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Antimicrobial Activity of Bio-enzyme Extract from *Garcinia mangostana* Peel, *Morinda citrifolia* Fruit and *Hibiscus sabdariffa* Petal

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Abstract. Bio-enzyme extracts are a mixture of juices prepared from several of fruits that can be consumed as nutritional or health supplements. These fruits, which include goji berries, maqui berries and acai berries, are difficult to source thus escalating the cost of the product. The purposes of this paper were to investigate the antimicrobial activity of the bio-enzyme extracts and the level of acceptance of the bio-enzyme drink when compared with a commercial brand bio-enzyme drink. The bio-enzyme extracts in this research were prepared from mangosteen peel, noni fruit and roselle petals. Disc diffusion method was used to determine the antimicrobial activity of the bio-enzyme extracts. Five microorganisms, namely *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli, Candida albicans* and *Pseudomonas aruginosa*, were used for the investigation. Thirty panelists were chosen for the sensory evaluation conducted through a hedonic test and a scoring test. The data obtained were analyzed using a paired sample t-test using "Statistical Package for Social Science" (SPSS) software. The bio-enzyme extracts showed inhibitory activity against the tested microorganisms. It had a better antimicrobial activity compared to a commercial bio-enzyme extract. The sensory evaluation showed no significant difference in overall acceptance between the bio-enzyme extract prepared and the commercial bio-enzyme extract. The findings of this research suggest that bio-enzyme extracts can prepared from selected local plant parts in Malaysia at a much lower cost and yet comparable to commercial bio-enzyme extracts in taste, aroma, colour, overall acceptance and antimicrobial acitivity.

Keywords: Bio-enzyme extract, antimicrobial activity, sensory test, mangosteen peel, noni and roselle.

1. Introduction

Bio-enzyme extracts are mixtures of juices prepared from plant parts and consumed as nutritional or health supplements. The extracts are generally perceived as functional foods with health and protective benefits to consumers particularly due to their high antioxidant content. A survey of several bio-enzyme extracts which incude brands such as Monavie, Exfuze Seven Plus, Superberry and Bio-N-zymes found the prices range from RM199 to RM250 per liter thus putting these extracts beyond the reach of most consumers. The high price tags of these extracts could be attributed to the fact that most of the ingredients found in these enzyme drinks are not easily sourced or readily available. These ingredients include acai berry from the Amazon forests, maqui berry from Chile, goji berry from Tibet and gac fruit from Vietnam. The main aim of this research was to explore the suitability of using locally sourced plant parts as alternatives to imported ingredients in the production of enzyme drinks. If locally sourced plant parts are good alternatives to imported ingredients as good sources of antimicrobial activity, the cost of producing bio-enzyme extracts will likely be lower thus making these extracts more affordable to the general public.

1.1. Problem Statement

Bio-enzyme extracts are costly due to ingredients that are hard to source in Malaysia which include acai berry from the Amazon forests, maqui berry from Chile and goji berry from Tibet. As a result, not many

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people are able to enjoy the antioxidant benefits of bi-enzyme extracts. If these imported and hard to source ingredients can be replaced by local plant parts with comparable antioxidant properties, then the cost of producing bio-enzyme extracts can be reduced. The general public will be able to benefit from consumption of more affordable bio-enzyme extracts.

1.2. Research Objectives

Determine the antimicrobial activity of bio-enzyme extracts.

Compare the organoleptic characteristics of the bio-enzyme extract produced with that of a commercial brand.

1.3. Research Questions/ Hypothesis

What is the antimicrobial activity of the bio-enzyme extracts using disc diffusion method?

Do the organoleptic characteristics of the bio-enzyme extract produced differ significantly with that of a commercial brand of bio-enzyme extract?

1.4. Scope

The selected local plants studied in the production of bio-enzyme extracts will be limited to mangosteen peels (*Garcinia mangostana*), noni fruits (*Morinda citrifolia*) and roselle petals (*Hibiscus sabdariffa*). This will be achieved by producing bio-enzyme extracts from mangosteen skins, noni fruit and roselle petals by modifying the formulation found in research of Masdar *et. al* (2012).[1] Jaroni et. al (2012) suggested the application of roselle extracts as potential antimicrobials in foods.[2] The antimicrobial activity will be determined using disc diffusion method. The sensory evaluation using the 7- scale hedonic test will be limited to the bio-enzyme extract produced and one commercial brand.

2. Experimental

2.1. Material and Instrument

Noni, roselle, mangosteen, and other ingredients were sourced at the local markets at Kuantan, Malaysia. The microorganisms were obtained from the culture collection at the Kulliyah of Science, International Islamic University Malaysia (IIUM), Kuantan campus. The gram positive bacteria include *Staphylococcus aureus* (IMR S-277) and *Bacillus cereus* (ATCC-11778). The gram negative bacteria include *Escherichia coli* (ATCC-29212) and *Pseudomonas aruginosa* (IMR C-353). The fungi selected is *Candida albicans* (ATCC-90028). Antimicrobial activity laboratory work was conducted in the Microbiology Laboratory at the Food Technology Department, Polytechnic of Sultan Haji Ahmad Shah (POLISAS), Malaysia.

