadly Ebola virus in its tracks in laboratory tests.

Adeleke *et al*, (2006) reported the antimicrobial properties of *Garcinia kola* seed extracts on some microorganism associated with throat infections and reported that of all the various extracts tested, the methanol extract of the seed was the most active on nearly all the organisms tested. Adeniyi *et al.*, (2004) worked on the *in vitro* anti-mycobacterial activities of three species of kola plants extracts (Sterculiaceae), *Kola acuminata, Kola nitida* and *Kola milleni* using the leaves, stem barks and roots of the three species and found only the methanol extracts of root of both *Kola nitida* and *Kola milleni* to be potent against both *M.bovis* and strains of *M.vaccae*.

In a bid to provide a stable and suitable dosage form for the proven crude extacts of the kola seeds, Onunkwo *et al.*, (2004) formulated powdered *Garcinia kola* seed and the crude aqueous extracts into tablets. It was considered worthwhile therefore, to confirm on a comparative basis, the antimicrobial activities of the methanolic extract concentrates of *G.kola, K acuminata* and *K. nitida*, before and after formulating each of the extract concentrates into liquid elixir. Such elixir formulations, hopefully, would be found convenient for use for patients who may have difficulty in swallowing the solid dosage form, like the elderly, or those that object to the bitter taste of the plant seeds, particularly that of *G.kola*.

Materials and Methods

Collection of plant materials:

The seeds of *Kola nitida, Kola acuminata* and *Garcinia kola* were obtained locally in Bodija and Oja Oba markets, both in Oyo State and authenticated at the Federal Research Institute of Nigeria, Ibadan. Oyo State.

Preparation of plant materials:

The three different plant seeds were weighed separately, and in the case of *Garcinia kola*, the seeds were first peeled before weighing. They were then sliced and air-dried to a

constant weight. The dried samples were pulverized with an electric blender into powder, labelled and stored at room temperature, 28°C, for further study.

Preparation of extracts

Soxhlet extraction:
The extraction of the active ingredients from the plant seeds was carried out in a soxhlet apparatus as described by Harbone (1973) using 100g of each of the pulverized seeds mixed with 250ml, methanol. Each extraction process lasted for 6 hours. The extracts obtained were concentrated by evaporation using electro-thermal water bath at 100°C and subsequently evaporated to dryness in an oven at a temperature of 60°C to give a constant weight of dry extracts.

Bacteria:
The pure cultures of the bacteria used and their sources are shown below:

Staphylococcus aureus	OOUTH206(Throat swab)
Staphylococcus aureus	NCIB 8588
Streptococcus pyogenes	*UCH320(Throat swab)*
Streptococcus pneumoniae	UIPHM (Sputum)
Streptococcus viridian	UIPHM (Unindicated)
Pseudomonas aeruginosa	ATCC 27853
Pseudomonas aeruginosa	UCHH189 (Wound swab)
Escherichia coli	OOUTH321 (H.Virginal wab)
Klebsiella pneumoniae	UCH898 (Pleural aspirate)
Klebsiella pneumoniae	UCH052(Urethra discharge)
Proteus mirabilis	OOUTH072 (Pharyngeal swab)
Proteus mirabilis	UCH130(Sputum)

Key:
OOUTH-Olabisi Onabanjo University Teaching Hospital
NCIB – National Centre for Industrial bacteria
UCH – University College Hospital
UIPHM – University of Ibadan Pharmaceutical Microbiology
ATCC – American typed culture collection.

Susceptibility Testing
The agar-cup diffusion method described by Singleton (1999) was used. A dilution of 10^{-2} was obtained in sterile distilled water from an overnight broth culture of each organism. Seeded plates were prepared from this dilution by inoculating 0.1ml into 20 ml molten and cooled Sensitest agar, which was then poured into a sterile culture plate. The seeded culture medium was allowed to set followed by drying inside an incubator at 37°C for 20 minutes. Thereafter, wells were dug in appropriate number using 6.5mm diameter cork borer (Size 3). A volume of 0.1ml from a calibrated Pasteur pipette was transferred from the dilutions prepared for each concentrate in 4%v/v methanol as well as 0.1ml of 40%v/v methanol and 4øg/ml Gentamicin as controls. Following a pre-incubation diffusion period of 2 hours, the plates were incubated at 37°C for 24 hours after which they were examined for zones of growth inhibition measured.

Minimum inhibitory concentration (MIC) determination:
The reconstituted crude extract concentrates were diluted with 40%v/v methanol in a doubling fold procedure in each container to obtain serial dilutions ranging from 60mg/ml to 0.12mg/ml in sterile test tubes. Agar-cup diffusion plates were prepared as described for susceptibility testing. Six wells were bored into the individual culture plates. Five of the wells were filled with the different concentrations of the test extracts and the sixth with the diluent, 40%v/v methanol, as control. After a pre-incubation period of 2 hours, the plates were incubated at 37°C for 24 hours. The diameters of zones of growth inhibition for each organism were then measured and recorded. The lowest concentration preventing growth was taken as the minimum inhibitory concentration (MIC) of the extract. The procedure was done in duplicates and the average values recorded.

