DETECTION OF A GENTAMICIN-RESISTANT BURN WOUND STRAIN OF PSEUDOMONAS AERUGINOSA BUT SENSITIVE TO HONEY AND GARCINIA KOLA (HECKEL) SEED EXTRACT

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SUMMARY. Studies on Staphylococcus aureus and Staphylococcus intermedius from dog and cat, and also on Staphylococcus aureus from wound and pyoderma infections, have shown a correlation between the site of microbial infection and antimicrobial susceptibility. Both the methanolic extract concentrate of Garcinia kola (Heckel) seeds and natural honey have been associated with activity on bacterial isolates from respiratory tract infections. In this study, selected bacteria belonging to genera from burn wound infection sites were treated with natural honey and methanolic extract concentrate of Garcinia kola in antimicrobial susceptibility tests separately and in combined form, and also with gentamicin and methanol as controls. The two natural products were found to be active on the bacterial isolates, excluding Klebsiella pneumoniae strains, all of which showed resistance to honey. Combination forms of the two natural products were active only on the strains of Pseudomonas aeruginosa. At 4 and 8 μg/ml, gentamicin was ineffective on the three strains of Klebsiella pneumoniae while 8 μg/ml was moderately active on only two strains of Pseudomonas aeruginosa. One strain of Pseudomonas aeruginosa, UCH002, was resistant to gentamicin beyond 1,000 μg/ml. Gentamicin at 4 μg/ml was inhibitory to one strain of Escherichia coli and two strains of Staphylococcus aureus. Though the antimicrobial activity of the two natural products tested had been previously reported against microbial agents of respiratory tract infection, it was also recorded in this study. The lack of activity of each of the three honey types used in this study against the Klebsiella pneumoniae strains tested underscores the need to exclude this organism from burn wound infections before embarking on treatment with honey. The sensitivity of one high-level gentamicin-resistant strain of Pseudomonas aeruginosa to honey and Garcinia kola seed extract was noteworthy considering the therapeutic failures of gentamicin and other antibiotics against Pseudomonas aeruginosa.

Keywords: honey, gentamicin, Garcinia kola, against Pseudomonas aeruginosa

Introduction

Honey, a natural secretory sweet product of the honey bee, Apis mellifera, originating from the nectar of flowering plants, is a viscous substance rich in glucose and fructose intended primarily as a food store for the three categories of bees. Garcinia kola (Heckel) is a tropically distributed plant of the Guttiferae family. Natural honey and Garcinia kola (Heckel) seed have certain properties in common: both are edible by man and are widely acclaimed for their traditional folklore curative effects on certain ailments. Garcinia kola seeds can be chewed raw or consumed as a concoction, while honey is consumed in its “raw” form or as a component of confectionery products and non-alcoholic beverages (honey drinks). Reports have also authenticated the curative effects of honey and Garcinia kola seed through the discovery of their antimicrobial properties. Essentially, both have been shown to inhibit various bacteria and fungi from various pathological sources.

In particular, both natural products were found to be active against some microbial agents of respiratory infection while the anti-pseudomonal property of honey has been reported. Many factors are known to influence antimicrobial susceptibility, including the site for microbial isolation.

Wounds, whether due to burns, surgical operation or trauma, constitute a pathological source for the isolation of different bacteria. It was therefore considered worthwhile to study, on a comparative basis, the antibacterial activities of natural honey and methanolic extract concentrate of Garcinia kola seed powder on selected bacteria isolated from burn wound infections.

Materials and methods

Ten bacterial isolates from four genera (Table I) were obtained on nutrient agar slants from the Routine Section of the Medical Microbiology Laboratory, University Col-
lege Hospital, Ibadan, Nigeria. They were confirmed by conventional biochemical tests and then preserved on fresh nutrient agar slants in a refrigerator at 4 °C.

Natural honey
Honey was obtained from three natural honey collection centres in Ibadan, South West Nigeria. Each stock was used undiluted and in 1:2 water dilution against the respective bacterial isolates tested.

Garcinia kola (Heckel) seeds
Garcinia kola (Heckel) seeds were obtained locally at a local market in Ibadan, Nigeria, and authenticated at the Federal Research Institute of Nigeria, Ibadan. The seeds were pulverized.

