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EFFICACY OF *AZADIRACHTA INDICA* AND *MOMORDICA CHARANTIA* AGAINST CLINICALLY IMPORTANT PATHOGENS

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Abstract – Microorganism have developed resistance against various antibacterial drugs, and to overcome this alarming situation medicinal plants are studied as the possible alternatives for the currently used antibiotics. Ethanolic extracts from the dried fruit of *Momordica charantia* and dried leaves of *Azadirachta indica* were tested against seventeen clinically important pathogens that cause various infections. Gram-positive bacteria (57%) that were tested in this study were found to be more susceptible compared to Gram-negative bacteria as all of the strains gave a promising zone of inhibition against the plant extracts as measured in the diameter of the zone of inhibition. The exception was *P. aeruginosa* which showed high susceptibility against both extracts. The results showed that these plants exhibit significant antimicrobial activity against Gram positive bacteria tested and *P. aeruginosa*. The resistance testing was also as promising as these bacterial isolates did not develop resistance towards the plant extracts in this assay. Thus, these plants have high potential to be developed as suitable alternatives to antibiotics to avoid the spread of resistance.

INTRODUCTION

Medicinal plants have been used therapeutically for centuries and are source of many potent and powerful drugs (Srivastava *et al.,* 1996). The World Health Organization (WHO) has stated that 80% of the community especially from developed countries use plant extracts as traditional medicine to provide continuous health treatment (Farnsworth *et al.,* 1985). In addition, WHO has approved the use of eight medicinal plants for use in Brazil (Palhares *et al.,* 2015). The main reason of using medicinal plants for therapeutic purposes is because conventional drugs have high content of chemicals and also indirectly due to misuse and overuse of antibiotics, as reported by Kazemipoor *et al.* (2012).

The need to find alternative agents with antibacterial activity has resulted in considerable research to obtain effective, safe and economical therapeutic agents. In addition, plant-based therapeutics are harmless to the environment, biodegradable, and are less expensive (Fullerton *et al.,* 2011). *Momordica charantia* and *Azadirachta indica* are economically important medicinal plants as they are used as traditional medicine to treat diabetes. It has been reported that these plants exhibit diverse biological activity such as antimicrobial, anti-hepatotoxic, antioxidant, antiviral and anti-ulcerogenic that could be attributes to an array of biologically active plant chemicals which are steroids, proteins and triterpenes (Grover and Yadav, 2004). *Momordica charantia* which belongs to the family *Cucurbitacae*, has been found to be rich in momordicin, tanins and glycosides, in both the leaves and fruits (de Oliveira *et al.,* 2018). The leaves of *Azadirachta indica*, have been shown to contain high levels alkaloids, flavonoids, and glycosides which contribute to significant antimicrobial activity (Dash *et al.,* 2017). Since plant extracts, in general, have shown some antimicrobial effects, the therapeutic properties of these compounds could have a significant effect on clinically important bacteria. Furthermore, the presence of efflux pump inhibitors in plant extracts could significantly contribute to the antibacterial effects of certain plants (Rao *et al.,* 2018).

In this study, we determined the antimicrobial activity of the dried fruit and leaf extracts of *Momordica charantia* and *Azadiratcha indica* respectively against clinically important bacterial strains which cause infectious diseases, including *Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Enterococcus faecalis, Citrobacter freundii, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Norcardia* sp., usingthe agar well diffusion method. The ability of these bacterial isolates to develop resistance against *Momordica charantia* plant extracts were also studied in this experiment.

MATERIALS AND METHODS

Preparation of bacterial cultures

Pure cultures of the seventeen bacteria tested were streaked onto different nutrient agar plates. These bacteria were obtained from the culture collection of INTI International University. The nutrient agar plates streaked with *E. coli, P. aeruginosa, P. vulgaris, K. pneumonia, P. mirabilis, Norcardia* sp., *S. aureus, E. faecalis, E. aerogenes, E. cloacae, C. freundii* and *B. subtilis* were incubated aerobically in an inverted position for 24 hours at 37 °C. The nutrient agar plates streaked with *S. aureus, S. epidermidis, P. acnes, S. pneumonia, K. pneumonia* and *P. vulgaris* were on the other hand, incubated anaerobically in a sealed gas jar in an inverted position for 24 hours at 37 °C. *S. marcescens* was incubated at room temperature. Confirmatory tests such as gram staining, IMViC (Indole, Methyl red, Voges Proskauer, Citrate tests) and catalase tests were then performed to confirm the identity of the pure cultures.

