Evaluation of the *In-Vitro* Combined Antimicrobial Activities of *Garcinia kola* Heckel and Honey

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Abstract

The antibacterial activities of Garcinia kola and honey were evaluated against pure cultures of Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa using the agar diffusion method. The combined antibacterial effects of various combination ratios of Garcinia kola extract and honey were further evaluated using a modified checkerboard method. Results show that Garcinia kola had minimum inhibitory concentration values of 0.25 mg/ml, 0.06 mg/ml and 0.25 mg/ml against S. aureus, B. subtilis and P. aeruginosa respectively while honey had MIC values of 1.8 mg/ml, 1.8 mg/ml and 0.45 mg/ml respectively. The results also showed higher sensitivity of the test isolates to a combination of Garcinia kola and honey than the individual agents. These results, therefore, indicate that combination of the Garcinia kola extract and honey could have some superior therapeutic benefits over the materials used singly.

Keywords: *Garcinia kola*, honey, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*

Introduction

Many plants are known to have medicinal effect. The specific constituent which imparts medicinal value on the plants can be derived from whole or part of the plant such as stems, leaves, fruits, flowers, seeds and roots. Apart from the importance placed by man on plants as a source of food, their greatest use has been in the area of medication. The medicinal use of plants and their products dates back to antiquity (Ogunyemi, 1979). From earliest times, man has used parts of plants to concoct healing potions, to eliminate pain, control suffering and counteract disease (Iwu, 1982). The World Health Organisation (WHO) has estimated that perhaps 80 percent of the inhabitants of the world rely chiefly on traditional medicine thus plants and plant products have been in use in the treatment of infectious diseases many centuries before the active principles in the plant products could be elucidated through the improvements in science and technology (Abonyi, 2000). Much success has been attained in the screening of plants for antibacterial and antifungal actions (Ogunlana, 1975; Levan, 1979). *Garcinia kola* Heckel (Family: Guttiferae) is a large tropical forest tree which is cultivated in southern Nigeria and some parts of tropical Africa (Hussain, 1982; Iwu, 1993). It is a spreading tree with a dense and heavy crown; the bark is greenish brown, smooth and thick, and yields a yellow sap when incised. The leaves are leathery in texture, elongated elliptic to broadly elliptic with short acute or short acuminate apex having very distinct resinous canals. The stalk is stout and finely hairy in young leaves about 8-10 mm (Iwu, 1993). Over thirty species of Garcinia have been studied phytochemically (Waterman, 1984; Iwu, 1982; Waterman, 1985; Hussain, 1982). The most important secondary metabolites isolated include xanthones, isoprenylated xanthones, benzophenones, biflavonoids and flavonone-chromones (Iwu, 1993). Hussain *et al* isolated a benzophenone (kolanone) from light petroleum extract of *Garcinia kola* fruit which possessed strong activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae* and *Candida albicans*. Beladi et al reported the inhibition of *Herpes virus hominis* and *Herpes virus suis* by a flavonoid-quercetin, an isolate from the leaves of the plant. Honey is a sweet fluid produced by honey bees (and some other species), and derived from the nectar of flowers (Crosby, 2004). Honey gets its sweetness from the monosaccharides; fructose and glucose and has approximately the same relative sweetness as granulated sugar (97 % of the sweetness of sucrose, a disaccharide). Most micro-organisms do not grow in honey because of its low water activity of 0.6 (Lansing *et al*, 1999). However, honey frequently contains dormant endospores of the bacterium *Clostridium botulinum*, which can be dangerous to infants as the endospores can transform into toxin-producing bacteria in the infant's immature intestinal tract, leading to illness and even death (Shapiro *et al,* 1998) Honey is made up of fructose 38.5 %, glucose 31.0%, sucrose 1.0%, water 17.0%, other sugars 9.0% (maltose, melezitose), ash 0.17%, others 3.38%. Honey has a density of about 1.36 kilograms per litre (36% denser than water) (Rainer, 1996). Antibacterial properties of honey are the result of the low water activity causing osmosis, hydrogen peroxide effect, and high acidity (Wahdan,1998). Honey is primarily a saturated mixture of two monosaccharides (Keast-Butler, 1980; Mossel, 1980). This mixture has a low water activity; most of the water molecules are associated with the sugars and few remain available for microorganisms, so it is a poor environment for their growth (Seymour, 1951; Sommerfield, 1991; Toyey, 1997). Hydrogen peroxidein honey is activated by dilution. However, unlike medical hydrogen peroxide, commonly 3% by

