JJEES

JJEES (2019) 10 (4): 252-255
ISSN 1995-6681

Jordan Journal of Earth and Environmental Sciences

# Antimicrobial Activity of Momordica cochinchinensis Seeds and Seeds Aril Extract

Qays Alkhafaji[1], Iqbal Mohammed[2], Shurook Saadedin[3], Salwa Al-Awadei[4], Taif Abedulhussein[3], Ghada AL-Jussani[5]

[1]Kamal al-Samarrai hospital, Fertility Center, infertility treatment and IVF, Iraq
[2]Ministry of Agriculture, office –Horticulture, Iraq
[3]University of Baghdad, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Iraq
[4]University of Al-Nahrain, College of Applied Biotechnology, Iraq
[5]The Hashemite University, Faculty of Medicine, Jordan

Received 16 July 2019; Accepted 2 September 2019

**Abstract**

In attempt to investigate the antimicrobial activity of the Gac fruit (Momordica cochinchinensis) seeds' aril, seed extract, and crude seed aril oil against some gram-positive and gram-negative bacteria using microdilution assay, seed arils were extracted using ethanol\ethylacetate (6:4), aqueous extraction of the seeds and crude seed aril oil were used. This study shows that the extract of seed arils exhibited the best antimicrobial activity. The minimum inhibitory concentration (MIC) of the ethanol\ethylacetate ranges between 0.391 and 3.125mg/ml. The extract showed significant inhibition against all bacteria Bacillus subtilis; Staphylococcus aureus; Micrococcus luteus; Klebsiella pneumonia, and Enterobacter aerogenes. The maximum antibacterial activities were observed against S. aureus by the seed aril extract (0.391 mg/ml), while the crude oil showed no antimicrobial activity in this study. The seed aqueous extract showed inhibition against S. aureus only with the concentration of (12.5 mg/ml). Therefore, the results of this study show that the antibacterial activity of the Gac fruit seed aril extracts can be beneficial for the development of alternative antibacterial sources.



**Keywords:** Antimicrobial activity, G+ bacteria, G- bacteria, Carotenoids, (Momordica cochinchinensis), Microdilution assay.

## 1. Introduction

Momordica cochinchinensis Spreng (gac) from the Cucurbitaceae family is commonly called "Gac fruit" which is an edible plant used as herbal medicine. This plant grows in tropical Asian countries, including Vietnam, Laos, Thailand, China, Bangladesh, and India (Kubola and Siriamornpun, 2011; Kubola et al., 2013). Fruits of M. cochinchinensis are big, denselyaculaeate, green in color and when ripe, become dark orange or red. It is composed of two main parts: a mesocarp and an endocarp. The mesocarp, which makes up nearly 50% of the weight of the fruit, is 1/2" thick, spongy, and orange in color. The endocarp is composed of the soft and sticky arils, with the thickness of about 1-3 mm. This part usually covers the black seed and accounts for around 25% of the fruit weight (Nguyen, 2014; Vuong, 2000).

The Gac plant is high in β-carotene and lycopene, and can be cultivated from seeds or root tubers. It grows as devious vines, and the fruit is a rich nutrient source (Tinrat, et al., 2014).

In recent years, antimicrobial resistance has become a major global problem (Raghunath, 2008). Posing an ever-increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. Among the factors contributing to microbial resistance are the indiscriminate use of antimicrobial agents by both healthcare professionals and patients. Therefore, there is a need for developing new antimicrobials drugs ( Okeke, et al. 1999; Mourad, et al., 1993). Scientific evidence supports the hypothesis that plants contain bioactive compounds with medical applications. Recent advancements in drug discanaloovery is based on designing compounds that are analogous to natural plant compounds. (Ghosh, et al., 2012; Begum, et al., 2012; Abreu, et al., 2012).

Innuna (2013) found that the seed aril ethanolic, ether, and aqueous extract had no effect on S. aureus, while the ethanolic extract had a high inhabitation effect on Micrococcus luteus, (Innuna, 2013).

