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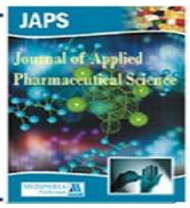

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Synergistic effect of menthol on in-vitro antibacterial activity of *Garcinia kola* against Gram positive Bacteria: A preliminary study

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ABSTRACT

In vitro antibacterial activity of aqueous and ethanol extracts of *Garcina kola* seeds and synergistic effect of menthol was investigated against selected Gram positive bacteria using agar diffusion method. The following bacteria were used as test microorganisms *Staphylococcus aureus, Streptococcus pneumoniae, Staphylococcus epidermidis and Micrococcus luteus*. Results obtained showed that only *S. epidermidis* was susceptible to aqueous extract while the remaining organisms did not show any susceptibility. It was also observed that *S. pneumoniae* did not show any susceptibility to ethanol extract. The Minimum Inhibitory Concentration (MIC) ranged between 25mg/ml and 200mg/ml. *M. luteus* showed the highest zone of inhibition (24mm). The least zone of inhibition (10mm) was observed in *S. aureus* and *S. epidermidis*. A different result was obtained when the organisms was tested with combinations of ethanol, aqueous and menthol. *S. aureus, S. epidermidis* and *M. luteus became* susceptible to ethanol extract when menthol was added likewise *S. aureus* and *M .luteus* became susceptible to aqueous extract with 5mm and 8mm as MIC respectively. Further study is required to ascertain the phytochemicals present in this plant seeds.

Keywords: Antimicrobial activity, *Garcinia kola,* plant extracts Gram positive bacteria.

INTRODUCTION

Globally, there have been various scientific records on antibiotic resistance. The discovery of antibiotics was thought to bring an end to microbes-originated diseases. This was proved wrong as a result of continuous resistance of pathogens to these chemotherapeutic agents although a huge source was recorded initially. Various factors have contributed to continuous incidence of microbial resistance to antibiotics. Among which are indiscriminate use of antibiotics and horizontal transfer of resistance gene between bacteria. World-wide increase in resistance to antibiotics has prompted scientists and researchers to seek for other possible potential antimicrobials (Mahesh and Satish, 2008). Due to this search, plants have been seen as a good source of antimicrobials (Del Campo et al., 2000), aside marine bacteria (Anand et al., 2006; Uzair et al., 2008; Burgess et al., 1991). This study therefore aims to preliminarily investigate the synergistic effect of menthol on antimicrobial activities of *G kola* gram positive bacteria.

MATERIALS AND METHOD

Sample preparation, extraction and sterilization

G. kola seeds were purchased from Mushin market in Lagos, Nigeria. The seeds were firstly peeled and then grated to coarse and fine particles. 20g of sun dried grated plant material

was weighed and the extraction was carried out using soxhlet apparatus with 200ml of distilled water as solvent to obtain aqueous extract. Extraction procedure was carried for 4 hours at 60^0C. The procedure was repeated for ethanol extract as it was carried out for aqueous extract except using ethanol as solvent. Each of the solutions was further concentrated by evaporating the solvent in water bath until a constant weight was obtained. The obtained extracts were later weighed into sterile McCartney bottles and then stored in refrigerator at 4^oC for further study. Both extracts were reconstituted with 1 ml of distilled water and 1ml of 70% ethanol respectively. Sterilization of the extracts was carried out using slightly modified method of Olukoya *et al*, (1993).

Test microorganisms

Tested pathogens - *S. aureus, S. pneumoniae, S. epidermidis, and M. luteus,* were obtained from the Molecular Biology and Biotechnology division of the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos.

Antimicrobial screening of extracts

The plant extracts were preliminarily screened on the test microorganisms using modified agar diffusion method according to the method of (Bauer et al., 1966). 0.2ml aliquot of a 24 hour broth culture of each test organism was aseptically inoculated and evenly spread using sterile glass rod on the surface of solidified Nutrient Agar. With the aid of a sterile cork borer, 3 wells (holes) of 6mm diameter were punched on the agar plates. 0.1ml of the various extracts was then seeded into the wells with the aid of sterile syringes. The plates were incubated for 24 hours at 37^oC. Zones of inhibition (clear zones) around the wells were noted as preliminary indication of antimicrobial activity of the plant against tested pathogens (Mbata et al., 2008).

Susceptibility test

Various concentrations of the plant extract (20mg/ml, 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml were seeded on sterile 5mm diameter filter paper discs (Olukoya et al.,1993). The discs were allowed to soak and absorb the extracts overnight before draining off excess extract and dried in the oven at 60^oC for 5 minutes (Olukoya et al., 1993). . Confirmatory test was carried out as described by (Bauer et al., 1966). Appropriate medium for each organism tested was used namely Blood agar for *S. pneumoniae*, Nutrient agar for *S. aureus* and *M. luteus.* Bacteria were cultured at appropriate growth conditions. Aqueous and ethanol extracts together with menthol were tested in parallel.

