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Direct and indirect antimicrobial effects of α-mangostin on pathogenic microorganisms

Nurul Huda Syed Ibrahim[1], Muhammad Taher[2*], Deny Susanti[3], Mohamed ZaffarAli Mohamed Amiroudine[2], Qamar Uddin Ahmed[4]

[1]*Department of Biomedical Science, Faculty of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia*

[2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia*

[3]*Department of Chemistry, Faculty of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia*

[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia*

PEER REVIEW

Peer reviewer
Juliana Md Jaffri, Assistant Professor, International Islamic University Malaysia.
Tel: +609-5704849
E-mail: juliana@iium.edu.my

Comments
This is a type of exploratory study. The manuscript is written according to the normal journal article format. The problem statement is also sufficiently described. Method chosen is according to standardize method for testing antimicrobial agents.
Details on Page 75

ABSTRACT

Objective: To test direct and indirect antimicrobial properties of α-mangostin towards a number of bacteria and fungi.
Methods: The experiment was carried out using broth microdilution and checkerboards methods. Activity of α-mangostin paired with an antibiotic was studied by calculating its fractional inhibitory concentration (FIC).
Results: The activity of all four bacteria towards ampicillin, penicillin G, streptomycin and tetracycline showed no interaction with the combination with α-mangostin where the FIC indexes were between the range of $0.5<FIC_{index}>4$. Activity of doxycycline on *Pseudomonas aeruginosa* fell into other set of range, $FIC_{index} \geqslant 4$ which is an antagonism.
Conclusions: The FIC index is far away in the range. The coupled antibiotic and α-mangostin is considered synergy in action if it lies in $FIC_{index} \leqslant 0.5$ and it was found that the isolated compound, α-mangostin revealed very low synergistic antimicrobial effects when coupled with antibiotics.

KEYWORDS
α-mangostin, Direct, Indirect, Antimicrobial, FIC index

1. Introduction

Microorganisms are widely spread and caused a broad spectrum of diseases. Resistance towards frequent used antibiotics has turned to ineffective cure of the infection. This has made the current researchers to think beyond the scope to invent variety of antimicrobial agents to bombard the hustle and bustle of these miniature creatures.

The emergence of new infectious diseases, the resurgence of several infections that appeared to have been controlled and the increase in bacterial resistance have created the necessity for studies directed towards the development of new antimicrobials with new mode of action and mechanism[1].

Both bacteria and fungi need to be fought specifically in order to get rid of the illness. In many commercially available antibiotics, combination is more effective. For example, Augmentin contains amoxicillin and clavulanate.

*Corresponding author: Muhammad Taher, Department of PharmaceuticalTechnology, Faculty of Pharmacy, International Islamic University Malaysia, JalanSultan Ahmad Shah, BandarInderaMahkota, 25200 Kuantan, Pahang, Malaysia.
Tel: 60-95704807
E-mail: mtaher@iium.edu.my; tahermuhammad@gmail.com
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It is prescribed for typical short term bacterial infections such as upper and lower respiratory tract infections, skin, soft tissue, bone and joint infections. According to the detailed prescribing information by Medical Information Management System Malaysia (MIMS), infections caused by amoxicillin-susceptible organisms are amenable to Augmentin treatment due to its amoxicillin content. On the other hand, mixed infections caused by amoxicillin-susceptible organisms in conjunction with Augmentin-susceptible β-lactamase producing organisms may also be treated with Augmentin. The joined actions of both are the one which give rise to more effective outcome[2].

Therefore, direct and indirect antimicrobial properties of α-mangostin towards a number of bacteria and fungi were performed to determine their action.

2. Materials and methods

2.1. Chemicals

The antibiotic ampicillin and penicillin G were purchased from Amresco (Ohio, USA). Doxycycline and nystatin were purchased from Fisher Scientific (Loughborough, UK). Streptomycin, tetracycline as well as Mueller Hinton and Sabouraud-2% broth were purchased from Merck (Darmstadt, Germany). Dimethylsulfoxide (DMSO) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA).