Tinrat et al. (2014) found that E. coli and P. aerginosa were the most susceptible to seed aril ethanoic extracts with a MIC value of 3.125 mg/ ml, while the same extract had a higher MIC value of 6.25 mg/ ml against S. aureus .

Tinrat and Sila-Asna, (2016) studied seed arils' different extracts and oil and found that the hexane extract was significantly better than the acetone and the methanolic extracts with a MIC value of 1.56- 12.50 mg/ml, MIC was 3.125 mg/ml for S. aureus and 1.56 mg/ml for K. pneumoniae, while the MIC for the oil was 100 mg/ml for S. aureus, and there was no effect on K. pneumoniae (Tinrat and Sila-Asna, 2016).

The fruit of M. cochinchinensis contains various bioactive compounds including flavonoids such as rutin, myricetin, luteolin, quercetin, Apigenin, and kaempferol (Raghunath, 2008). carotenoids such as α, β-carotene,zeaxanin, lycopene, lutein, and phenolic compounds such as gallic acid, vanillic acid, ferulic acid, caffeic acid, and proto catechuic acid . The

* Corresponding author e-mail: ghadahmed2000@gmail.com

aim of this study is to evaluate the antibacterial activity of seed arils, seed extract, and crude seed aril oil of the Gac fruit using microdilution assay.

## 2. Materials and Methods

### *2.1 Preparation of Plant Extract*

*2.1.1 Plant Materials:*

The Momordica cochinchinensis seed aril oil with 0.4% lycopene. The Gac seeds were imported from Sabiwe Vietnam Co., Ltd- Vietnam. Seed arils from the Gac fruit were cultivated in Iraq by the researchers.

*2.1.2 Materials Extraction*

The seeds of Gac fruit were ground into a fine powder. Then, 50 g were weighed. The powder was boiled in one liter of water for one hour. The extract was filtrated, and the aforementioned step was repeated twice with the residue. All of the filtrated collection was mixed together. The extract was dried to powder using the Minispray Dryer B-290\ Switzerland modified method.

The seeds' aril was prepared and extracted with Ethanol: Ethyl acetate (6:4v/v) according to the procedures of Saadedin (Saadedin, et al., 2017).

### *2.2 Test Microorganisms*

The antimicrobial activity of the Gac fruit extracts were tested against five bacterial strains (B.subtilis ATCC 6633, S. aureus ATCC 25923, M. luteus ATCC 10240, E. aerogenes ATCC 13048, and K. pneumoniae ATCC 31488).

### *2.3 Preparation of the Inoculum:*

Stock bacterial cultures were maintained at room temperature for two hours. Each strain was streaked on a Müller-Hinton agar plate, and incubated at 37 ºC for twenty-four hours. The inoculum was prepared by emulsifying a minimum of three colonies from those plates in sterile 0.9% NaCl (w/v) till $10^8$ colony forming units (CFU) per ml (0.5 McFarland scale) are formed. For the agar dilution assays, the suspensions were diluted with sterile 0.9% NaCl (w/v) until they reach $10^7$ CFU per ml (working bacterial suspensions) (National Committee for Clinical Laboratory Standards, 2000).

### *2.4 Antibiotics*

The antibiotics were prepared by dissolving standard antibacterial powders of tetracycline from Sigma and vancomycine from Lilly in dimethylsulphoxide (DMSO) (Merck) (Tinrat, 2014).

### *2.5 Sterilization*

The Gac fruit extracts and antibiotics were filtered through a 0.22-l µm membrane filter before use for sterilization (Sousa, et al., 2007).