Preparation of the Elixir formulations of the extracts:
The formula for the elixirs of the three extracts were obtained based on stability and solubility studies and is given below:-

Powdered extract	1.5g
Ethanol (96%v/v)	7ml
Water (Sterile distilled)	13ml
Syrup (B.P)	80ml

The weighed powered extract was added to the ethanol in a suitable glass bottle with an air tight cover and 13ml sterile distilled water was added gradually to ensure that the extract totally dissolved. The 80ml Syrup B.P was then added and shaken to give a clear preparation of 15mg/ml concentration of each elixir preparation containing 6.72%v/v ethanol. The elixirs were kept in the refrigerator at 4°C for the antimicrobial screening. A similar preparation but without the addition of the extract was also prepared for use as control.

Antimicrobial screening of the elixir formulations:
Molten Sensitest agar (20 ml) was seeded with 0.1ml a 10^{-2} dilution of an overnight broth culture of the different clinical isolates and poured into sterile culture plates and allowed to set. Drying of the seeded plates and digging of wells were done as described for susceptibility testing. 0.1 ml of each elixir formulation was introduced each in 0.1ml into labelled wells. The extract-free elixir was also introduced as negative control, while Gentamicin 4øg/ml was used as positive control. The plates were left for one hour at room temperature to allow pre-incubation diffusion within the medium after which they were incubated at 37°C for 24 hours. Zones of growth inhibition were measured and recorded. This was done in duplicates and the average values recorded.

Results
With the exception of *7.5mg/ml* of *K. nitida* seed extract against *Strep. viridians*, the three extracts at the concentrations used inhibited the growth of the Gram-positive bacteria in varying degrees but most especially that of *Staph aureus*, and the *G. kola* extract (concentrate) exhibited the most outstanding result. It was remarkable that the three extracts were very active on *Streptococcus pneumoniae* growth, a capsulated Gram-positive bacterium. *Strep viridians* and *Step pyogenes* were the other Gram-positive bacteria whose growth were

inhibited (Table 1).

Among the Gram-negative bacteria tested, the three extracts were reasonably active on *Pseud aeruginosa, Proteus mirabilis;* and on one strain each of *E. coli* (UCH1065). *G. kola* did not show any inhibitory activity against *Kleb. pneumoniae* (UCH8998) . None of the three extracts at the concentrations of 30, 15, and *7.5mg/ml* tested was active on the strain of *E. coli* (OOUTH321) and only the 30mg/ml of each extract was active on *Kleb. pneumoniae* (UCH052). Again, it was remarkable that *Pseud. aeruginosa* was the most sensitive to the three extracts particularly *G. kola.* It is noteworthy also that gentamicin was active on all the bacterial isolates used as against the lack of inhibitory activity observed for the diluent, 40%v/v methanol. The individual elixir formulations from *G. kola, K acuminata* and *K. nitida* behaved in a similar way in their inhibitory activity as the extract concentrates, particularly with respect to all the Gram-positive bacteria, *Pseud. aeruginosa* and *Proteus mirabilis.* Remarkably none of the elixirs showed any inhibitory activity on *Esch. coli* and *Kleb. pneumoniae* (Table 2).

Table 1: The antimicrobial activity of the methanolic extracts concentrates of the seeds of *Garcinia kola, Kola acuminata* and *Kola nitida*, on some bacterial isolates.

EXTRACTS CONCENTRATIONS (mg/ml)										CONTROLS	
ORGANISMS	*G.kola*			*K.acuminata*			*K.nitida*			GEN	METH
	30	15	7.5	30	15	7.5	30	15	7.5	4lg	40%v/v
ZONES OF GROWTH INHIBITION (mm)											
S. aureus OOUTH206	19^{S}	18^{S}	17^{S}	15^{S}	14^{S}	13^{S}	15^{S}	13^{S}	11^{S}	20^{S}	-
S aureus NCIB8588	35^{S}	34^{S}	27^{S}	19^{S}	15^{S}	12^{S}	16^{S}	14^{S}	12^{S}	35^{S}	-
S pneumoniae UIPHM	18^{S}	15^{S}	12^{S}	13^{S}	11^{S}	10^{S}	13^{S}	11^{S}	9^{S}	20^{S}	-
S.viridians UIPHM	16^{S}	14^{S}	10^{S}	12^{S}	11^{S}	10^{S}	12^{S}	10^{S}	-R	12^{S}	-
S pyogenes UCH320	22^{S}	20^{S}	18^{S}	20^{S}	18^{S}	16^{S}	22^{S}	21^{S}	16^{S}	25^{S}	-
P.aeruginosa UCH189	15^{S}	13^{S}	12^{S}	14^{S}	13^{S}	R	13^{S}	10^{S}	8S	23^{S}	-
P.aeruginosa ATCC27853	16^{S}	14^{S}	12^{S}	16^{S}	15^{S}	12^{S}	14^{S}	13^{S}	12^{S}	28^{S}	-
E.coli OOTH321	R	R	R	R	R	R	R	R	R	20^{S}	-
E.coli UCH065	13^{S}	12^{S}	11^{S}	13^{S}	12^{S}	R	12^{RM}	11^{RM}	11^{RM}	15^{S}	-
K.pneumoniae UCH898	11^{S}	-R	-R	11^{RM}	10^{RM}	9^{RM}	11^{RM}	10^{RM}	9^{RM}	27^{EL}	-
K.pneumoniae UCH052	11^{S}	-R	-R	11^{S}	-R	-R	11^{RM}	-R	-R	15^{S}	-
P.mirabilis OOUTH072	11^{RM}	R	R	11^{RM}	10^{RM}	9^{RM}	11^{RM}	10^{RM}	9^{RM}	27^{RM}	-
P.mirabilis UCH130	12^{RM}	-R	-R	18^{RM}	18^{RM}	17^{RM}	18^{RM}	18^{RM}	14^{RM}	27^{EL}	-