Preparation of ethanolic extracts of *Momordica charantia* and *Azadirachta indica*

Leaves of neem were collected from trees growing near INTI International University, Nilai campus. The collected neem leaves were thoroughly washed with water, and rinsed with 70% ethanol to remove dirt, before being air dried. Dried slices of *M. charantia* fruits were obtained from a Chinese Medicinal shop in Johor Bahru. Ten grams each of *A. indica* leaves and dried *M. charantia* fruits were then ground well by using an electrical grinder. The powders were separately extracted overnight with 20 mL Absolute ethanol (80%) at room temperature before being filter sterilized. The extracts were stored on ice and used within two hours.

Agar well diffusion assay.

A suspension of each bacteria tested was compared to the McFarland standard, 0.5. Approximately 50 µL of the inoculum was used to obtain 5 ×10^5 CFU/mL was spread onto Mueller-Hinton agar plates by lawning technique using sterile cotton swabs (Citron *et al*., 2005). The plate was then allowed to dry for 15 mins. A well of six mm diameter was punctured in the middle of each of the four quadrants in every agar plate using a sterile micropipette tip. Approximately 100 to 200 µL of plant extract was dispensed into each well of the quadrants. The diameter (mm) of the inhibition zone was measured after incubation at 37 °C, overnight. The agar well diffusion assay was done in triplicates to obtain more accurate results.

Data statistical analysis.

The mean value and standard deviation were calculated by using Microsoft Excel 2010. The IBM Statistical Package for Social Sciences (SPSS Version 20) were used to conduct. Two-way ANOVA test with a significance level of $P \leq 0.05$ was used on the data collected.

Resistance testing using linear gradient plate

Resistance testing was carried out using the linear gradient plate method by Liu *et al.* (2011), where the concentration of plant extract (*M. charantia* fruitand *A. indica* leaves) gradually increased in proportion with the axis of gradient forming the concentration gradient from low to high. Observation was done to determine the presence or absence of colonies on the gradient plates with the higher plant extract concentration.

RESULTS AND DISCUSSION

Anitimicrobial activity of *A. indica* and *M. charantia* against Gram positive and Gram negative bacteria. In this study, testing of the antimicrobial activity for *M. charantia* dried fruit against 17 clinically important pathogens in all the three screening showed that all the Gram positive microorganisms had a zone of inhibition, while the Gram negative isolates, *P. mirabilis, E. aerogenes, K. pneumonia, C. freundii, E. cloacae, P. vulgaris* and *E. coli* showed no zone of inhibition. However, the largest zone of inhibition was observed for *P. aeruginosa* which is a Gram negative bacteria with average diameter of 24.7 mm. According to BSAC (British Society of Antimicrobial Chemotherapy) (Wootten, 2013), if

the zone of inhibition analysed were more than or equal to 14mm it is susceptible and if less that 14mm it shows resistance. Studies has reported that, these plants contain high concentrations of secondary metabolites including alkaloids, glycosides, saponins and many more and also compounds such as momorchanins, charantin and galacturonic acids which exhibit antimicrobial activity and have been applied to treat many diseases (Leelaprakash, 2011). These compounds play an important role in antimicrobial activity by disrupting the cell membrane or protein synthesis of an organism which could contribute to the effectiveness of *M. charantia* against bacteria. Previous studies have also reported that *M. charantia* exhibit high antimicrobial activity against *S. aureus* and *P. aeruginosa*. Hence, extracts from this fruit can be used as alternative to treat mostly skin diseases and nosocomial infections caused by these pathogens (Mwambete, 2009).

