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Antimicrobial Activities of Bitter Kola (*Garcina Kola*) Extract on *Salmonella typhi*

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ABSTRACT

This research work was carried out to determine the antimicrobial activity of the extract of bitter kola (Garcinia kola) on *Salmonella typhi*. Different concentrations of plant extracts were obtained and disc diffusion assay was used to determine the antimicrobial activity of the plant extract against Salmonella typhi. The results revealed that the minimum inhibitory concentration of ethanol extract (150mg/ml) on the test organism was signficantly higher than that of aqueous extract (200mg/ml) respectively (p=0.009). Further observations showed that Minimum bactericidal concentration of' ethanol extracts (200mg/ml) was higher than that ofaqueous extract (250mg/ml). This study revealed the importance of this plant as a source of antimicrobial agents due to the increasing drug resistance among microorganisms. The extracts from the plant can therefore be considered by pharmaceutical industries in the production of affordable and easily accessible drugs for the cure of infections caused by the test organism.

Key words: Garcina kola; Antimicrobial; Salmonella typhi, Extract

INTRODUCTION

Typhoid fever is a bacterial infection of the intestinal tract and blood stream symptoms can be mild or severe and include sustained fever as high as 39◦C-40◦C, malaise, anorexia, headache, constipation or diarrhoea, rose coloured spot on the chest area and enlarged spleen and liver. Most people show symptoms 1-3 weeks after exposure. Typhoid is caused by the bacteria *S. typhi* growing in the intestine and Typhoid is spread by eating or drinking food or water contaminated with the faeces of an infected. The risk factors include poor sanitation and poor hygiene [1, 2].

In 2015, About 12.5 million new cases were recorded globally, with children having the highest prevalence. The diseases were reported to be responsible for over 149,000 deaths worldwide [1]. Typhoid can be treated with antibiotics, although resistance to these antibiotics has made treatment disease more difficult.

Garcina kola popularly known as bitter kola belongs to the family *Guttifarae*, it is a tropical plant widely distributed around West African countries including Nigeria [2]. The plant was named after *Garcin*, who lived and wrote about the plant in the 18th century [3]. The seeds are used as in folk medicine and herbal formulations. *Garcina kola* is reported to contain phytochemicals such as kolaviron, flavonoids, saponins, resins and tannins [4]. The extract is effectively against hepatitis, gram-negative and gram-positive bacteria.

The quest for new antimicrobial agents is closely linked with the problem of emergence of strains that are resistant to most synthetic antibiotics. It is therefore very necessary that the search for newer antibiotic sources be a continuous process. The aim of this research is to determine the antimicrobial activity of *Garcina kola* seed extract on *Salmonella typhi*.

MATERIALS AND METHODOLOGY

Collection of Samples

The medicinal plant *Garcina kola* was bought in the market from the bitter kola sellers. The plant was washed in water and allowed to dry for two weeks. Then ground using mortar and pestle and sieved using a mesh cloth.

Collection of Test Organism

The test organism was collected from the General Hospital Mubi as a pure culture and sub cultured in the school laboratory microbiology unit, the department of Biological Science Federal Polytechnic Mubi.

Extraction of Plant Material

The method of [5] as modified by [6] was used for ethanol and aqueous solvents extraction as described by [6].

Ethanol Extraction

12g of the grinded plant was soaked in 100ml of 100% ethanol for 24 hours at room temperature with occasional stirring. The content was filtered and was allowed to evaporate using a water bath at 78°C, the extract collected was stored in the refrigerator at 4°C

Aqueous extraction

12g of the grinded plant was soaked in l00ml of aqueous into a conical flask for 24 hours. The solution was filtered using a filter paper, the filtrate was evaporated to dryness using a water bath at l8°C then stored in a refrigerator at 4°C

Plant Extracts Disc Preparation

The plant extract disc was prepared from membrane filter paper by punching it, using a cork borer of 6mm in diameter. The disc was autoclaved at 121°C for 15 minutes. Different concentration of the plants extract was prepared as follows 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml, and 250mg/ml respectively.

For 50mg/ml, 0.5g of the extract was diluted with l0ml of distilled water to give the concentration of 50 mg/ml.

For 100mg/ml, 1g of the extract was diluted with 10ml of water to give the concentration of 100mg/ml.

For 150mg/ml, 1.5 g of the extract was diluted with l0ml of water to give the concentration of 150mg/m.

For 200mg/ml, 2g of the was diluted with l0mI of water to give the concentration of 200mg/ml

For 250mg/ml, 2.5g of the extract was diluted with l0ml of water to give the concentration of 250mg/ml.

The discs were added to the different concentrations allowed to absorb, then air dried at room temperature.

