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EC EMERGENCY MEDICINE AND CRITICAL CARE
Research Article

Antimicrobial Effects of *Bryophyllum pinnatum* and *Garcinia kola* Juice against Selected Uropathogens

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Received: November 12, 2020; **Published:** February 27, 2021

Abstract

This study was aimed at determining the antimicrobial activities of *Garcinia kola* and *Bryophyllum pinnatum* juices on some selected uropathogens. The antimicrobial activities of the individual juice were tested and the synergistic activities of the combined neat juices of *Garcinia kola* and *Bryophyllum pinnatum*. The dilutions of the juices at concentrations of 75%, 50%, 25% and 10% were tested to determine their antimicrobial activities on the selected organisms: *Escherichia coli*, *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus* and *Candida albicans.* Agar well diffusion method was used on Muller-Hinton agar to check the susceptibility pattern. The juice of *Garcinia kola* showed the large zone of inhibition on *E. coli* with zone diameter of 22 mm at 100% concentration (neat), *Garcinia kola* was moderately sensitive to *K. pneumoniae*, *P. mirabilis* and *C. albicans* at the concentrations of 100% (neat) and 75%. *S. aureus* was resistance to *Garcinia kola* juice at all concentrations. *Bryophyllum pinnatum* showed antimicrobial activity against *E. coli*, *K. pneumoniae*, *P. mirabilis*, *S. aureus* but *Candida albicans* was resistant to it. The combination of *Bryophyllum pinnatum* and *Garcinia kola* juices showed some remarkable synergistic activities compared to when the juices were used individually. *Staphylococcus aureus* which was resistant to *Garcinia kola* and *Candida albicans* that was resistant to *Bryophyllum pinnatum* juice became susceptible to their combined juices at the concentrations of 75% and 100%.

Keywords*: Antimicrobial Activity; Garcinia kola; Bryophyllum pinnatum; Juice; Uropathogens*

Introduction

The high rate in antimicrobial resistant strains of clinical important pathogens have resulted in the existence of microorganisms with multi-resistant character, the unavailability and high cost of recent antimicrobial drugs with limited effective span have resulted in increased morbidity and mortality [1]. There is need to source for more alternative means with proven antimicrobial activities. Consequently, this has led to the search for more effective antimicrobial agents from materials of plant origin, with the hope to discover potentially useful active components that may serve as sources for the production of new antimicrobial drugs [2-4].

Bryophyllum pinnatum belongs to the plant family *Crassulaceae*. This is used in ethnomedicine for the treatment of many diseases such as ear-ache, cough, diarrhea, dysentery, abscesses, ulcers etc. [5,6]. In Southern Nigerian, *Bryophyllum pinnatum* is used to facilitate the dropping and healing of placenta wounds of the new born babies [6]. The leaves of *Bryophyllum pinnatum* contain bryophyllin, malate, potassium, ascorbic, citric acids and malic [6,7]. The plant is rich in macro and micro elements, vitamins, calcium, phosphorus, ascorbic acid, inulin [6,8]. Other compounds like saponin, flavonoids, anthraquinones, xanthones, bryophyllin A and B [6,9]. Anti-inflammatory,

hypoglycaemic, anti-diabetic and anti-cancer properties have been reported [5]. Antimicrobial activities of *Bryophyllum pinnatum* have been reported.

Garcinia kola is a species of flowering plant in the Clusiacea or Guttiferae family. It is used in most parts of West Africa as a social beverage and offered to guests as kola in many Nigerian cultural settings [10]. *Garcinia kola* is valued for its medicinal effects. It is used in traditional medicine for treatment of Laryngitis, general inflammation, bronchitis, viral infections and diabetes. It is also used as rejuvenating agents, adaptogen and general antidotes [10]. This study was conducted to determine the antimicrobial activities of the above named plant extracts and their synergistic effects on some selected uropathogens.

Materials and Methods

Source of materials

Fresh leaves of *Bryophyllum pinnatum* were obtained from Mgbuoduohia community, Rumuolumeni in Obio/Akpor Local Government Area of River State, Nigeria, while *Garcinia kola* were obtained from the tree source in Obiohia community in Omuma Local Government Area of Rivers State, Nigeria.

Preparation of juices

The fresh leaves of *Bryophyllum pinnatum* obtained from the plant were properly washed in clean running tap water and air dried. Then the leaves were squeezed to press out the fluid content, the greenish fluid were preserved in refrigerator in sterile bottle for further use. The brown covers of the *Garcinia kola* seeds were peeled using table knife, the seeds were chopped into smaller pieces. The pieces were further grinded using mechanical blender. The crushed *Garcinia kola* was placed in a fine sieve clot and sieved-squeezed to obtain sticky, milky fluid. The fluid was stored in sterile bottle in the refrigerator for subsequent uses.