2.2. Microorganism selection

The microorganisms cultured and tested include both Gram-positive, negative bacteria and fungi. Gram-positive used were *Staphylococcus aureus* and *Bacillus cereus* while the Gram-negative bacteria selected were *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli*. Fungi were *Candida albicans* (*C. albicans*) and *Candida tropicalis* (*C. tropicalis*). In this particular research, only the minimum inhibitory concentration (MIC) was used as to test the synergistic effect of the antimicrobial agents.

2.3. Culturing the microorganisms

Each selected microorganism was impregnated onto Mueller Hinton (MH) agar for bacteria while Sabouraud-2% dextrose (SD) agar was used for fungi. Inclusive striking of bacteria onto the medium was performed in order to get hold of single colonies on the agar. Later after 24 h of incubation period, a single colony was picked and dips into the broth for subculture. The subculture strains were again incubated for 18-24 h at 37 °C designed for bacteria, at the same time as 48-72 h at 30 °C for fungi. Sequentially, three colonies from each of the microbes were transferred into subsequent MH or SD broth and were let to incubate. The suspensions obtained were then measured for optical density (OD) in order to estimate the number of cells in a suspension (CFU/mL). The spectrophotometric method was employed to demonstrate the OD of inocula[3]. This method is used to measure the turbidometric of microbial concentrations directly, ranging from 10^4-10^5 CFU/mL (OD range from 0.4-0.5) for bacteria and 10^5 CFU/mL (OD range from 0.9-1.7) for fungi via spectrophotometer. In general, the spectrophotometer should be set at a wavelength of 420-660 nm[4]. The suspension should be used immediately or maintained at 4 °C if not used.

2.4. McFarland standard solution preparation

McFarland standard solution was prepared by mixing the H_2SO_4 solution (1% in broth) and $BaCl_2$ solution (1% in broth). H_2SO_4 (1 mL) was mixed in broth (100 mL) while $BaCl_2$ (1 g) was dissolved in broth (100 mL). The mixture of H_2SO_4 (9.95 mL) and $BaCl_2$ (0.05 mL) is equivalent to 150×10^6 colony/unit[5].

2.5. Media preparation

2.5.1. Mueller Hinton agar

A total of 34 g of the powder was suspended in 1 L of distilled water and sterilized by heating in the autoclave 121 °C for 15 min.

2.5.2. Sabouraud Dextrose agar

A total of 65 g of the powder was suspended in 1 L of distilled water and sterilized by heating in the autoclave 121 °C for 15 min.

2.6. Broth preparation

2.6.1. Mueller Hinton broth

A total of 21 g of the powder was suspended in 1 L of distilled water and sterilized by heating in the autoclave 121 °C for 15 min.

2.6.2. Sabouraud Dextrose broth

A total of 30 g of the powder was suspended in 1 L of distilled water and sterilized by heating in the autoclave

121 °C for 15 min.

2.7. Antibiotic solution preparation

The antibiotic solution is fresh prepared ahead of every single test done as directed by the manufacturer based on a formula which is:

$$\left(\frac{1000}{P}\right)\times V\times C=W$$

Where, P=potency of the antibiotic base, V=volume in mL required, C=final concentration of solution, W=weight of the antimicrobial to be dissolved in V.

The stock solution should be frozen at −20 °C or −60 °C[6]. The antibiotics used include ampicillin, doxicycline, penicillin G, streptomycin, tetracycline and nystatin with different potency and activity. All six antibiotics worked against the microorganism were freshly prepared before each test was carried out. The dose used was 1 mg/mL.

2.8. Sample preparation

α-Mangostin was isolated from the stem bark of *G. malaccensis*[7] and was weighed and dissolved in a light sensitive solvent of 100% DMSO. And 10 mg of α-mangostin was dissolved in DMSO to make 10 mg/mL α-mangostin solution.

2.9. Antibiotic resistance testing

Disk diffusion method was carried out where a standard quantity of microorganism was spread onto an agar plate. The antibiotic solution was cast onto 6 mm disk and impregnated on the media.