The extracts of *A. indica* showed a larger zone of inhibition against *S. marcescens, S. epidermidis, B. subtilis, P. acnes, S. aureus, P. aeruginosa, S. pneumonia, E. faecalis* and *Norcardia* sp. compared to *M. charantia* dried fruit. More than 50% of the Gram positive isolates exhibited zones of inhibition and showed susceptibility to the *M. charantia* fruit extracts, however the larger zone of inhibition was against *P.aeruginosa* (Gram negative) with average diameter of 29mm. Studies also shown that *A. indica* was also effective as antimicrobial agent, as it contains phytochemicals such as alkaloids and glycosides similar to *M. charantia.* This plant has already been used as gel formulation to treat psoriasis which is a skin related disease which caused by the clinically important pathogens (Fatima *et al*., 2014). The concentrated oil extracts from *A. indica* leaves have been shown to be highly effective against *B. cereus* (Upadhyay 2011). These leaves have shown great antimicrobial activity against oral microorganism because of the high content of antimicrobial or bioactive compound such as nimbidin which was effective against Staphylococci and Salmonella species (Kumari *et al*., 2014).

When comparing both the plant extracts used in this study, we found that *A. indica* conferred higher antimicrobial activity compared to *M. charantia* against *P. acnes, B. subtilis, S. epidermidis* and *Norcardia sp*., which constituted 57% of the Gram positive bacteria tested. Furthermore, this study also showed that only one out of the eight Gram negative bacteria tested was susceptible to both *M. charantia* and*A. indica*, which was *P. aeruginosa.* This could be due to the fact that one of the main mechanisms of antimicrobial activity in *P. aeruginosa* is via the efflux pump mechanism, which is MexAB-OprM. Plant extracts are known to have efflux pump inhibitors which could contribute to the susceptibility of these pathogens to medicinal plants (Kumar and Patial, 2016).

In this study, the combination of *M. charantia* and *A. indica* crude plant extract in a 1:1 ratio was also tested. This combination demonstrated synergistic effects against Gram positive pathogens and *P. aeruginosa*. However, the zone of inhibition was observed to be intermediate when compared with the individual testing of the crude plant extracts as shown when tested against *Norcardia* sp. (Figure 1).

As for the synergistic effects of both extracts, *S. epidermidis* was indicated to have larger zone of inhibition, 20mm compared to other bacterial strains. These results were analysed using ANOVA and the results were tabulated in a histogram (Figure 2). However, the individual activity of *M. charantia* against *P. aeruginosa* was higher than the combined extracts (Figure 3). From this test, there is enough evidence demonstrating a significance difference between testing of the plant extracts against each microorganism tested.

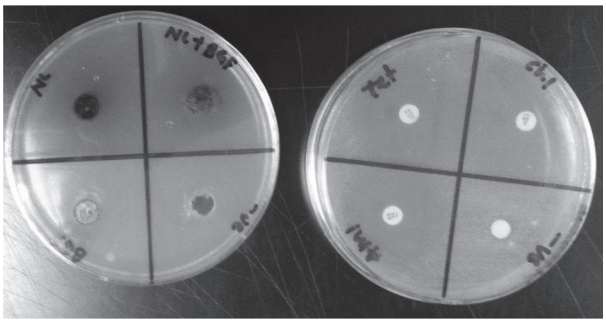

Fig. 1. The agar well diffusion (left picture) and disc diffusion (right picture) assay of *Nocardia* sp .

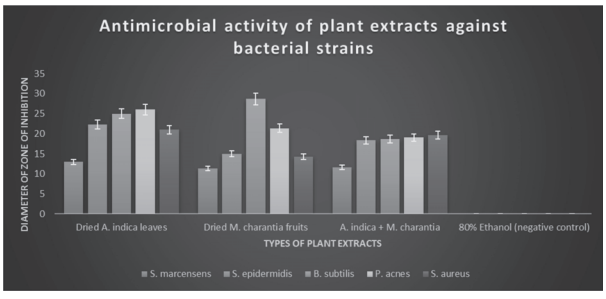

Fig. 2. Antimicrobial effects (individual and in combination) of *A. indica* and *M. charantia* against *S. marcescens, S. epidermidis, B. subtilis, P. acnes* and *S. aureus.*

