Vol. 14(11), pp. 579-582, November, 2020 DOI: 10.5897/JMPR2020.6958 Article Number: 92978C765111 ISSN 1996-0875 Copyright © 2020 Author(s) retain the copyright of this article http://www.academicjournals.org/JMPR



Journal of Medicinal Plants Research

Full Length Research Paper

Phytochemical screening and antimicrobial activity of methanol extract of *Garcinia Kola* Heckle fruit mesocarp

Ali Ibeabuchi Jude¹*, Adonu Cyril Chekwube² and Okorie Ndidiamaka Hannah¹

¹Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Enugu State Nigeria. ²Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology Enugu, Enugu State, Nigeria.

Received 6 April, 2020; Accepted 14 October, 2020

The phytochemical screening and antimicrobial activities of the methanol extract of *Garcinia* kola fruit mesocarp were investigated against four bacteria and two fungi in the study. The extract was investigated for the presence of phytochemicals using standard phytochemical screening methods. The results revealed the presence of flavonoids, tannins, alkaloids, terpenoids, cardiac glycosides, and reducing sugar. The percentage of alkaloids, flavonoids and tannins are 24, 27.5 and 4%, respectively. The extract exhibited effective antimicrobial activity against the test organisms. The minimum inhibitory concentration ranged from 275.4 to 691 μ /ml for bacteria and 346.7 to 318.2 μ /ml for fungi respectively compared to chloramphenicol and ketoconazole [standards drugs] which exhibited a minimum inhibitory concentrations (MIC) of 14.31-31.62 and 2.66-2.99 μ g/ml. These findings suggest that the fruit mesocarp of *G. kola* could be a potential source of compounds used against microorganism infection.

Key words: Phytochemical screening, antimicrobial activity, Garcinia kola heckle fruit extract.

INTRODUCTION

Nigeria is blessed with large number of diverse types of medicinal plants grown in different parts of the country. Medicinal plants are generally known to produce certain bioactive compounds used against microbial invasion and play an important role in protecting human diseases (Muhammadi et al., 2018). In recent time, there is growing global concern on the rising cost of buying synthetic drugs, assessing their toxicological profile and their periodic side effect and unstable efficacy (Gupta et al., 2016). These anomalies lead researchers to explore alternative source of drug from medicinal plants with little or no side effect in treatment of microbial infections.

Garcinia kola heckle belongs to the family Guittiferae. It is a dicotyledonous plant found in moist rain forests and swamps and grows as a medium sized tree up to a height of about 12 m high (Burkill, 1985). The bitter kola plant is found in Nigeria and other western Africa countries. Across the places where it grows it is known by various names such as bitter kola, in English, Orogbo in Yoruba, Aku-ilu in Igbo and Namijingoro in Hausa. Traditionally, the seeds and bark are used in folklore remedies for treatment of gastric and liver disorders. The seeds are

*Corresponding author. E-mail: ibeabuchi.ali@esut.edu.ng. Tel: 08064535760.

Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> chewed to suppress headaches, laryngitis, bronchitis, malaria, and gonorrhea (Iwu, 1993; Anna et al., 2019), while dried ground kernels can be mixed with honey to prepare a traditional paste against cough. The plant exhibits very potent pharmacological activities such as antioxidant, antibacterial, antiviral, antifungal and antiinflammatory properties (Adegboye et al., 2008). The antimicrobial potential of bitter kola is based mainly on the phytochemical components of the plant: tannins, saponins, alkaloids, cardiac glycosides, terpenonids and flavonoids (Ibedu et al., 2018). The phytocompounds can be derived from leaves, stem bark, roots, fruits, seeds and flowers. As a result of their versatile application, Garcinia kola plants have potentials as the richest source of drugs in traditional and modern medicine. The aim of the study is to carry out the phytochemical screening and antimicrobial activities of methanol extract of Garcinia kola heckle fruit mesocarp.

MATERIALS AND METHODS

The ripped fruits of *Garcinia* kola were harvested in the bush at Owerreze Orba in Udenu Local Government Area on geographical coordinates: 6° 51' 0" N, 7° 27'0" E, Nsukka, Enugu State, Nigeria, in August 2014. The ripped fruits of *G. kola* were authenticated by Mr. Ozioko Alfred of the herbarium Department of the International Centre for Ethnomedicine and Drug Development, 110 Aku Road, Nsukka, Enugu State.

Plant sample treatment

The ripped fruits were peeled off and bisected longitudinally to extricate the seeds. The fresh mesocarp was sliced into smaller size, sun dried at room temperature for 14 days. The dried mesocarp was pulverized using electric blender and made ready for extraction.

Preparation of the extract

The extraction was done successfully using cold maceration method. A 600 g of the pulverized sample was correctly weighed and transferred into a plastic container with lid. A 2.5 L of 100 % methanol was poured into the container and made air tight with the lid. The contents were agitated intermittently and allowed to be extracted for 72 h at room temperature. The extract was filtered first using cotton wool then with Whatman number 1 filter paper to obtain a clear extract. The extract was concentrated using rotary evaporator under pressure to afford methanol extract.

Phytochemical screening of methanol crude extract

The phytochemical screening was carried out by standard phytochemical methods (Soforowa, 1993; Trease and Evans, 1989) for testing phytochemical compounds.

Quantitative phytochemical determination

The quantitative determination of alkaloids was determined using the alkaline precipitation gravimetric method (Harborne, 1998); flavonoids by the Ethyl acetate precipitation method (Bohm and Kocipai, 1994) and tannins by the Follins-Dennis spectrophotometric method (Peason, 1974).