Preparation of dilutions

Distilled water was used for dilutions in the preparation of different concentrations of the juices; 2 ml of working solution were prepared from the stock of each juice. 100% of the juices constitute the neat without dilution, 75% was prepared by adding 0.5 ml of distilled water to 1.5 ml of each juice from the neat, 50% dilutions of the working solution of each juice were prepared by adding 1 ml of distilled water to 1 ml of each juice; 25% made by 0.5 ml juice to 1.5 ml of distilled water and 10% dilutions were made by adding 0.2 ml of each juice to 1.8 ml of distilled water respectively.

Test organisms

Pure cultures of the uropathogens were obtained from microbiology laboratory of Braithwait Memorial Specialist Hospital. They were further subjected to chemical and biochemical tests for confirmation. The isolates were: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus and Candida albicans*.

Antimicrobial susceptibility testing: Agar well diffusion method was used for susceptibility testing of the extracts on the pathogens. The pure cultures were aseptically inoculated into bijou bottles containing 3 ml of peptone water. The inoculum was standardized by matching the turbidity with 0.5 McFarland standards. The suspensions were incubated at ambient temperature. Then the suspensions were poured onto the surface of Muller-Hinton agar in petri dish, which was immediately decanted after allowing it to spread through the surface of the Muller-Hinton agar by gentle rotation. Using sterilized cork borer with diameter of 10 mm, agar wells were made on the seeded Muller-Hinton agar plates at seven positions already marked on the back of the petri dish. Then 100 µl of the extracts were

dispensed into wells using micropipette. The first batch contains 100% of the extracts (neat) of *Garcinia kola* and, *Bryophyllum pinnatum* respectively; and combinations of *Garcinia kola* and *Bryophyllum pinnatum*. The plates were incubated in upright position, at temperature of 37ºC for 18 - 24 hours. The diameters of the zones of inhibition were measured in millimeter (10 mm is equivalent to zero) and the results were recorded. The processes were repeated for different concentration as follows: 75%, 50%, 25% and 10%. Each organism tested was seeded on a plate separately.

Result

The antimicrobial activities of *Garcinia kola* (bitter kola) and *Bryophyllum Pinnatum* (air plant) extracts on the uropathogens tested at concentrations 75% dilution and 100% (neat) were: *E. coli* 15 mm, 22 mm, *K. pueumoniae* 13 mm, 18 mm, *P. mirabilis* 12 mm, 16 mm, *S. aureus,* 10 mm, 10 mm and *Candida albicans* 14 mm, 19 mm respectively. The average mean ± standard deviation (Ẋ ± SD) in millimeter (mm) and zero inhibition zones were recorded as 10.00 mm which is equivalent to the well size.

Test Organisms	Concentration of Juices (%)					Ẋ ± SD
	10	25	50	75	100	
	Diameter of Inhibition Zone (Mm)					
E. coli	10.0	10.0	10.0	15.0	22.0	13.4 ± 5.273
K. pneumonia	10.0	10.0	10.0	13.0	18.0	12.2 ± 3.493
P. mirabilis	10.0	10.0	10.0	12	16	11.6 ± 2.608
S. aureus	10.0	10.0	10.0	10.0	10.0	10.0 ± 0.000
C. albicans	10.0	10.0	10.0	14.0	19.0	12.6 ± 3.975

Table 1: *The antimicrobial activities of Garcinia kola on some uropathogens.*

Bryophyllum pinnatum (air plant) juice demonstrated evidence of antimicrobial activities against the uropathogens tested, except *C. albicans* at the concentrations 75% and 100%. *E. coli* had inhibition zones of 16.00 mm 75%, 20.00 mm at 100% with Ẋ ± SD of 13.20 ± 4.60 mm, *K. pneumoniae* had inhibition zones of 18.00 mm at 75% and 26.00 mm at 100% with average Ẋ ± SD of 14.80 ± 7.16 mm, *P. mirabilis* had inhibition zones of 12.00 mm at 75% and 15.00 mm at 100% with Ẋ ± SD of 11.40 ± 7.64 mm. *S. aureus* had inhibition zones of 19.00 mm at 75% and 25.00 mm at 100% with Ẋ ± SD of 14.80 ± 6.91 mm respectively. *Candida albicans* was resistant to *Bryophyllum pinnatum* juice at all concentrations and no zone of inhibition was recorded at concentrations of 10%, 25% and 50%.