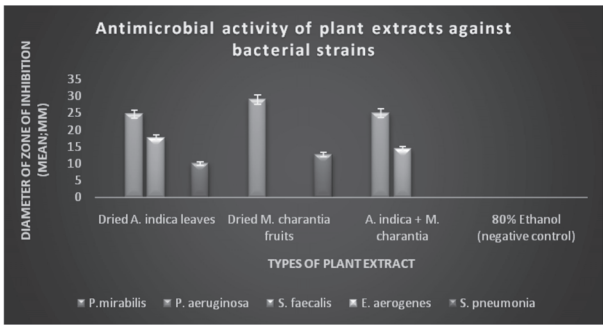

Fig. 3. The diameter of zone of inhibition of dried *A. indica* leaves, dried *M. charantia* fruits and combination of *A. indica* and *M. charantia* against *P. mirabilis, P. aeruginosa, S. faecalis, E. aerogenes* and *S. pneumonia.*

Resistance testing using the linear gradient plate

Crude medicinal plant extracts are promising alternative sources which has the potential as a resistance modifying agent. In order to ensure the delivery of antimicrobial agent by plant extracts, production of efflux pump inhibitors is the most effective way to prevent resistance of bacteria.

In this test, *M. charantia* and *A. indica* crude plant extract were tested for resistance by seventeen bacterial cultures. Isolates that showed absence of colonies at the higher concentration of plant extracts were *P. acnes* (Figure 4) and *P. aeruginosa* (data not included). This preliminary study indicates the possibility of resistance modifying agents and efflux pump inhibitors in preventing the development of resistance in these bacteria against *M. charantia* and *A. indica*. These finding exhibits that both these plant extracts have a potential to be used as antimicrobial agents.

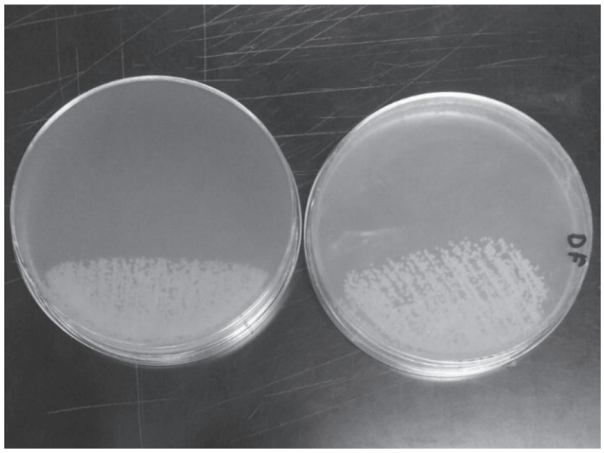

Fig. 4. Example of resistance testing with *Momordica charantia* and A*zadiratcha indica* plant extracts against *P. acnes.*

CONCLUSION

The antimicrobial effectiveness of *M. charantia* and *A. indica* were tested in combination and individually. From our results, it can be concluded that the antimicrobial activity of *A. indica* was effective against *P. aeruginosa, Norcadia sp.* and the Gram positive bacteria tested, both individually and in combination with *M. charantia.* The results suggest the potential use of *A.indica* and the combination of plant extracts as alternative therapeutic agents against these pathogens. In addition, the resistance testing showed that these pathogens showed a lower probability to develop resistance against these plant extracts which could be a significant advantage over antibiotics. Furthermore, these extracts can be used to develop healthcare products exhibiting antimicrobial activity that can be used to help prevent the dissemination of pathogenic bacteria, and thereby aid in preventing the spread of antibiotic resistance.

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REFERENCES

Citron, D.M., Warren, Y.A., Fernandez, H.T., Goldstein, M.A., Tyrrell, K.L. and Goldstein, E.J. 2005. Broth microdilution and disk diffusion tests for susceptibility testing of *Pasteurella* species isolated from human clinical samples. *Journal of Clinical Microbiology.* 43 (5) : 2485-2488.

Dash, S. P., Dixit, S. and Sahoo, S. 2017. Phytochemical and Biochemical Characterizations from Leaf Extracts from *Azadirachta Indica:* An Important Medicinal Plant. *Biochemistry and Anallytical Biochemistry.* 6 : 1 – 4.

