Original Article

Antimicrobial activity of *Garcinia kola* against human upper respiratory tract pathogens.

Olumide Adedokun ODEYEMI\(^1\) \(^*\) and Solakunmi O. OLUWAJOBA\(^2\)

\(^1\) Marine Microbiology Laboratory, School of Biosciences & Biotechnology, Faculty of Science & Technology, Universiti Kebangsaan Malaysia 43600, UKM Bangi, Malaysia

\(^2\) Department of Biological Science, School of Applied Sciences, Yaba College of Technology, Yaba, Lagos, Nigeria.

\(^*\) Email: oluodeyemi@gmail.com, Phone: +60163087064

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Abstract

The antibacterial activity of aqueous and ethanolic extracts of the seeds of *Garcinia kola* was investigated. Agar diffusion and paper disc methods were used for the investigation. The tested organisms: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Micrococcus luteus* were obtained from the Molecular Biology and Biotechnology Division of the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos. *Salmonella typhi* was obtained from the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos. Results obtained show that 75% of the tested organisms were susceptible to ethanolic extract only. 37.5% showed susceptibility to aqueous extract of the plant. It was also observed that 62.5% of the organisms tested were inhibited by mixture of ethanolic extract. Menthol showed inhibitory effect on 62.5% of the tested organisms. The diameter of the zones of inhibition of the mixture of ethanolic extract and menthol were larger than that of ethanolic extract only. The Minimum Inhibitory Concentration (MIC) ranged between 50mg/mL and 150mg/mL. *E.coli* showed the highest zone of inhibition (20mm). The least zone of inhibition (5mm) was observed in *S. aureus* and *S. pneumoniae*. *P. aeruginosa* exhibited resistance to all the extracts.

Key words: Antimicrobial, Chemotherapeutic agents, Minimum Inhibitory Concentration

INTRODUCTION

The development and wide spread of resistance of microorganisms to existing antibiotics calls for increased efforts in the development of new antibiotics for treatment of microbial infections and diseases. Although, there is a wide range of antibiotics for the treatment of these infections and diseases, the development of resistance to chemotherapeutic agents are increasingly becoming a serious and global problem. Globally, the last two decades has witnessed an unprecedented increase of drug resistance by pathogenic microorganisms as well as the appearance of undesirable side effects of certain antibiotics. Other limitations of modern chemotherapeutic drugs are their high cost and non-availability, especially in rural areas. As a consequence, it is necessary to search new organic molecules with antimicrobial activity, which in addition could be potential sources for starting materials for the semi-synthesis of new drugs [1]. It is estimated that there are over 65000 species of flowering plants that have medicinal properties [2]. African plants, in particular medicinal plants constitute a rich but still largely untapped pool of natural products. Many countries from the developing world are still dependent on medicinal plants for treating the sick among them. The Nigerian climate favours a great array of plant species many of which have varied medicinal and antimicrobial potentials [3]. Traditional herbalist in Nigeria uses a variety of herbal preparations to treat different kinds of ailments including many microbial infections such as gonorrhea, sore throat, skin infections like eczema. This has been the case ever before the introduction of antibiotics and other modern drugs into Africa [4]. A number of plants that have medicinal and antimicrobial properties in Nigeria have been identified and documented [5, 6]. Some of the active ingredients of the extracts of some
plants have been isolated, tested and documented [7]. The bark of *G. kola* can be taken orally for fever, cough, inflammation, respiratory tract disease and as an antihelmintic [8]. The dried root soaked in alcohol is taken orally for the treatment of cough, inflammation, liver cirrhosis, tooth decay and gonorrhea [9]. The seed enjoys a folk reputation in Africa as a poison antidote, additionally, the plant possesses anti-hepatotoxic [10, 11], antioxidant [12], hypoglycemic [13]. This research work therefore aims to investigate the antimicrobial activities of *G. kola* on human upper respiratory tract pathogens.

**MATERIALS AND METHOD**

**Source of *Garcinia kola* seeds**

*Garcinia kola* seeds were obtained from a public market in Mushin, Lagos, Nigeria and then transported to the laboratory. Seeds were grated to fine particles and sun dried for 24 hours to evaporate moisture.

**Aqueous and Ethanol extracts preparation**

Twenty grams of the powdered plant materials was loaded into soxhlet apparatus containing 200 mL of 75% v/v ethanol in a round bottom flask. Phytochemical constituents were extracted for 4 hours at 60°C. The solution was concentrated by evaporating the solvent. The extract was weighed into sterile universal bottles and stored at 4°C in a refrigerator. 20 g of the powdered plant materials was loaded into soxhlet apparatus containing 200 mL of distilled water in a round bottom flask. This was placed in a heating mantle. The constituents were exhaustively extracted at 60°C for 4 hours. The solution was concentrated by evaporating the distilled water (solvent) in a water bath. The extract was weighed into sterile universal bottles and stored at 4°C in a refrigerator.