Soxhlet extraction
The seed powder, weighing 200 g, was extracted in a Soxhlet apparatus using 500 ml of methanol for 6 h. The hot extract was concentrated by evaporation at 100 °C on an electrothermal water-bath and evaporated to dryness in an oven at 60 °C into a constant weight of 3 g.

Gentamicin
Gentamicin sulphate BP, produced by Greenfield Pharmaceutical, China, was obtained in ampoule vials (2 ml each) from a local pharmacy store. This served as a standard antibiotic against the bacterial isolates tested.

Phytochemical screening
The seed powder was screened for the presence of secondary metabolites, namely saponins, tannins, alkaloids, cardenolides, and anthraquinone glycosides, as previously described.20,21

Antimicrobial susceptibility testing
The agar-cup diffusion method was employed on a 10³ aq dilution of each bacterial isolate to obtain the susceptibility pattern against each honey type and also 4 and 8 μg/ml of gentamicin, as well as 30, 15, and 7.5 mg/ml of the methanolic extract concentrate of Garcinia kola (Heckel) seed and methanol. The antibiogram of the Gram-negative bacterial isolates was determined by the disk-diffusion method.19

Results

The phytochemical screening revealed the presence of saponins, tannins, alkaloids and cardenolides, as secondary metabolites. The antibiogram showed complete resistance by all the bacterial species tested to augmentin® and amoxicillin while ofloxacin, gentamicin, nitrofurantoin, nalidixic acid, tetracycline, and cotrimoxazole were effective in descending order of activity against the bacterial species. Notably, the three isolates of Pseudomonas aeruginosa were resistant to amoxicillin, cotrimoxazole, and nitrofurantoin, while one of them was resistant to all the antibiotics tested.

With the methanolic extract of Garcinia kola seed and honey in separate experiments, all the bacterial isolates were sensitive at varying levels to each of the 30, 15, and 7.5 mg/ml of the methanolic extract concentrate of Garcinia kola (Heckel) seed and methanol. The antibiogram of the Gram-negative bacterial isolates was determined by the disk-diffusion method.19

Table I - Antimicrobial activity of Garcinia kola and honey

<table>
<thead>
<tr>
<th>Organism</th>
<th>Garcinia kola</th>
<th>Honey</th>
<th>Met</th>
<th>Gen</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>15</td>
<td>7.5</td>
<td>Ao</td>
</tr>
<tr>
<td>Pseud. aeruginosa</td>
<td>UCH 001</td>
<td>15</td>
<td>-</td>
<td>10</td>
</tr>
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<td>UCH 002</td>
<td>15</td>
<td>13</td>
<td>12</td>
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<tr>
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<td>UCH 003</td>
<td>11</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Kleb. pneumoniae</td>
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</table>

Key: Ao, Bo, Co = undiluted honey; Met = methanol; Gen = gentamicin; UCH = University College Hospital, Ibadan, Nigeria
a = zone of growth inhibition (antibacterial activity observed)
b = no zone of growth inhibition (lack of antibacterial activity)
erately sensitive to 8 mg/ml gentamicin, while the third isolate, *Pseudomonas aeruginosa* (UCH002), was resistant to both 4 and 8 mg/ml as well as beyond 1000 mg/ml of the antibiotic (Table I).

The combination of honey (undiluted and in 1:2 dilution) with each of the 30, 15, and 7.5 mg/ml of the methanolic seed extracts was active remarkably only on the three isolates of *Pseudomonas aeruginosa* (Table II). The minimum inhibitory concentrations of the methanolic seed extract varied from the highest of 7.5 µg/ml for *Klebsiella pneumoniae* to 0.47 mg/ml for *Staphylococcus aureus* (Table III).

Table II - Antimicrobial activity of the combination of methanolic extract of *Garcinia kola* seed and honey

<table>
<thead>
<tr>
<th>Organism</th>
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<th>30 µg/ml</th>
<th>15 µg/ml</th>
<th>7.5 µg/ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
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<td>A</td>
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<td>a 16</td>
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</tr>
<tr>
<td><em>Kleb. pneumoniae</em></td>
<td>UCH 001</td>
<td>b</td>
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<tr>
<td><em>Esch. coli</em></td>
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</table>