2.10. Antimicrobial testing

Broth micro dilution method was carried out in order to test the direct and indirect antimicrobial effects. From this particular test, the MIC was determined by observing the lowest concentration of samples that gave effects to bacteria and fungi[8]. The MIC value is the smallest amount of the drug that inhibits the growth and reproduction of the pathogen. It is determined through a broth dilution test, in which a standardized amount of bacteria is added to a serial dilution of antimicrobial agents in tubes or wells containing broth. In addition, the MIC is the lowest concentration of anti-microbial agent at which there is no visible growth. For this purpose least amount of anti-microbial agent should be used which can inhibit visible growth of an organism after overnight incubation. The uses of determination of MIC include measuring the anti-microbial sensitivities of slow-growing organisms, test for patients with serious infectious and when equivocal result are obtained with disk diffusion test[9]. After incubation, the turbidity (cloudiness) appearance indicates bacterial growth. This technique was performed in 96 micro-plates and serially diluted according to the preferred test.

In this step, the most top well contains the solutions to be serially diluted. Well 1 to well 6 was the positive control. Well 1 contains α-mangostin (20 μL) while well 2 to well 6 were filled up with the antibiotic solutions (20 μL). On the other hand, well number 7 is the negative control containing the DMSO (20 μL). Aiming the objective of the study, the remaining wells (well 8 to 12) integrated perfectly the α-mangostin (20 μL) and the prepared commercial antibiotic solution (20 μL). Each well was allocated with the bacterial suspension.

Next, the mixture (90 μL) from well 1 to well 7 was transferred from the first well to the second well. This twofold serial dilution was continued downward/vertically. This was done to increase the dilution while at the same time decrease the concentration. Well number 8 till 12 have dissimilar amount of transfer (100 μL).Only one microorganism was used per micro-plate in order to avoid contamination. Then, the culture was incubated for 24 h at 37 °C for bacteria and 30 °C for fungi. Later after the incubation period is over, the micro plate was taken for observation. Small degrees of turbidity point demonstrate the inhibition of bacteria/fungi, while the clear solution specifies that the microorganism were killed. The microbial growth dictated by the presence of turbidity was determined by the formation of a pallet at the bottom of the well[10]. Tables 1 and 2 summarize the template used for bacterial and fungi MIC.

Table 1
Template for bacterial MIC.

Well no.	Mixture
1	α-mangostin+bacterial suspension
2	Ampicillin+bacterial suspension
3	Doxycicline+bacterial suspension
4	Penicillin G+bacterial suspension
5	Streptomycin+bacterial suspension
6	Tetracycline+bacterial suspension
7	DMSO+bacterial suspension
8	α-mangostin+Ampicillin+bacterial suspension
9	α-mangostin+Doxycicline+bacterial suspension
10	α-mangostin+Penicillin G+bacterial suspension
11	α-mangostin+Streptomycin+bacterial suspension
12	α-mangostin+Tetracycline+bacterial suspension

Table 2
Template for fungal MIC.

Well no.	Mixture
1	α-mangostin+fungal suspension
2	Nystatin+fungal suspension
3	α-mangostin+Nystatin+fungal suspension
4	DMSO+fungal suspension
5-8	Duplicate
9-12	Triplicate

The MIC gives rise to the fractional inhibitory concentration (FIC) where the activity and relationship of the isolated compound with the antibiotic can be observed. It can be calculated with the given formula[11,12].

$$FIC_{index}=FIC_{Antibiotic}+FIC_{\alpha-mangostin}$$

$$=\frac{\text{MIC antibioticin combination}}{\text{MIC antibiotic alone}}+\frac{\text{MIC }\alpha\text{-mangostinin combination}}{\text{MIC }\alpha\text{-mangostin alone}}$$

3. Results

Quantitative analysis on the antimicrobial properties was obtained through the resilience of bacteriostatic and bactericidal concentrations against α-mangostin. Appendix A and B had the view on the MIC of the pure compound and the antibiotics both mathematically and visually. Experiment was carried out in triplicates for both bacteria and fungi. Each plate contained only one type of bacteria or fungi during one run. Results were categorized based on the antibiotic. Table 3 is the overall result from the test where there is no synergistic effect between α-mangostin and the commercialized antibiotic from the shelf in combating the selected bacteria and fungi.