Test microorganism

The microorganisms were collected from the clinical isolate of bacteria and fungi obtained from the stock organism in the Department of Pharmaceutics Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. A total of four bacterial cultures: one gram positive (*Staphylococcus aureus*) and three gram negative (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia*) and two fungi cultures (*Candida albicans* and *Aspergillus niger*) were used in this study.

Antimicrobial evaluation

The antimicrobial assay was performed using the agar well diffusion method (Ali et al., 2017). The nutrient agar was prepared according to manufacturer specifications, then distributed in 20 ml portions using MacArthucy bottle capped with stopper. It was sterilized using autoclave at 121°C for 15 min. A 0.1 ml of 24h broth culture of the test organism suspension was aseptically transferred into Petridishes and thereafter covered with 20 ml molten nutrient agar mixed thoroughly and allowed to solidify. After solidification, 5 wells were made in the seeded plates using cork-borer. From the prepared concentrations of the extract 12500-781.2 µg/ml, a 0.2 ml of each was transferred into the wells using a micropipette. A prediffusion time of 1h was allowed before incubating at 37 and 25°C for bacteria and fungi (Muhammadi et al., 2018) respectively. Likewise controls drugs: chloramphenicol of concentration 6250-10000 µg/ml and ketoconazole of concentration 6250-10000 µg/ml were carried out respectively. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of extract against the square of inhibition zones diameter (Vincent, 2005). The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC values (Vincent, 2005).

RESULTS

The result of the qualitative phytochemical analysis of the extract revealed the presence of alkaloids, flavonoids, cardiac glycosides, terpenoids, tannins and reducing sugar similar to previous reports on the phytoconstituents of sembark and seeds (Muhammadi et al., 2018; Okwulehie et al., 2017; Nnaji et al., 2017) as shown in Table 1. Table 2 shows the results of the quantitative determination of some phytocompounds as 24% alkaloids, 27.5% flavonoids and 4% tannins. The result is in agreement with previous researcher that quantifies the same phytocompounds in the seed of the plant¹⁷. This indicates that just like other parts of the plant the fruit mesocarp is loaded with phytocompounds with potential therapeutic properties.

Result of antimicrobial evaluation

The test micro-organisms showed susceptibility at different concentrations of the extract indicating the

Table 1. Qualitative phytochemical screening of methanol extract.

Phytocompounds	Alkaloids	Flavonoids	Terpenoids	Reducing sugar	Tannins	Cardiac glycosides
Result	+++	+++	++	++	+	+

+++, more abundant; ++ moderate abundant; + least abundant.

Table 2. Percentage quantitative determination of some phytoconstituents.

Phytocompounds	Flavonoids	Alkaloids	Tannins
% yield	27	24	4

Table 3. MICs exhibited by the extract and control drugs against test organisms.

Microorganisms	Crude extract (μg/ml)	Chloroamphenicol (µg/ml)	Ketoconazole (µg/ml)
E. coli	275.4	14.13	-
P. aeruginosa	691.8	31.62	-
K. pneumonia	630.9	31.62	-
S. aureus	316.2	22.39	-
C. albicans	346.7	-	2.66
A. nigger	318.2	-	2.99

-, not tested

potential of the extract as putative drug for infectious diseases. This corroborates with antimicrobial activity of seed extract as previously reported (lbedu et al., 2018; Okwulehie et al., 2017; Muhammadi et al., 2018). The MICs of the methanol extract ranged from 275.4-891.8 and 346.7-318.2 µg/ml for the test organism respectively. On the other hand, the MIC of the control drugs varied from 14.13-31.62 and 2.66-2.99 µg/ml for chloroamphenicol and ketoconazole respectively as shown in Table 3.

DISCUSSION

The phytochemical screening and antimicrobial activities of methanol extract of *Garcinia* kola heckle fruit mesocarp were investigated. The methanol extract of dried fruit mesocarp of *Garcinia* kola exhibited antimicrobial activities against test organisms. The observed antibacterial activities were more on *E. coli* and *S. aureus* than *P. aeruginosa* and *K. pneumonia*. This suggests that the plant can be used to treat diseases caused by *E. coli* and *S. aureus*. The impact of antifungal activities on C. *albicans* and A. *niger* are relatively similar. Several other researchers have attributed the efficacy of *G. kola* parts to the presence of phytochemical constituents in line with the present findings. This accounts for the antimicrobial activity against bacteria and fungi. Tannins are metal chelators and can form complexes with macro molecules. Through this process essential substrates co-factor and enzymes of microorganism are depleted leading to cell death (Nnaji et al., 2017). The typical astringent taste of Garcinia kola seed and fruit is caused by tannins, known for their natural treatment of intestinal disorders such as diarrhea and dysentery. Flavonoids which are present in Garcinia kola fruit mesocarp exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, and thus improving health (Ferguson, 2001). Flavonoids also exhibit anti-inflammatory, anti-allergic effects, analgesic and antioxidant properties (Hodek et al., 2002). The antimicrobial properties of Garcinia kola are attributed to benzophenones and flavanones which consist of biflavanones GB1, GB2, and kolaflavanone (Tchimene et al., 2015). Therefore, the plant extract has antimicrobial properties needed for future development of potent antimicrobial drugs with minimal or no adverse effect.

Conclusion

The present study has shown that *Garcinia kola* heckle fruit extract contains promising antimicrobial agent. Therefore, the use of the plant's part as antimicrobial agent in folklore has been authenticated. However, there is need to isolate and characterize the active phytoconstituent of the plant's part.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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