### *2.6 Minimum Inhibitory Concentration (MIC) Determination*

The antibacterial activity of different parts of the Gac friut extracts was studied using a microdilution method according to the method of (Tinrat, 2014), and MIC values were determined for the bacterial strains. The extracts were dissolved in 10% DMSO, and diluted with a culture broth to a concentration of 100 mg/ml. Further two-fold serial dilutions were performed by the addition of a culture broth to reach the required concentrations18. Ten dilutions (100 - 0.195 mg/ml) were prepared from each extract in test tubes. Then, 0.5 ml of a bacterial suspension ($10^7$ CFU/mL) of the pathogenic strains was added to each concentration of the extracts. The same tests were done at the same time for growth control (Nutrient Broth + inoculums) and sterility control (Nutrient Broth + test sample) and DMSO.

100 µl of each dilution was distributed in 96-well plates, as well as a sterility control and a growth control and antibiotics. All experiments were performed in triplicate and the microdilution 96-well plates were incubated at 37ºC for twenty-four hours. The well with the least concentration of the extract that completely inhibits the growth after twenty-four hours of incubation was recorded as the MIC. The growth of pathogenic strains was identified by optical density (ELISA reader, CLX800-BioTek Instruments). The results were expressed in (mg/ml).

## 3. Results and Discussion

### *3.1 Antimicrobial Activities of Plant Extracts by MIC*

The antimicrobial activities of the Ethanol\ethyl acetate extracts of M. cochinchinensis against five bacterial species were evaluated by observing the microdilution method. The antimicrobial activities of the extracts are shown in Table 1. It was found that all Ethanol\ethyl acetate extracts showed antimicrobial activity against all five bacterial strains with MIC values varying from 0.391 to 3.125 mg/ml . Among the gram-positive ones, the ethanol\ethyl acetate extract of aril had the lowest MIC value (0.391mg/mL) against S. aureus ATCC 25923, and among the gram- negative ones, the lowest MIC value was (1.563mg/mL) against K. pneumoniae ATCC 31488. On the basis of MIC values, S. aureus ATCC 25923, B. subtilis ATCC 6633, M. luteus ATCC 10240 were more sensitive to the extract than E. aerogenes ATCC 13048, K. pneumoniae ATCC 31488. The seed water extract had no microbicidal activity against all pathogenic strains except S. aureus ATCC 25923 with MIC values of 12.5 mg/ml. The MIC values for tetracycline were found at a concentration of 2.0-3.0µg/ml, while that of vancomycine was between 3.125 and 11.12 µg/ml.

**Table 1.** MIC of (Momordica cochinchinensis) extracts against tested microorganisms by microdilution assay.

| Momordica cochinchinensis | Solvent Fraction | | MIC (mg/ml) | | | |
|---|---|---|---|---|---|---|
| | | *Bs+* | *Sa+* | *Ml+* | *Ea-* | *Kv -* |
| **Seeds' aril** | Ethanol\ethylacetate(6:4) | 0.781 | 0.391 | 0.781 | 3.125 | 1.563 |
| **Seed** | aqueous | No effect | 12.5 | No effect | No effect | No effect |
| **Seeds' aril oil** | Crude | No effect | No effect | No effect | No effect | No effect |
| **Tetracycline** | - | 2.32 | 2.0 | 2.34 | 2.71 | 3.0 |
| **vancomycine** | - | 7.89 | 3.125 | 8.01 | 11.12 | 7.58 |

*(B s= Bacillus subtilis); (S a= Staphylococcus aureus); ( Ml= Micrococcus luteus); (Kv= Klebsiella pneumoniae) and (Ea) Enterobacter aerogene*

A comparison of the susceptibility of the extracts towards bacterial strains, Figure (1), shows that S. aureus appeared to be more susceptible to aril extracts than the other strains tested. The different cell-wall composition of each species may be responsible for the different antimicrobial susceptibilities. The cell wall of S. aureus (gram positive bacteria) is composed of peptidoglycan layers combined with the teichoic acid molecules. In gram-negative cell wall, the peptidoglycan layer is much thinner, and there is no teichoic acid. Moreover, an outer membrane closely overlies the peptidoglycan layer so that the membrane and layer comprise the cell wall (Alcamo, 2001).