Farnsworth, N. R., Akerele O., Bingel, A. S., Soejarto, D. D. and Gu, Z. G. 1985. Medicinal plants in therapy. *Bulletin of the World Health Organization*. 63 (6) : 965-981

Fatima, G.X., Joan, V.R., Rahul Raj, S., Shanmuganathan, S. and Chamundeeswari, D. 2014. Formulation *in vitro* evaluation and UV protective study of polyherbal sunscreen gel. *World Journal of Pharmaceutical Research.* 3(4) : 2196-2203.

Fullerton, M., Khatiwada, J., Johnson, J.U., Davis, S. and Williams, L.L. 2011. Determination of Antimicrobial Activity of Sorrel (*Hibiscus sabdariffa*) on *Esherichia*

coli O157:H7 isolated from Food, Veterinary, and Clinical Samples. *Journal of Medicinal Food.* 14 (9) : 950-956.

Grover, J.K. and Yadav, S.P. 2004. Pharmacological actions and potential uses of *Momordica charantia*: A review. *Journal of Ethnopharmacology.* 93 : 123-132.

Kazemipoor, M., Mohamed Radzi, C. W. J. W., Cordell, G. and Yaz, I. 2012. Potential of traditional medicinal plants for treating obesity: a review. *International Conference on Nutrition and Food Sciences*. 39.

Kumari, R., Prakash, R., Suman, P. K. and Padmadeo, S. R. 2014. Hepatoprotective Activity of *Azadiractha indica* Leaves on Alloxan Induced Diabetic Swiss Albino Mice. *International Journal of Basic & Applied Science Research.* 1(1) : 28-34.

Kumar, K. and Patial, S. J. P. 2016. A review of efflux pump inhibitors on Gram positive and Gram negative bacteria from plant sources. *International Journal of Current Microbiology and Applied Sciences*. 5 (6) : 837-855.

Leelaprakash G. 2011. *In-vitro* Antimicrobial and Antioxidant Activity of *Momordica charantia* Leaves. *Journals of Pharmacophore*. 2 (4) : 244-252.

Liu, Y. Q., Li, J. R., Du, J. F., Hu, M., Bai, H., Qi, J. and Gao, P. J. 2011. Accurate assessment of antibiotic susceptibility and screening resistant strains of a bacterial population by linear gradient plate. *Science China Life Science.* 54 (10) : 953-960.

Mwambete, K. D. 2009. The *in vitro* antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia:* A Tanzania medicinal plant. *African Health Sciences.* 9 (1) : 34–39.

De Oliveira, M.S., da Costa, W.A., Fernanda Wariss Figueiredo Bezerra, Araújo, M. E., Ferreira, G.C. and de Carvalho, R. N. Jr. 2018. Phytochemical profile and biological activities of *Momordica charantia* L. (Cucurbitaceae): A review. *African Journal of Biotechnology.* 17 (27) : 829-846.

Palhares, R. M., Drummond, M. G., dos Santos, B. Brasil, A. F., Cosenza, G. P., Brandão, M. G. L. and Oliveira, G. 2015. Medicinal Plants Recommended by the World Health Organization: DNA Barcode Identification Associated with Chemical Analyses Guarantees Their Quality. *PLoS ONE.* 10 (5): 1-29.

Rao, M., Padyana, S., Dipin, A.M., Kumar, S., Nayak, B.B. and Varela, M.F. 2018. Antimicrobial compounds of plant origin as efflux pump inhibitors: new avenues for controlling multidrug resistant pathogens. *Journal of Antimicrobial Agents* 4 (1) DOI: 10.4172/2472-1212.1000159.

Srivastava, Lambert and Vietmeyer, V. 1996. Medicinal Plants: An expanding role in development. *World Bank Technical Paper*. No. 320.

Upadhyay, R. K. 2011. Plant natural products: their pharmaceutical potential against disease and drug resistant microbial pathogens. *Journal of Pharmacy Research*. 4 (4) : 1179-1185.

Wootton, M. 2013. BSAC standardized disc susceptibility testing method. *Journal of Antimicrobial Chemotherapy*. Version12.