Keys: S-Sensitive, R-Resistant, GEN Gentamicin, METH-Methanol, RM-Resistant mutants, EL-Enzyme inactivation, IM Intermediate.

Table 2: The antimicrobial activity of the elixir formulations of the extract concentrates of *Garcinia Kola*, Kola *acuminata* and *Kola nitida*

FORMULATIONS (15mg/ml)	G.k ELIXIR	K.a ELIXIR	K.n ELIXER	CONTROLS	
ZONES OF GROWTH INHIBITION (mm)					
CLINICAL ISOLATES				BE	GEN 4µg/ml
S.pyogenes UCH320	13^S	11^S	13^S	10^S	25^S
S.pneumoniae UIPHM	16^S	13^S	14^S	10^S	20^S
S.viridians UIPHM	14^S	12^S	13^S	10^S	12^S
S.aureus NCIB8588	42^S	40^S	40^S	20^S	35^S
S.aureus OOUTH206	35^S	22^S	22^S	-R	20^S
Ps.aeruginosa ATCC27853	16^S	15^S	15^S	-R	27^S
Ps.aeruginosa UCH189	14^S	14^S	12^S	-R	24^S
E.coli OOUTH321	-R	-R	-R	-R	20^S
E.coli UCH065	-R	-R	-R	-R	15^S
K.pneumoniae UCH898	R	R	R	R	23^S
K.pneumoniae UCH052	-R	-R	-R	-R	16^S
P.mirabilis OOUTH072	16^S	11^S	13^S	-R	27^{EL}
P.mirabilis UCH130	18^S	11^S	12^S	-R	28^{EL}

Key: G.k –*Garcinia kola* , K.a- *Kola acuminata* , K.n – *Kola nitida*, BE-*Blank Elixir preparation*, GEN-*Gentamicin*, S-*Sensitive*, R-*Resistant*, EL-*Enzyme inactivation*.

Discussion

The findings of this study further demonstrates the efficiency of methanol in the extraction of *G. kola, K acmninata,* and *K .nitida* plant parts (seed, leaves, stem bark or root), agreeing with the previous reports of Adeniyi *et al.* (2004) and Adeleke *et al.* (2006). Similarly, the antimicrobial activities of the three plant seeds as previously reported by Adeniyi *et al.* (2004) and Adeleke *et al,* (2006) were also confirmed. *Pseud. aeruginosa* is noted for its recalcitrance in its response to treatment with antibiotics and other drugs (Cheesbrough, 1999) and *Strep. pneumoniae,* a Gram-positive bacterium responsible for pneumonia disease, possesses capsules with antiphagocytic properties and which shields the organism from the effects of antimicrobial agents. These two organisms along with *Proteus mimbilis* (Gram-negative) were reasonably inhibited along with *Staph. aureus* and *Strep. pyogenes,* by both the diluted extract concentrates and their elixir formulations. *Staph. aureus* and *Strep. pyogenes* are associated with a good number of suppurative and other diseases (Cheesbrough, 1999). In a similar manner as Onunkwo (2004) reported the formulation of G. *kola* seed powder and crude aqueous extract into tablets, the study described here reports the formulation of the methanolic extract concentrate of each of the seed powder of *G. kola, K. acuminata* and *K. nitida* into elixir formulations for use orally against susceptible respiratory tract infections. These elixir formulations would provide hopefully, convenient alternatives for patients who may have difficulty in swallowing the solid dosage form (tablets) or object to the bitter taste of the plant seeds, particularly that of *Garcinia kola.*

Conclusion

This study reported the formulation of methanolic seed extract concentrates of *G. kola*, *K. acuminata*, and *K. nitida* into elixirs. The elixirs which showed similar antimicrobia activity as the methanolic extract concentrates could serve as alternative dosage forms for patients presenting with throat infections, especially the elderly, who may have objections to the use of the solid dosage form or the bitter taste, particularly that of raw G. *kola* seed.

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