**Sterilization of the reconstituted extracts**

The reconstituted extracts (ethanol and aqueous) were sterilized using 0.45μm membrane filter paper [4].

**Source of tested pathogens**

Microorganisms used for this research were obtained from the Molecular Biology and Biotechnology Division of the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos and Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria respectively.

**Microbiological media**

Nutrient Agar (NA), Nutrient Broth (NB), Chocolate Agar (CA), Blood Agar (BA) and MacConkey Agar (MA, Oxoid, England).

**Maintenance, activation and standardization of stock microbial cultures.**

The stock microbial cultures were maintained on nutrient agar slants at 4°C. In order to activate these cultures; subcultures were freshly prepared and inoculated at 37°C for 18-24 hours before use.

**Preliminary antimicrobial screening of extracts**

The preliminary tests of the plant extracts were carried out on the test microorganisms using agar-gel diffusion inhibition method. In the agar-gel diffusion method, 0.2mL of a 24 hour log phase broth culture of each organism was aseptically introduced and evenly spread using sterile glass rod on the surface of the gelled nutrient agar [14]. Three wells (holes) of 6 mm in diameter were aseptically punched on each agar plate using sterile cork borer, allowing at least 30 mm distance between adjacent wells and the edge of the plates. 0.1 mL of the various extracts was then seeded into the wells in the plates using sterile insulin syringes. The plates were incubated at 37°C for 24 hours. Clear zones around the wells were known as Preliminary indication of antimicrobial activity of the plant against the inoculated microorganisms.

**Susceptibility testing (Confirmatory test)**

Various concentrations of the plant extract (200mg/mL, 150mg/mL, 100mg/mL, and 50mg/mL and 25mg/mL were seeded with sterile 5mm diameter filter paper disc [4]. The disc were allowed to soak and absorb the extracts for overnight before draining off the excess extract and drying in the oven at 60°C for 5 minutes [4]. Appropriate medium for each organism tested was used namely blood agar for *S. pneumoniae*, Chocolate agar for *K. pneumoniae*, Pseudomonas Based Agar for *P. aeruginosa*, Nutrient agar for *S. aureus, M. luteus* and *E. coli*. Salmonella-Shigella agar was used for *S. typhimurium*. Appropriate growth condition was provided for the organisms. Both aqueous and ethanol extracts were tested in parallel. Positive results were retested as described by [15].

**Determination of Minimum Inhibitory Concentration (MIC) using the Disc method**

The minimum inhibitory concentration (MIC) was carried out using the disc method [4]. Minimum inhibitory concentration is defined as the lowest concentration of antimicrobial that is able to inhibit visible microbial growth.

**RESULTS AND DISCUSSION**

Results obtained showed that 75% of the tested organisms were susceptible to ethanolic extract only 37.5% show susceptibility to aqueous extract of the plant. It was also observed that 62.5% of the tested organisms were inhibited by mixture of ethanolic extract and menthol. Susceptibility to mixture of aqueous extract menthol was 37.5% while mixture of aqueous extract and menthol showed 37.5% susceptibility. Menthol alone inhibited 62.55% of the tested organisms. The diameter of the zones of inhibition of the mixture of ethanol extract and menthol were larger than that of ethanolic extract only. The mixture of aqueous extract and menthol were equally larger than aqueous extract only. The minimum inhibitory concentration ranged between 50mg/mL and 150mg/mL. *E.coli* showed the highest zone of inhibition (20 mm). The least zone of inhibition (5mm) was observed in *S. pneumoniae*. **
aureus and S. pneumoniae. P. aeruginosa exhibited resistance to all the extracts. The crude ethanol extract was the most active of the extracts showing activity against all the isolates except P. aeruginosa. The crude extract of G. kola singly and in combination with menthol did not exhibit any in vitro inhibition on the growth of P. aeruginosa as can be seen in Table 1. Similar result was reported by [16] who investigated the antimicrobial activity of extracts of local cough mixtures on upper respiratory tract pathogens. E. coli showed the highest susceptibility with ethanol extract only (Table 1). Table 2 shows that the aqueous extract does not inhibit the growth of S. aureus. More so, some organisms were not susceptible to the aqueous extract only of G. kola. However it was inhibitory to S. typhi, S. epidermidis and E. coli. The zones of inhibition of ethanolic extracts were larger in diameter than that of aqueous extracts on the three organisms. Many published reports showed the effectiveness of traditional herbs against pathogenic organisms. As a result, plants are one of the bedrocks for modern medicine. Many accomplishments have been achieved on medicinal plants. Research is still being carried out on the properties and uses of this plant. Large numbers of plants are employed in Nigeria for the treatment of diverse kinds of diseases of microbial origin. A lot of information about these plants has been provided in ethno-medicine however, a major setback of tradio-medicine is their inability to standardize their concoctions and lack of proper hygiene.

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REFERENCES

Table 1: Disc diffusion Method of Ethanol

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<td></td>
<td>200mg/mL</td>
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Table 2: Disc Diffusion Method of Aqueous Extract

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