Research Paper

Antimicrobial Activities of *Garcinia kola* Seed Oil against Some Clinical Microbial Isolates

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Abstract

Oils, fats including their hydrolytic products (glycerol and fatty acids) are widely used as raw materials in food, cosmetics, chemical and pharmaceutical industries. In this study, the seed oil of *Garcinia kola* was investigated for its antimicrobial properties. The seed oil, extracted with *n*-hexane by cold maceration was assessed for the presence of secondary metabolites and thereafter tested against some bacterial (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumoniae*), fungal (*Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium notatum* and *Candida albicans*) clinical isolates. Qualitative phytochemical tests as well as thin layer chromatographic profile of the oil indicated the presence of some dissolved secondary metabolites such as phenols and alkaloids. The oil was found to possess a broad-spectrum of activity which is concentration-dependent against the Gram-positive and Gram-negative bacteria isolates as well as the fungi isolates. The oil could find applications in the formulation of antimicrobial creams and lotions for treating skin diseases, in food preservation and in folk medicine.

*Keywords:* *Garcinia kola* Seed Oil, Antimicrobial, Secondary Metabolites, Clinical Microbial Isolates

1. Introduction

*Garcinia kola* Heckel, (*Guttiferae*) tree, grows in forest and cultivated for its economic importance, is widely distributed in the tropics especially West and Central Africa. The seeds of *G. kola* commonly known in commerce as “bitter kola” are widely consumed in West Africa as a masticatory and adaptogen (Iwu, 1993). The seeds are used in folk medicine, many herbal formulations and have therapeutic benefits largely due to the presence of various secondary metabolites such as complex mixtures of phenolic compounds (flavonoids, biflavonoids and tannins), xanthones, benzophenones, triterpenes and saponins (Iwu & Igboko, 1982; Waterman & Hussain, 1983 and Geiger & Quince, 1988). Scientific evaluations of the seed extractives have demonstrated antidiabetic (*Iwu* et al, 1990b), antibacterial (*Akinpelu* et al, 2008) and hepato-protective activity against hepato-toxicity induced by phallolidin, amanita, 2-acetylaminofluorene, carbon tetrachloirde, paracetamol, thioacetamide, aflatoxin, dimethyl nitrosamine in rodents (*Iwu* et al, 1990a; *Farombi* et al, 2000; 2005 and 2009).

The proximate analysis of *G. kola* seed revealed the following composition: crude protein, lipid, ash, carbohydrate and crude fibre (*Eleyinmi* et al, 2006 and *Adesuyi* et al, 2012). In one of the studies, lysine and valine were found to be the dominant essential amino acids, while the dominant saturated, monounsaturated, and polyunsaturated fatty acid were palmitic (31.55 mg/kg), oleic (38.36 mg/kg) and linoleic acids (36.16 mg/kg) respectively (*Eleyinmi* et al, 2006).

Oils and fats as well as their hydrolytic products (glycerol and fatty acids) are widely used as raw materials in food, cosmetics, pharmaceutical industries, soap production, synthetic detergents, greases, and several other products (Gunstone, 2004). Oils possessing antimicrobial activities...
are useful in treatment of wound, formulations of antimicrobial creams and lotions for treating skin diseases, as well as in the area of food preservation. For instance, Shittu & Bwala (1988) reported the proven efficacy of the seed of mahogany (Kyuya ivoricensis) against dermatitis associated with mange and dermatophilosis. In addition, the agouróladó (green olive oil produced by crushing the olives without the use of hot water) was, and still is, one of the most important natural medicines for various illnesses among the inhabitants of the Mediterranean (Polymerou-Kamilakis, 2006).

While various studies had been reported on the G. kola seed extractives, there is no report on antimicrobial activity of the seed oil. This paper reports the assessment of the antimicrobial activity of G. kola seed oil. The seed oil could be useful in the field of pharmaceutical in the formulations of antimicrobial creams and lotions for treating skin diseases and infected wounds as well as in folk medicine.

2. Materials and Methods

2.1. Plant Material

Seeds of G. kola were bought at Oja-Oba market, Ibadan, Oyo State. It was identified at the Botany Department, University of Ibadan. The seeds were chopped into tiny pieces with the use of a stainless steel knife. It was then sun-dried for a week. Thin coverings were peeled off after drying and subsequently milled into powder.