volume, it is present in a concentration of only 1 mmol/L in honey. Honey chelates and deactivates the free iron, which starts the formation of oxygen free radicals produced by hydrogen peroxide and the antioxidant constituents in honey help clean up. When used topically (as, for example, a wound dressing), hydrogen peroxide is produced by dilution with body fluids. As a result, hydrogen peroxide is released slowly and acts as an antiseptic. The purpose of this work is to evaluate the *in vitro* combined antibacterial effect of *Garcinia kola* and honey against selected bacterial isolates.

Materials and Methods

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Collection of samples: *Garcinia kola* and honey were collected from Obollo-afor, in Enugu State, Nigeria. Botanical identification was carried out by Mr. J.M.C. Ekekwe of the Department of Botany, University of Nigeria, Nsukka and voucher specimen were deposited in the University Herbarium. *Garcinia kola* was milled and bottled in an airtight container.

Test microorganisms: Clinical isolates of *Staphylococcus aureus*, *P. aeruginosa* and *Bacillus subtilis* were obtained from the diagnostic laboratory of Bishop Shanahan Hospital, Nsukka.

Culture media: Blood agar, nutrient agar, cetrimide agar, deoxycholate agar and MacConkey agar were from Oxoid, England.

Extraction: Approximately 50 g of *Garcinia kola* was milled. Then 50 ml of water was added and this was soaked for 24 h. It was filtered and the extract was stored in a closed container in a refrigerator.

Maintenance, purification and standardization of stock microbial cultures: Gram staining was carried out on all the isolates collected, for the purpose of characterization. Isolation and purification of the organisms were done by sub-culturing in selective media for the different microorganisms. *Staphylococcus aureus* was selectively cultured in mannitol salt agar. *P. aeruginosa* was grown in cetrimide agar. *Bacillus subtilis* was selectively grown in blood agar. The organisms were activated before use by successive sub-culturing in 5 ml sterile nutrient agar for three consecutive days. The inoculum size was standardized to contain 1.0 x 10^5 cfu/ml by comparison with McFarlands 0.5 standard.

Phytochemical tests: Phytochemical screening of the extract was carried out using standard procedures (Trease and Evans, 1989)

Preliminary antimicrobial screening of the *Garcinia kola* extract: Preliminary antimicrobial screening of the extracts was done using the cup-plate agar diffusion method. Sterile cork borer having a diameter of 8 mm was used to bore holes into the plate containing 20 ml each of solidified agar and seeded with the respective microorganisms. A 0.4 ml volume of the *Garcinia kola* extract was added into the labeled hole using a sterile pipette. The experiment was repeated for all the test microorganisms. Growth was examined after incubation at 37^{o}Cand the inhibition zone diameter measured. The tests were performed in triplicates.

Determination of minimum inhibitory concentrations (MICs) of the extract: The minimum inhibitory concentrations (MICs) were determined by the agar diffusion method. After seeding nutrient agar plates with the test microorganisms respectively, wells were bored on the plates using a sterile cork borer. Two-fold serial dilutions of the stock solutions (1mg/ml) of the *Garcinia kola* extract in sterile distilled water was prepared and introduced into each of these wells and placed on the bench for 30 min to allow for pre-diffusion. Thereafter, the nutrient agar plates were incubated at 37^{o}C for 24 h. This procedure was repeated with the fructose, glucose and sucrose respectively. After the incubation period, the inhibition zone diameters surrounding each well were measured and the minimum inhibitory concentration estimated.

Evaluation of the combined antibacterial effect of the extract of *Garcinia kola* and honey: Stock solutions of *Garcinia kola* (1 mg/ml) and honey (1.8 mg/ml) were prepared for the evaluation of their combined effect against the test microorganism. The two agents were mixed in accordance with a modified continuous variation checkerboard method. A standardized inoculum of the test isolates was seeded into sterilized molten nutrient agar and allowed to set. Holes of known diameter were bored in the nutrient agar plates and each of the dilutions introduced into the respective holes and incubated at 37^{o}C for 24 h. the inhibition zone diameters were measured and used to estimate the MIC of the *Garcinia kola* extract and honey combinations.