Key: 30 mg/ml - 7.5 mg/ml = concentrations of *Garcinia kola* seed extract.
UCH = University College Hospital, Ibadan, Nigeria
a = zone of growth inhibition (antibacterial activity observed)
b = no zone of growth inhibition (lack of antibacterial activity)
A, B, C = undiluted honey types

Discussion and conclusion

The detection of secondary metabolites in *Garcinia ko- la* (Heckel) seeds was consistent with a previous report, and the seeds may be in part responsible for their antimicrobial properties. Past reports have indicated curative and antimicrobial properties of honey and of the methanolic extract of *Garcinia kola* (Heckel) seed. Specifically, the antimicrobial activity of the seed’s methanolic extract concentrate against microbial agents of respiratory tract infections has been reported. Interestingly, in this study, the seeds’ methanolic extract and the three honey types tested were equally active on burn wound isolates of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* but not *Klebsiella pneumoniae*, thereby negating the previous report on the variation of antibiotic activity with the site of microbial infection, probably because the seed extract and honey are both natural products.

The total resistance of the three *Klebsiella pneumoniae* isolates to every honey tested, due possibly to their capsule, is noteworthy. Hence, the possibility of burn wound contamination by this organism should be excluded before applying honey. The improved antipseudomonal activity exhibited by the combination of methanolic extract concentrate of *Garcinia kola* seeds and honey in this study is of therapeutic relevance, particularly in cases where either of the two is ineffective alone against *Pseudomonas aeruginosa*. This organism is a recognized major burn pathogen involved in the aetiology of conjunctivitis, endocarditis, meningitis, and urinary tract infections. The high-level resistance of *Pseudomonas aeruginosa* UCH002 to over 1000 µg/ml gentamicin but its sensitivity to honey and *Garcinia kola* seed extract are remarkable.
RÉSUMÉ. Les études sur le *Staphylococcus aureus* et le *Staphylococcus intermedius* du chien et du chat, comme aussi sur le *Staphylococcus aureus* et les infections des lésions corréllées aux pyodermites, ont montré un rapport entre le site de l’infection microbienne et la sensibilité antimicrobienne. Soit le concentré de l’extrait méthanolique des graines de *Garcinia kola* (Heckel) soit le miel naturel ont été associés à l’action sur les isolats bactériens provenant des infections des voies respiratoires. Dans cette étude, certaines bactéries appartenant aux genres observés dans les sites de l’infection des lésions ont été traitées avec du miel naturel et le concentré de l’extrait méthanolique de *Garcinia kola* dans le contexte de tests de sensibilité antimicrobienne séparément et sous forme combinée, et aussi avec la gentamicine et le méthanol comme témoins. Les deux produits naturels se sont montrés actifs sur les isolats bactériens avec l’exclusion des souches de *Klebsiella pneumoniae*, qui ont montré une résistance au miel. Les formes combinées des deux produits naturels ont été actives seulement sur les souches de *Pseudomonas aeruginosa*. A la dose de 4 et 8 µg/ml la gentamicine a été inefficace sur les trois souches de *Klebsiella pneumoniae* tandis que, à la dose de 8 µg/ml, elle a été modérément active seulement sur deux souches de *Pseudomonas aeruginosa*. Une souche de *Pseudomonas aeruginosa*, UCH002, était résistante à la gentamicine au-delà de 1000 µg/mL. La gentamicine à la dose de 4 µg/ml a été inhibitrice vers une souche d’*Escherichia coli* et deux souches de *Staphylococcus aureus*. L’activité antimicrobienne des deux produits naturels testés avait été déjà signalée contre les agents microbiens de l’infection des voies respiratoires, mais elle a été également enregistrée dans cette étude. L’absence d’activité manifestée par chacun des trois types de miel utilisés dans cette étude contre les souches testées de *Klebsiella pneumoniae* souligne la nécessité d’exclure cet organisme parmi les infections causées par les brûlures avant d’entreprendre un traitement basé sur le miel. La sensibilité au miel et à l’extrait des graines de *Garcinia kola* manifestée par une souche de *Pseudomonas aeruginosa* douée de haute résistance à la gentamicine était remarquable, vu les échecs thérapeutiques de la gentamicine et d’autres antibiotiques contre *Pseudomonas aeruginosa*.

Mots-clés: miel, gentamicine, *Garcinia kola*, contre *Pseudomonas aeruginosa*

BIBLIOGRAPHY