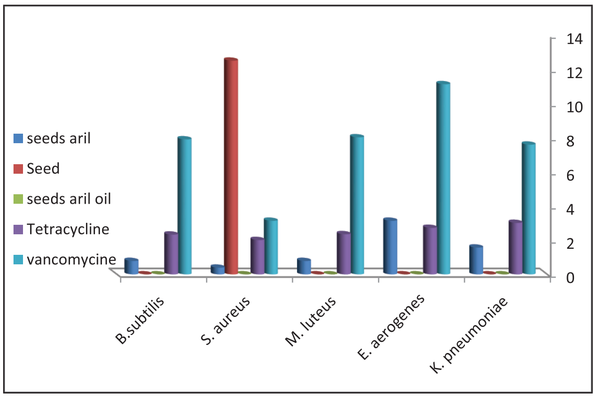


**Figure 1.** Comparison of Antimicrobial Activities of seeds' aril, aqueous seed extract, crude oil and antibiotics against different microorganisms. MIC values varying from 0.391 to 3.125 mg/ml for the plant extract and 2.0-3.0μg/ml, for tetracycline, while that of vancomycine was 3.125 to 11.12 μg/ml.

The data of this study clearly show that the Gac seed aril extract had antibacterial activity against both types of bacteria. The MIC values show that the antibacterial activity depends on the extract concentration. The ethanol\ethyl acetate extracts may contain effective compounds such as asluteolin, quercetin, apigenin, and kaempferol [14] which showed strong antibacterial properties against a broad range of pathogenic strains[13]. This finding comes in agreement with other studies showing that the ethanolic extract from other species of Cucurbitaceae has antimicrobial activities (Tang, et al., 2010; Badmanaban and Patel, 2009; Bhattacharya, et al., 2010; Kumar and Kammaraj, 2010).

## 4. Conclusions

The antibacterial activity of different extracts of Momordica cochinchinensis Spreng. (Gac fruit) was evaluated using the minimum inhibitory concentration (MIC) method. The seed aril extraction with ethanol\ ethyl acetate showed good activity against the five tested pathogenic strains, including gram-positive and gram-negative bacteria. Staphylococcus aureus ATCC 25923 had the most susceptibility to this extract, while there was no effect of the seed aril crude oil. The aqueous seed extract showed activity against Staphylococcus aureus ATCC25923 only. On the basis of these data, and the data of other researchers, it becomes clear that the activity of the extracts largely depends on the type of the solvent used. This study supports the idea that the Momordica cochinchinensis fruit is likely to be a new antimicrobial source for the pharmaceutical industry.

**Acknowledgement**

In this work, the authors would like to thank the Hamdi Mango Center for Scientific Research (HMCSR)/ Department of Cell Line and Culture, University of Jordan for their assistance, guidance, and hosting the research activities within their facilities.

## References

Abreu, A.C., McBain, A.J., Simoes, M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents, Nat. Prod. Rep., 29: 1007–1021.

Alcamo, I.E. (2001). Fundamentals of microbiology. Jones and Bartlett Publishers, Inc., New York, 6th edition.

Badmanaban, R., and Patel, C.N. (2009). Study on anthelmintic and antibacterial activity of the leaf extracts of Lagenaria siceraria. Journal of Global Pharma Technology, 2 (4): 66-70.

Begum, S., Naqvi, S.Q.Z., Ahmed, A., Tauseef, S., Siddiqui, B.S. (2012). Antimycobacterial and antioxidant activities of reserpine and its derivatives, Nat. Prod. Res., 26: 2084–2088.

Bhattacharya, B., Samanta, M. Pal P., Chakraborty, S., Samanta, A. (2010). In vitro evaluation of antifungal and antibacterial activities of the plant Coccinia grandis (L.) voigt (Family-Cucurbitaceae). Journal of Phytology. 2: 52-57.

Ghosh, S., Chisti, Y., Banerjee, U.C. (2012). Production of shikimic acid. Biotech. Advances, 30: 1425–1431.