Table 3
Antimicrobial activity of α-mangostinand all control.

Microorganism	Evaluation
Staphylococcus aureus	No Interaction
Bacillus cereus	No Interaction
Pseudomonas aeruginosa	No Interaction with all antibiotics, antagonism with doxicycline
Escherichia coli	No Interaction
C. albicans	Antagonism
C. tropicalis	No Interaction

4. Discussion

The development of antibiotics remains one of the most significant advances in modern medicine. Antibiotics have saved countless lives and continued to be a mainstay of therapy for bacterial infections. The indications for combination of antimicrobial agents are to obtain potentiation, delay development, and broaden the spectrum of activity and to reduce severity of incidence of adverse reaction. In combination, lower therapeutic dose of each drug reduces adverse reaction. There are many factors responsible for the development of resistance of microorganisms to antimicrobial agents. Inappropriate selection of antimicrobial agent in the absence of bacteriological culture sensitivity results plays an important role in the development of resistance. Under such circumstances, it becomes essential for the physician to have knowledge of the common pathogenic microorganism which would be involved in a particular disease and the specific antibiotics which could be used in order to obtain desired response[13].

Drug synergy occurs when drugs can interact in ways that enhance or magnify one or more effects of those drugs. In this approach of drug alliance, a second drug is administered alongside the principal drug. The role of the second drug is to guard or assist the principal drug. Usually, the second drug inhibits an enzyme that metabolizes the principal drug. For example, clavulanic acid inhibits the enzyme β-lactamase and is therefore able to protect penicillins from that particular enzyme in the form of sentry drug. "Augmentin" is one of the good examples to explain this phenomenon. It is the amalgamation of "Amoxicillin" and clavulanic acid. This combination results in an antibiotic with an increased spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β-lactamase. This perfect combination is a brilliant scheme as a remedy since amoxicillin is susceptible to degradation by β-lactamase-producing bacteria and are resistant to a broad spectrum of β-lactam antibiotics, such as penicillin. For this reason, it is often combined with clavulanic acid, a β-lactamase inhibitor. This increases effectiveness by reducing its susceptibility to β-lactamase resistance. This drug acts by inhibiting the synthesis of bacterial cell walls. It inhibits cross-linkage between the linear peptidogly can polymer chains that make up a major component of the cell walls of both Gram-positive and Gram-negative bacteria.

Amoxicillin/clavulanate has been available for clinical use in a wide range of indications for over 20 years and is now used primarily in the treatment of community-acquired respiratory tract infections. It was developed to provide a potent broad spectrum of antibacterial activity, coverage of β-lactamase-producing pathogens and a favorable pharmacokinetic and pharmacodynamic (PK/PD) profile. These factors have contributed to the high bacteriological and clinical efficacy of amoxicillin/clavulanate in respiratory tract infection over more than 20 years. This is against a background of increasing prevalence of antimicrobial resistance. In addition to high efficacy, it has a well known safety and tolerance profile based on its use in over 819

million patient courses worldwide[14].

The pure compound *viz.*, α-mangostin, isolated from *G. malaccensis* tested in this research has the same focal idea of Augmentin. It was combined with the commercial antibiotic checking the feasibility of it in the remedy locale. The principle is to bond together the natural product with a synthetic one and witnessing the upshot of it. α-mangostin was combined with other six natural and semi synthetic antibiotics which are among the resistance antibiotics namely the ampicillin, doxicycline, penicillin G, streptomycin, tetracycline and nystatin.

The untainted α-mangostin reacted differently on each antibiotic and microorganism tested. Even though it gave rise to different numbers of inhibitory concentration index (FIC_{index}) between the ranges, but on the whole, there was no reaction in the midst of it for all four bacteria except for *P. aeruginosa*.