Test Organisms	Concentration of Juices (%)					Ẋ ± SD
	10	25	50	75	100	
	Diameter of Inhibition Zone (Mm)					
E. coli	10	10	10	16	20	13.2 ± 4.605
K. pneumonia	10	10	10	18	26	14.8 ± 7.155
P. mirabilis	10	10	10	12	15	11.4 ± 2.191
S. aureus	10	10	10	19	25	14.8 ± 6.907
C. albicans	10	10	10	10	10	10.0 ± 0.000

Table 2: *The antimicrobial activities of Bryophyllum pinnatum on some uropathogens.*

Combination of *G. kola* (bitter kola) and *B. pinnatum* (air plant) juices on *E. coli* 17 mm 75%, 24 mm neat; *K. pneumoniae* 19 mm 75%, 25 mm neat; *P. mirabilis* 20 mm 75%, 26 mm neat*; S. aureus* 13 mm 75%, 17 mm neat and *C. albicans* 15 mm 75%, 30 mm neat respec-

tively. Other concentrations (10%, 25% and 50%) did not show zones of inhibition to the tested uropathogens. The combination of these juices exhibited promising synergistic effects on some tested organisms. The organisms had the following inhibition mean of *E. coli*: 14.20 ± 6.26 mm, *K. pneumoniae*; 14.80 ± 6.91 mm, *P. mirabilis*; 15.20 ± 7.43 mm, *S. aureus*; 12.00 ± 3.08 mm, *C. albicans*; 11.40 ± 2.19 mm.

Test Organisms	Concentration of Extracts (%)					$\dot{X} \pm SD$
	10	25	50	75	100	
	Diameter of Inhibition Zone (mm)					
E. coli	10.0	10.0	10.0	17.0	24.0	14.2 ± 6.261
K. pneumonia	10.0	10.0	10.0	19.0	25.0	14.8 ± 6.907
P. mirabilis	10.0	10.0	10.0	20.0	26.0	15.2 ± 7.420
S. aureus	10.0	10.0	10.0	13.0	17.0	12.0 ± 3.082
C. albicans	10.0	10.0	10.0	15.0	30.0	11.4 ± 2.191

Table 3: *The antimicrobial synergistic activities of the combined extracts of Garcinia kola and Bryophyllum pinnatum on some uropathogens.*

Discussion

The result of this study had shown that the juices of *Garcinia kola* and *Bryophyllum pinnatum* had antimicrobial activities on the organisms tested against them, as well as the combination of the extracts. The extract of *Garcinia kola* showed the highest zone of inhibition against *E. coli* with zone diameter of 22 mm at 100% concentration (neat), this is in line with previous reports that *Garcinia kola* has good antimicrobial properties [9,11]. On the other hand, it is contradictory to the result given by Indabawa and Arzai [12], the difference in susceptibility may be as a result of different method of extraction of the extracts of *Garcinia kola* and preservation. *Garcinia kola* moderately inhibited *K. pneumonia*, *P. mirabilis*, and *C. albicans* at100% concentration (neat) and 75%. *S. aureus* was resistance to *Garcinia kola* extract at all concentrations, this is in line with a previous report that *Garcinia kola* did not show a significant activity against *Staphylococcus* species [13].

The lack of susceptibility of *Garcinia kola* to *Staphylococcus aureus* contradicts the previous report that alcohol extract of *Garcinia kola* was active against *Staphylococcus aureus* [12], the difference in the activity may as a result of the presence of alcohol used to extract the juice, which may have acted as an adjuvant to the active ingredient or the antimicrobial activity of alcohol.

Bryophyllum pinnatum extract showed inhibitory activity to all the organisms, tested against it, except *C. albicans* at 100% concentrations (neat) and 75%. It was active against *E. coli*, *K. pneumonia*, *P. mirabilis*, *S. aureus* but not *Candida albican*s (Table 2). This result correlates with previous report that there was no antimicrobial activity observed against *C. albicans* with extracts of *Bryophyllum pinnatum* and *Kalanchoe crenata* [4]. *Bryophyllum pinnatum* extract showed significant antimicrobial activities on some Gram positive and Gram negative organisms [4,8].

The combination of *Bryophyllum pinnatum* and *Garcinia kola* extracts showed some remarkable synergistic effect compared to the individual plant extracts. *Staphylococcus aureus* was resistant to *Garcinia kola* and *Candida albicans* was resistant to *Bryophyllum pinnatum* extract, but the combination of the two extracts showed remarkable zones of inhibition against *Staphylococcus aureus* and *Candida albicans* at the concentrations of 75% and neat extracts of both [14].

Conclusion

The extracts of *Garcinia kola* and *Bryophyllum pinnatum* have antimicrobial properties, the combination of *Garcinia kola* and *Bryophyllum pinnatum* exhibited synergistic effect on *Staphylococcus aureus* and *Candida albicans*. Hence these medicinal plant extracts are promising templates for production of future antimicrobial drugs.

Conflict of Interest

The authors have declared no conflict of interest.

Authors Contributions

Conception and design of the study; (NNS and ASE); collection, testing, data collation (NNS); analysis and interpretation of data (AUU); manuscript write up (AUU and NNS); oversight of all the stages of the research (ASE) and All authors read through and approved the final manuscript.

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