2.2. Materials

Precoated thin layer chromatography aluminium sheets (0.2 mm, silica gel 60, F_254, Merck, Darmstadt, Germany), gentamycin injections (80mg/2ml) (Laboratory Pharmaceutical, India), ketoconazole reference standard, peptone water (Acumedia, England), acetone, absolute ethanol, methanol, n-hexane, toluene, ethyl acetate, iodine, ferric chloride, concentrated Hydrochloric Acid (HCl), vanillin (all are British Drug Houses (BDH), UK., analytical grade) nutrient broth, nutrient agar and sabouraud dextrose agar (Biotin).

2.3. Oil Extraction

Oil extraction was conducted following Idowu et al (2009). Briefly, 3kg of powdered seeds were packed in a cotton cloth bag and extracted exhaustively by cold maceration using n-hexane. The solvent was evaporated in a vacuum rotary evaporator at 40 °C. The recovered oil was filtered through glass wool, while the dark-brownish sediments were discarded. The oil was dried in vacuo at 40 °C in a vacuum oven for 24 hours. Afterwards, it was poured into a glass container and stored in a fridge for subsequent use.

2.4. Preparation of Cultures of Microbial Organisms

Each test organism (Bacterial-Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi, Klebsiella pneumoniae and Pseudomonas aeruginosa; Fungi-Aspergillus niger, Rhizopus stolonifer, Penicillium notatum and Candida albicans) was obtained from the clinical isolates laboratory stock of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria.

An overnight culture of each bacterial organism was prepared. Each organism in the stock was sub-cultured into freshly prepared nutrient broth aseptically using a flamed wire loop and then incubated at 37 °C for 24 hours in the incubator. For the fungal test organism, sterile wire loop was used to transfer a loopful of fungi spore to the surface of Sabouraud Dextrose Agar (SDA) and streaked. They were then incubated at room temperature i.e. 28 °C, until growth occurred and produced spores in 5-7 days. The spores were harvested and used for sensitivity test.

2.5. Preparation of Culture Media

Nutrient broth was prepared. 5ml volumes were distributed into test tubes bottles and sterilized by autoclaving at 121 °C for 15 minutes. The nutrient agar was similarly prepared following the manufacturer’s instructions. This was distributed into 20 ml universal bottles and sterilized in an autoclave at 121 °C for 15 minutes. The SDA was also prepared following the manufacturer’s instructions. It was also distributed into 20 ml aliquots into universal bottles and sterilized in an autoclave at 121 °C for 15 minutes.

2.6. Preparation of Standard Drug Solution

0.5 ml of gentamycin injection (80 mg/2 ml) was measured into a volumetric flask and diluted to make 200 µg/ml by making up the volume with sterile distilled water to 100 ml. 5 ml of this solution was further diluted to 100 ml with sterile distilled water to give 10 µg/ml solution. 10 mg of ketoconazole reference standard was dissolved in about 6 ml ethanol. This was then transferred into a 10 ml volumetric flask and made up to volume with more ethanol to make a 1 mg/ml i.e. 1000 µg/ml solution. This solution was used as the standard drug for the fungal isolates.

2.7. Preparation of G. kola Oil for Sensitivity Test

5 ml of the seed oil was used as 100% undiluted stock. Series of dilutions of the seed oil were carried from this stock, while making up to volume with acetone, to give lower concentrations of 50, 25, 12.5, 6.25 and 3.125%.
2.8. Preliminary Tests

2.8.1. Qualitative Phytochemical Test

This was used to detect the presence of secondary metabolites such as phenols and alkaloids. For the phenols, a drop of the oil was put on filter paper, followed by a drop of ferric chloride solution. A blue-black colouration is indicative of a positive test. Alkaloid detection was carried out by extracting 0.5 ml of the oil, dissolved in 2 ml n-hexane, with 2 ml of an equal mixture of methanol and 2 M HCl. 1 ml of the acid-methanol extract was placed in two separate test tubes and few drops of Meyer's and Wagner's reagents added. Appearance of turbidity or precipitation indicated that the test was positive.

2.8.2. Thin-Layer Chromatography (TLC) of the Oil

Aliquots of the seed oil extract was dissolved in ethyl acetate and spotted on precoated TLC plates and developed in an already saturated tank containing toluene/ethyl acetate solvent system (8:2). After development, one of the TLC plates was examined in iodine saturated tank while another was sprayed with vanillin-HCl reagent solution (0.1 g vanillin, 28 ml methanol, 1 ml HCl) and examined in daylight after heating at 105 °C for 5 minutes.