α-mangostin alone does not execute as a good antimicrobial agent. This kind of pure and isolated compound may not exhibit the best in fighting microbes. It may be due to the deficiency of support by other compound as for in the crude extract. In favor of attesting the synergistic effect of α-mangostin as an antibacterial agent, it turned out to be that ampicillin, penicillin G, streptomycin and tetracycline on all four selected bacteria had no interaction. This showed that the existence of α-mangostin as an antimicrobial agent did not finish the task. As for what is expected, the combination should have given a better effect in either distorting the bacterial activity or kill it anyhow. For instance, it did not enhance the antibiotic in performing their task significantly.

Conversely, the performance of doxicycline on *P. aeruginosa* came into sight in a different way. It is antagonistic-opposition in physiological action. Instead of declining the bacterial growth, the combination endorsed the activity. The combination of the antibiotic and the natural product hada false hope where the mixture did not barrage the bacterial activity. It is better off for the antibiotic to work alone even though the bacterial fighting depends on the sequential rise of the dose. The naked eye scrutinized that the turbidity of the well appeared at the upper level of the 96-well microplates.

C. albicans and *C. tropicalis* are species of yeast in the genus of *Candida*. Biofilm formation is a major virulence attribute of *C. albicans* and is directly associated with therapeutic failure. One method by which *Candida* acquires antifungal resistance is the expression of drug-resistance genes. This study aimed to evaluate the transcriptional regulation of several genes associated with antifungal resistance of *C. albicans* under planktonic, recently adhered and biofilm growth modes and in *C. albicans* biofilms in response to antifungal agents[15].

Another species of *Candida*, *C. tropicalis* has been identified as the most prevalent pathogenic yeast species of the Candida-non-albicans group. Historically, *C. albicans* has been the major species responsible for causing candidiasis in immunocompromised and immunocompetent patients. However, infections (candidiasis) due to *C. tropicalis* have increased dramatically on a global scale thus proclaiming this organism to be an emerging pathogenic yeast. The reasons for this organism's dominance and its resistance to fluconazole have been difficult to elucidate. In addition, the mechanism of this organism's pathogenicity and the consequent immune response remain to be clarified[16].However, in this particular study, *Candida* species of fungi have two distinct output when assessed with the combination of α-mangostin and an antifungal agent namely nystatin. The end result of *C. albicans* with the antibiotic recipe was observed in the form of antagonism while no reaction was displayed for *C. tropicalis*.

As a conclusion, based on the antimicrobial study that has been investigated, it was observed that α-mangostin alone has a very low antimicrobial property. As to be combined with five commercially antibiotics, none of the antibiotics worked synergistically with α-mangostin. The combination with tetracycline would be a promising synergistic effect if the dose is increased. Moreover, it has the nearest FIC_{index} values approaching the synergy category. On the other hand, doxicyclineis out of the rail acting antagonistically when mixed with this pure compound. As for the fungi species, *C. albicans* has the need to be fixed with other kind of antifungal. It is strongly suggested sincethe $FIC_{index}$was found to be far away in the range. In corroborating the hypothesis and objective of the study, it is proven that the isolated compound α-mangostin (10 mg/mL) from the stembarks *G. malaccensis* has small synergistic antimicrobial effects when coupled with antibiotics (1 mg/mL).

Conflict of interest statement

We declare that we have no conflict of interest.

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04960) and the e-science fund from the Ministry of Science, Technology and Innovation of Malaysia (Grant No. 02-01-08-SF0110) for the financial support.

Comments

Background

Sufficiently written and well justified. There is an urgent need for new antibiotic development as the existing ones are at facing high risk of resistance.

Research frontiers

Exploration of an alternative antimicrobial, which should always be a continuous effort, is present in this paper. The compound tested (α-mangostin) is newly discovered and has never been tested for antimicrobial properties.

Related reports

The study employs standard methods on the culturing of microbes and testing of the compound/antibiotics for antimicrobial activity. This is important to ensure the reliability of the findings.