Results and Discussion

Sensitivity test: The results of the sensitivity of the microorganisms to the test samples are presented in Tables 1, 2 and 3. The results showed that *Staphylococcus aureus* was most susceptible to *Garcinia kola* and also to honey respectively.

Table 1: Sensitivity of the test microorganism to *Garcinia kola*

Test sample	Microorganism	Zone of inhibition (mm)(IZD)
Garcinia kola	*Staph. aureus*	13
	Ps. aeruginosa	12
	B. subtilis	10

Table 2: Sensitivity of the test microorganism to honey

Test sample	Microorganism	Zone of inhibition (mm)(IZD)
Honey	*Staph. aureus*	15
	Ps. aeruginosa	12
	B. subtilis	15

Table 3: Sensitivity of the test microorganism to components of honey

Test samples	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
Fructose	-	-	-
Sucrose	-	-	-
Glucose	-	-	-

Table 4: Minimum inhibitory concentration (MIC) of *Garcinia kola*

Test sample	Microorganism	MIC (mg/ml)
Garcinia kola	*Staph. aureus*	0.25
	Ps. aeruginosa	0.25
	B. subtilis	0.06

Table 5: Minimum inhibitory concentration (MIC) of Honey

Test sample	Microorganism	MIC (mg/ml)
Honey	*Staph. aureus*	1.8
	Ps. aeruginosa	0.45
	B. subtilis	1.8

Table 6: The Combined Effect of *Garcinia kola* and Honey against test microorganisms

Combination ratio of GK : H (mg/ml)	*Staph. aureus* IZD (mm)	*Ps. aeruginosa* IZD (mm)	*B. subtilis* IZD (mm)
1.0 :1.8	23.0	26	17
0.50:0.9	21.0	22	13
0.25:0.45	18.0	20	11
0.125:0.23	16.0	18	10
0.06: 0.11	5.0	14	-

GK = Garcinia kola; H = Honey

Honey gave a higher inhibitory effect than *Garcinia kola* against the clinical isolates. Honey has been reported to have an inhibitory effect to approximately 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives (Molan, 1992). An antifungal action has also been observed for some yeast and species of *Aspergillus* and *Penicillium* (Molan, 1992) as well as all the common dermatophytes (Brady *et al*, 1997). It has often been assumed that this is due entirely to the osmotic effect of its high sugar content (Bose, 1982; Condon, 1993; Green, 1988). The combination of *Garcinia kola* and honey showed a higher zone of inhibition against the clinical isolates than the individual agents. *Pseudomonas aeruginosa* showed the most susceptibility to the combination than *Staphylococcus aureus* and *Bacillus subtilis*. Fructose, glucose and sucrose had no significant antimicrobial effect. The importance of the additional antibacterial activity of honey is demonstrated in comparisons between the therapeutic effects of honey and sugar. In an experimental study, conducted on burns created on the skin of pigs (Postmes *et al*, 1997), there were fewer bacterial colonies seen histologically in wounds treated with honey compared with those treated with sugar, fewer micro-pustules in the neo-epidermis, and fewer bacteria seen in the scar of the honey-treated wounds. There has also been a clinical case report of a discharging deep pressure sore not responding to various treatments, including dressing with sugar, which was completely healed in six weeks when dressed with honey (Hutton, 1966). The results of the MIC of *Garcinia kola* and honey against the test organisms are represented in Tables 4, 5 and 6. The results confirm that a combination of *Garcinia kola* and honey gave lower inhibitory concentrations than the individual agents and could be affirmed as synergistic in most of the ratios against the test organisms. This goes to confirm the folkloric use of *Garcinia kola* and honey in the treatment of some bacterial infections.

Conclusion: From the results, it can be deduced that a combination of *Garcinia kola* and honey had synergistic effect and may be useful in the treatment of mild infections caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively, though further investigations may be necessary to validate the claims.

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