RESULTS AND DISCUSSION

Increase in bacteria resistance to antibiotics worldwide has made research for new antimicrobials either from plants or bacteria intensified. It is imperative that more and effective antimicrobials are discovered. From the results obtained, some of the tested pathogens showed resistance to the extracts. *S. aureus*, *S. pneumoniae* and *M. luteus* were resistant to aqueous extract only while *S. epidermidis* was susceptible to it with minimum inhibitory concentration (MIC) of 10mm at 100mg/ml *as* seen in Table 1. The minimum inhibitory concentration ranged between 50mg/ml and 150mg/ml. The result also show that *M. luteus, S. epidermidis and S. pneumoniae* were susceptible to ethanol extract of the plant with *S.epidermidis* having the highest MIC as seen in Table 2. However, a different result was obtained when the organisms was tested with combinations of ethanol, aqueous and menthol. From Table 3, it can be seen that *S. aureus*, *S. epidermidis* and *M. luteus* are susceptible to ethanol extract and menthol. *S. aureus* and *M. luteus* became susceptible to aqueous extract in combination with menthol with 5mm and 8mm as MIC respectively. This can be attributed to synergistic effect of menthol on the extract. The combination extract was not effective against, *S. epidermidis and S. pneumoniae* as seen in Table 4. Many published reports showed the effectiveness of traditional herbs against pathogenic organisms (Del Campo et al., 2000; Mahesh and Satish, 2008). As a result, plants are one of the bedrocks for modern medicine. Many accomplishments have been achieved on medicinal research plan. *G. kola* as so far been described as one of the potential medicinal plants in this generation. Further study is required to ascertain the phytochemicals present in this plant and menthol respectively.

Table 1: Antimicrobial activity of aqueous extract.

TESTED PATHOGENS	ZONES OF INHIBITION (mm)					
	25mg/ml	50mg/ml	100mg/ml	150mg/ml	200mg/ml	MIC
S. aureus	-	-	-	-	-	-
S. pneumoniae	-	-	-	-	-	-
M. luteus	-	-	-	-	-	-
S. epidermidis	-	-	10	11	12	10

Antimicrobial activity of aqueous of the plant seeds were tested against selected pathogens. It was observed that only *S. epidermidis* susceptible to the extract at 100mg/ml, 150mg/ml and 200mg/ml respectively.

Table 2: Antimicrobial activity of ethanol extract.

TESTED PATHOGENS	ZONES OF INHIBITION (mm)					
	25mg/ml	50mg/ml	100mg/ml	150mg/ml	200mg/ml	MIC
S. aureus	-	-	-	-	-	-
S. pneumoniae	-	5	6	7	9	5
M. luteus	-	8	9	10	10	8
S. epidermidis	-	11	12	11	14	11

Ethanol extract inhibited growth of tested pathogens with larger zone of inhibition when compare to effect of aqueous extract

Table 3: Synergistic effect of menthol on ethanol extract.

TESTED PATHOGENS	ZONES OF INHIBITION (mm)					
	25 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	MIC
S. aureus	10	12	14	16	20	10
S. pneumoniae	-	-	-	-	-	-
M. luteus	12	14	17	20	24	12
S. epidermidis	10	9	10	12	14	10

Combination of menthol and ethanol showed a corresponding result. In comparison with ethanol extract only. Hence menthol has synergistic effect on the extract

Table 4: Synergistic effect of menthol on aqueous extract.

TESTED PATHOGENS	ZONES OF INHIBITION(mm)					
	20mg/ml	50mg/ml	100mg/ml	150mg/ml	200mg/ml	MIC
S. aureus	-	-	5	7	9	5
S. pneumoniae	-	-	-	-	-	-
M. luteus	-	-	8	9	10	-
S. epidermidis	-	-		-	-	-

In addition to synergistic effect of menthol on ethanol extract of the plant seeds, it also contributed to the efficacy of aqueous extract. Aqueous extract when applied singly, inhibited growth of only one pathogen while combination aqueous extract

and menthol inhibited growth of *S. aureus* and *M. luteus*. However, the combination did not inhibit growth of *S. pneumoniae* and *S. epidermidis*. The later pathogen was inhibited by aqueous extract.

CONCLUSION

Plants as source of antimicrobials have received attention from researchers worldwide. However, due to indiscriminate use of antibiotics, some of the discovered plant antimicrobials are becoming ineffective in the control of diseases. Various solvents such as water, ethanol, methanol, chloroform and phenol have been used to extract phytochemicals from plants. A combination of these extracts with other potential antimicrobials such as menthol, a soothing agent can bring about great difference in the effectiveness of these extracts against pathogens.

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