Innovations and breakthroughs

This is an exploratory study on α-mangostin, which has never been performed before. The author also explored the possibility of synergistic effect between α-mangostin and various antibiotics.

Applications

With new findings, the study may be expanded to the development of a new product.

Peer review

This is a type of exploratory study. The manuscript is written according to the normal journal article format. The problem statement is also sufficiently described. Method chosen is according to standardize method for testing antimicrobial agents.

References

[1] Valgas C, de Souza SM, Smania EF, Smania A. Screening methods to determine antibacterial activity of natural products. *Braz J Microbiol* 2007; **38**: 369-380.

[2] Medical Information Management System. Augmentin, detailed prescribing information. Malaysia: Medical Information Management System; 2012. [Online] Available from: http://www.mims.com/Malaysia/drug/info/Augmentin/?type=full#Indications [Accessed on 8 February 2012].

[3] Guarro J, Pujol I, Aquilar C, Llop C, Ballard JF. Inoculum preparatiomfor *in-vitro* susceptibility testing of filamentous fungi. *J Antimicrob Chemother* 1998; **42**: 385-387.

[4] Sutton S. Measurement of cell concentration in suspension by optical density. [Online] Available from: http://www.microbiol.org/resources/monographswhite-papers/measurement-of-cellconcentration- in-suspension-by-optical-density [Accessed on 27 April, 2012]

[5] Rogers EH, Grant MH. The effect of the flavonoids, quercetin, myricetin and epicatechin on the growth and enzyme activities of MCF7 human breast cancer cells. *Chem Biol Interact* 1998; **116**: 213-228.

[6] Lalitha MK. *Manual on antimicrobial susceptibility testing*. USA: NCCLS Publication; 2004.

[7] Taher M, Susanti D, Rezali MF, Zohri FS, Ichwan SJ, Alkhamaiseh SI, et al. Apoptosis, antimicrobial and antioxidant activities of phytochemicals from *Garcinia malaccensis* Hk.f. *Asian Pac J Trop Med* 2012; **5**: 136-141.

[8] Isenberg HD. Antimicrobial susceptibility testing. In: Hinder JF, Munro S, editors. *Clinical microbiology procedures handbook*. 2nd ed. Washington D.C: ASM Press; 2004, p. 5.1.1-5.18.12.

[9] Jorgensen JH, Turnidge JD. Susceptibility test methods: diluton and disk diffusion methods. In: Murray PR, Baron EJ, Pfaller MA, Jorgensen JH, Yolken RH, editors. *Manual of clinical microbiology*. 8th ed. Washington D.C: ASM Press; 2003, p. 1108-1127.

[10] Taher M, Susanti D. *Natural product research-basic techniques and applications*. Selangor: IIUM Press; 2011, p. 3-13.

[11] Okusa PN, Penge O, Devleeschouwer M, Duez P. Direct and indirect antimicrobial effects and antioxidant activity of *Cordia gilletii* De Wild. *J Ethnopharmacol* 2007; **112**: 476-481.

[12] Palaniappan K, Holley RA. Use of natural antimicrobial to increase antibiotic susceptibility of drug resistant bacteria. *Int J Food Microbiol* 2010; **140**: 164-168.

[13] Purohit SS, Saluja AK, Kakrani HN. *Pharmaceutical microbiology*. India: Agrobios; 2003.

[14] Anthony W, Clive K, James P, Rienk P, Gary W, Brian W. Augmentin® (amoxicillin/clavulanate) in the treatment of community-acquired respiratory tract infection: a review of the continuing development of an innovative antimicrobial agent. *J Antimicrob Chemother* 2004; **53**: 3-20.

[15] Watamoto T, Samaranayake LP, Egusa H, Yatani H, Seneviratne CJ. Transcriptional regulation of drug-resistance genes in *Candida albicans* biofilms in response to antifungals. *J Med Microbiol* 2011; **60**: 1241-1247.

[16] Kothavade RJ, Kura MM, Valand AG, Panthaki MH. *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole. *J Med Microbiol* 2010; **59**: 873-880.