Culturing and Sensitivity Test

The test organism *Salmonella typhii* that was collected as a pure strain from (General Hospital , Mubi) and sub-cultured in the school laboratory of The Federal Polytechnic Mu hi, the department of Biological Science/Micro biology unit. The media was prepared by dissolving 28g of nutrient agar in 1 litre of water, autoclave at 121°C for 15 minutes in order to achieve sterilization. The media was poured into the petri dishes and was allowed to get set. The Inoculums of the sub-cultured test organisms was introduced into the petri dish using the streaking method. The prepared plant extract disc of different concentration was placed in the cultured plates (Disc Diffusion Method) using a sterile forceps, and then kept in the incubator for 24 hours at 37°c. After 24 hours the zone of inhibition was measured in millimeter using a meter rule. The minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] of the plant extract was determined from the concentration of the different plant extract against the test organism.

Statistical Analysis

Non parametic Mann Whitney test was used to analyse data obtained in the study.

RESULTS AND DISCUSSION

Table 1 revealed that ethanol extract of bitter Kola at 250mg/ml recorded the highest inhibition zone of 13.0mm which was followed by 200mg/ml with 12.0mm then 150mg/ml with l.0mm inhibition zone, 50 mg/ml had the least with 10.0mm inhibition zone followed by 100mg/ml which recorded 10.0mm inhibition zone. The results also revealed that Aqueous extracts of bitter kola at 250mg/ml recorded the highest inhibition zone of 9.0mm followed by 200mg/ml with 6.5mm then 150mg/ml with 4.0mm inhibition zone, it also revealed that at 50mg/ml and 100mg/ml there was no inhibition, furthermore, the results also showed the MIC and the MBC of both the ethanolic and aqueous extracts determined after inoculating the swab taken from the inhibition zone of the highest concentration of ethanolic extract which was recorded at the concentration of 150mg/ml with the inhibition zone of 11.0mm and then followed by 200mg/ml with the inhibition zone of 12.0mm, therefore the MIC for the ethanolic extract was found to be 150mg/ml since the swab inoculated on fresh nutrient agar medium yielded growth and the one from 200mg/mi yielded no growth which represent theMBC. Table 2 showed the MIC and MBC of aqueous extract determined after inoculating the swab taken from the inhibition zone of the higher concentration of 200mg/ml with the inhibition zone of 6.5mm then

followed by 250mg/ml, therefore the MIC for the aqueous extract was found to be 200mg/ml since the swab inoculated on fresh nutrient agar medium yielded growth and the one from 250mg/ml yielded no growth representing the MBC. Statistical analysis using Non parametic Mann Whitney test was used to determine if there was any significant between the two different extracts which showed that the antibacterial activity of ethanol extract is significantly higher than that of aqueous extract with the level of difference (P=0.009).

Table 1: the antimicrobial activity of different concentrations of ethanol and aqueous extract of bitter kola (G. kola) on *Salmonella typhii*

s/n	Concentration mg/ml	Ethanolic extracts inhibition zone (mm)	Aqueous extract inhibition zone (zone)
1.	50	10.0	-
2.	100	10.5	-
3.	150	11.0	4.0
4.	200	12.0	6.5
5	250	13.0	9.0

Key: (-) indicates no inhibition zone

Table 2: the MIC and MBC of ethanol and aqueous extract of G. kola on *Salmonella typhii*

	Ethanolic extract (mg/ml)	Aqueous extract (mg/ml)
MIC	150	200
MBC	200	250

The result of the study as shown in table 1; revealed that bitter kola extract of both ethanol and aqueous where able to inhibit the growth of *Salmonella typhii*. It was evident that ethanolic extract has more effect than that of the aqueous extract which is in line with the result of [7] which also revealed the antibacterial potency of the plant extract studied. On the solutions used for the extractions, the result for MIC and MBC in table2; revealed that ethanol was a better extraction agent than aqueous since the ethanolic extracts is more effective in producing zone of inhibition of 13.0mm against the organism Salmonella lyphi than the aqeuous extract and this also agree with the findings of some authors [8]. Therefore, the inlbition of the test organism by the extracts of bitter kola agrees with the claim by traditional practioners and other investigators that the seed and leaf of G. kola have antimicrobial activity and could be administered as a lòrm of therapy for the treatment of related diseases of the test organism. Further pharmacological evaluations, toxicological studies and possible isolation of the active therapeutic ingredients will be of

immense advantage in overcoming the menace of these bacterial diseases. The successful inhibition of these bacteria is a good development especially when we consider the records of multiresistance to various conventional antibiotics by bacteria over the years. The findings justify the traditional uses of this plant for therapeutic purposes.

CONCLUSION

From the research findings both ethanolic and aqueous extracts of bitter kola (Garcinia kola) prepared at different concentration were capable of inhibiting 'the growth of Salmonella lyphi which could be used as alternative to orthodox antibiotics. This work will also go a long way to reduce the challenges threatened by the antibiotics. This work will also go long way to reduce the challenges threatened by the antibiotics resistance exhibited by pathogenic microbial agent as well as other bacterial infection found in our country.

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