Innuna, A. (2013) Antimicrobial activity of Gac (Momordica cochinchinensis), Proceeding, Science and Engineering, (2013) 1-6.

Kubola, J., and Siriamornpun, S. (2011). Phytochemical and antioxidant activity of different fruit fraction (Peel, pulp, aril and seed) of thai gac (Momordica cochinchinensis spreng). Food Chemistry. 127(3): 1138-1145.

Kubola, J., Meeso, N., Siriamornpun, S. (2013). Lycopene and beta carotene concentration in aril oil of gac (Momordica cochinchinensis Spreng) as influenced by aril-drying process and solvents extraction Food Research International. 50: 664-669.

Kumar, S.S., and Kammaraj, M. (2010). Analysis of phytochemical constituents and antibacterial activities of Cucumis anguri L. against clinical pathogens. American-Eurasian Journal of Agricultural and Environmental Sciences, 7: 176-178.

Mourad, A.S., Metwally, M., Nour, E.L., Deen, A., Threlfall, E.J., Rowe, B., Mapes T., Hedstrom, R., Bourgeois, A.L., Murphy, J.R. (1993). Multiple-drug resistant Salmonella typhi. Clin and Infect Dis., 17(1):135-136.

National Committee for Clinical Laboratory Standards (2000). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard Fifth Edition. NCCLS document M7-A5. NCCLS: Wayne, PA, USA.

Nguyen, P.M. (2014). Investigation the ratios of antioxidant supplementation into the mixture of GAC (Momordica Cochinchinensis spreng) and carrier to get the highest total carotenoid content during drying. International Journal of Multidisciplinary Research and Development, 1(3): 34-40

Okeke, I.N., Lamikaure, A., Edelman, R. (1999). Socioeconomic and Behavioural Factors Leading to Acquired Bacterial Resistance to Antibiotics in Developing Countries. EmergInfect Dis., 5(1): 1-9

Raghunath, D. (2008). Emerging antibiotic resistance in bacteria with special reference to India. J Biosci., 33(4): 593-603.

Saadedin, S.M. K., Mohammed, I.H., Abdullah, S.J. (2017). Solvents extraction efficiency for lycopene and β-carotene of GAC fruit (Momordica cochinchinensis, Spreng) cultivated in Iraq. Bioscience Research, 14(4): 788-800.

Sousa, M., Ousingsawat, J., Seitz, R., Puntheeranurak, S., Regalado, A., Schmidt, A. (2007). An extract from the medicinal plant Phyllanthus acidus and its isolated compounds induce airway chloride secretion: a potential treatment for cystic fibrosis. Mol Pharmacol, 71: 366-76.

Tang, J., Meng, X. , Liu, H., Zhao, J., Zhou, L., Qiu, M., Zhang, X., Yu, Z., Yang, F. (2010). Antibacterial activity of sphingolipids isolated from the stems of cucumber (Cucumis sativus L.). Molecules, 15: 9288-9297.

Tinrat, S. (2014) Comparision of antioxidant and Antimicrobial activities of unripe and ripe fruit extracts of Momordica cochinchinensis Spreng (Gac fruit). Int. J. Pharm. Sci. Rev. Res., 28(1): 75-82.

Tinrat, S., Akkarachaneeyakorn, S., Singhapol, C. (2014). Evaluation of antioxidant and antimicrobial activities of Momordica Cochinchinensis Spreng (Gac fruit) ethanolic extract. International Journal of Pharmaceutical Sciences and Research. 5(8): 3163-3169.

Tinrat, S., and Sila-Asna, M. (2016) Antimicrobial and synergistic effects with antibiotics of Momordica cochinchinensis Spreng (Gac fruit) aril against pathogenic bacteria, Int. J. Pharm. Sci. Rev. Res., 39(2): 286-294

Vuong, L.T. (2000). Underutilized β-carotene-rich crops of Vietnam. Food Nut.Bull., 21: 173-181.