2.9. Antimicrobial studies

0.1 ml of the test organism was added into 9.9 ml of sterile distilled water to give 10 ml at 1:100 (1 in 100) dilution. From this dilution of a bacteria organism, 0.2 ml was taken into the sterile molten nutrient agar at 45-50 °C. This was aseptically poured into the sterile plates and allowed to set on the bench for about 30 minutes. The wells were made on the set plates. Inside each well, the graded concentrations of the oil samples were poured into the wells using a sterile Pasteur pipette. Acetone was used as control and gentamycin as drug standard. The plates were then left on the bench for about 2 hours. This was done to allow the pre-diffusion to take place.

For the fungi plates, the SDA was poured inside the plates, the plates were allowed to set for 45-50 minutes and then the 0.2 ml of 1 in 100 dilution of a fungal organism was spread on the plates. Ketoconazole was used as the drug standard, while acetone was used as the control. The wells were made and the other process followed as described above. The bacterial plates were incubated for 18-24 hours at 37 °C, while the fungi plates were incubated for 2-5 days at 26-28 °C. The zones of inhibition were then measured and recorded in millimetre. Each determination was done in duplicates.

3. Results and Discussion

3.1. Oil Extraction

The extraction of the powdered seed with n-hexane by cold maceration yielded dark-brownish oil with sediment after the solvent was distilled off. Filtration of the recovered oil through glass wool gave clear dark-brownish oil.

3.2. Qualitative Phytochemical Test

The qualitative phytochemical test was positive for phenols with a blue-black colouration resulting from the test. For alkaloids the result was also positive indicating the presence of alkaloids in the oil. This is in consonance with earlier works that showed the presence of these secondary metabolites in the seed (Iwu & Igboke, 1982; Waterman & Hussain, 1983; Geiger & Quince, 1988 and Adesuyi et al, 2012).

3.3. Thin-Layer Chromatography (TLC) of the Oil

Figure 1 shows the image of the TLC profile of the oil showing various secondary metabolites present as dissolved components in the oil.

![Figure 1](image-url)
Staining with iodine, Figure 1a, shows various unsaturated compounds present, while this was corroborated by the vanillin-HCl reagents solution which like iodine is a general locating reagent. These molecules are possibly responsible for any bioactivity the oil might exhibit. The polar phenols present may also be contributing to the stability of the oil as observed in Olive oil which showed good correlation of stability of Olive oil and total or individual phenol content (Tsimidou et al, 1992 and Gutiérrez et al, 2001).

3.4. Antimicrobial Studies

The results of the antimicrobial studies of the G. kola seed oil are shown in Tables 1 and 2.

Table 1. Diameter of Zones of Inhibition (mm) of Bacterial Isolates by Different Concentrations of the Oil

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Concentration of Oil (%)</th>
<th>Gentamycin (10µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>28</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2. Diameter of Zones of Inhibition (mm) of Fungal Isolates by Different Concentrations of the Oil

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Concentration of Oil (%)</th>
<th>Ketoconazole (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Rhizopus stolonifer</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Penicillium notatum</td>
<td>20</td>
<td>16</td>
</tr>
</tbody>
</table>

From the results presented in Table 1, the oil showed activity against both Gram-positive and Gram-negative organisms used in the investigation. This activity was, however, concentration-dependent with the least activity observed at 3.125 % concentration of the oil. The highest activity was recorded against K. pneumoniae followed by S. aureus and P. aeruginosa while the least was against S. typhi. The antifungal activity of the seed oil is presented in Table 2. The oil appeared to have relatively equal activity against the fungal organisms used in the study. This activity was also concentration-dependent with the least activity being observed at 6.25% concentration of the oil. Overall, the antimicrobial activity of the oil is lower as compared to the standard drugs used in the study (i.e. gentamycin and ketoconazole). This is so because these are pure synthetic drugs compared to the oil that contains minor dissolved secondary metabolites. However, in comparison to Khaya senegalensis seed oil (Audu-Peter et al, 2006) and Leucaena leucocephala seed oil (Aderibigbe et al, 2011) the G. kola seed oil gave a broader spectrum of activity as the former were inactive against the fungal organisms used in the studies.

4. Conclusion

The study showed that Garcinia kola seed oil had a concentration-dependent antimicrobial activity against both bacterial and fungal organisms used in the study. Findings from this study are significant in the sense that apart from the healthful benefits which could result from the consumption of the oil and its usage in food products formulation (due to its high content of unsaturated fatty acids such as oleic and linoleic acids); the oil could also
find applications in other areas. This includes usage in the field of pharmaceutical in formulation of antimicrobial creams and lotions for treating wounds and skin diseases, especially those caused by susceptible organisms such as, \textit{S. aureus}, a Gram-positive bacterium, which is implicated in several skin ailments such as boils, abscesses, burns and infected wounds. Also, the oil could be useful in preserving meat, spiced cheese and vegetables.

References