The sensory evaluation tests were conducted in the Sensory Laboratory at the Food Technology Department, POLISAS. Two set of questionnaires were used, namely a 7-scale hedonic test and and a 10-scale scoring test. A scale of 1 corresponding to *Dislike very much* and ascale of 7 corresponding to *Like very much*. were used to test for the attributes of taste, colour, aroma and overall acceptance. For scoring test, three attributes were tested namely, colour (from very light to very dark), taste (from not sweet to very sweet) and aroma (from no smell to very strong smell). Thirty semi-skilled panellists who were Diploma undergraduates from the Food Technology Department, POLISAS, Malaysia were chosen for this sensory test.

2.2. Preparation of bio-enzyme extract

Four types of bio-enzyme extracts were prepared in this study. Three were prepared solely from one type of fruit, peel or petal. These are noni fruit *(Morinda citrifolia)* bio-enzyme extract, mangosteen peel *(Garcinia mangostana)* bio-enzyme extract and roselle petal *(Hibiscus sabdariffa)* bio-enzyme extract. The fourth bio-enzyme extract was prepared from a mixture of noni fruit, mangosteen peel and roselle petal. All four bio-enzyme extracts were tested for their antimicrobial activity. The sensory test however only compared the bio-enzyme extracts of the mixture of noni fruit, mangosteen peel and roselle petal against a commercial brand bio-enzyme.

2.3. Test of Antimicrobial Activity

Antimicrobial activity test was based on bacteria maintained on stock culture agar inoculated in nutrient broth. The antimicrobial assay was carried out by disc diffusion method. Sterile discs were impregnated with extracts, dried and then placed on the inoculated agar. The clear zone of inhibition around the disc was measured in millimeters. The assays were conducted in triplicates.

Five microbial strains were used in this study consisting of two gram positive bacteria, two gram negative bacteria and one fungi. The control used was a commercial bio-enzyme extract.

2.4. Media preparation

Nutrient agar was used as the agar media for bacteria while potato dextrose agar was used for fungi. The media were autoclaved at 121^{o}C and 20 psi for 30 minutes. The media were left to cool to about 55^{o}C before being poured aseptically into sterile plastic petri dishes and allowed to solidify.

2.5. Preparation of Inoculums

Firstly, each species of bacteria was cultured on nutrient agar in triplicates while the fungi was cultured on potato dextrose agar plates. The bacterial plates were incubated for 24 hours at 37 ℃ while the fungi plates were incubated for 48 hours at 30 ℃.

About 3-5 colonies of the same morphology were selected from the subcultured plates and transferred into universal bottles which contain nutrient broth for the bacteria and sabaroud broth for the fungi. The inoculums were incubated for 24 hours at 37 ℃ while the fungi plates were incubated for 48 hours under room temperature.

2.6. Preparation of Disc

Bio-enzyme of mangosteen peel, noni fruit and roselle petals were extracted. Extracts in liquid form were collected. Sterile discs (diameter 6 mm) was impregnated with 20 µL of extract and allowed to dry under a laminar flow cabinet. Discs impregnated with 20 µL of a commercial bio-enzyme served as the control in this study.

2.7. Antimicrobial Disc diffusion Test

Disc diffusion method was performed using a 24 h culture (approximately 10^5 CFU/mL) at 37 ℃ in Broth). Five hundred microliters (µL) of the suspensions were spread over sterile plates containing Mueller-Hinton agar using a sterile glass L-shape hockey stick in order to get a uniform microbial growth on both control and test plates.

The discs impregnated with 20 µL concentrations of the bio-enzyme extracts were placed on the inoculated agar. Disc impregnated with commercial bio-enzyme was used as a control.

All petri dishes were sealed with sterile parafilm to avoid eventual evaporation of the samples. The plates were left for 30 min at room temperature to allow the diffusion of extract, and then incubated at 37 ℃ up to 24 hours for bacteria and at 30 ℃ for 24 – 48 hours for fungi.

The results were recorded by measuring the zones of growth inhibition surrounding the disc with a ruler. Clear inhibition zones indicated the presence of antimicrobial activity.

According to Bauer *et. al* (1966), an inhibition zone for a high antimicrobial activity are around 14 mm or greater.[3] Diameter of inhibition zones were measured manually in triplicate according to Nagshetty *et. al*, 2010 and Mothana *et. al*, 2005. [4][5]

3. Result and Discussion

The result of the antimicrobial activity on bio-enzyme extract is shown in Table 1.

Bio-enzymes extract of noni show the highest antimicrobial activity to *E. coli*, with an inhibition zone of 18 mm. This result is comparable to the findings of Usha *et. al* (2010) which showed that noni leaf extracted with petroleum ether showed an inhibition zone of 20mm against *E. coli*.[6] Usha *et. al* also found that noni leaf extracted with water had an inhibition zone of 18mm against *S. aureus*.[6] The bio-enzyme extract of noni in this finding also presented the highest antimicrobial activity against *S. aureaus*, where the inhibition zone was 16 mm. Extracts of mangosteen peel presented the highest inhibition zone to *Candida albicans*,

where the inhibition zone was 13 mm. This finding agrees with a previous study by Ragasa *et. al* (2010) who found that that α-mangostin, gartanin and 3-isomangostin from freeze-dried pericarp of mangosteen exhibited inhibition zones fo 12 – 14 mm against *C. albican*.[7] On the whole, this study found that the bio-enzyme extracts exhibited zones of inhibition equal to or greater than the zone on inhibition of bio-enzyme from the selected commercial brand.

Table 1. Growth inhibition zone (mm)

No		Growth inhibition zone (mm)				
	Bio-enzyme / Bacteria / Fungi	Mangosteen peel (340 mg/ml)	Roselle (543 mg/ml)	Noni (381mg/ml)	Mixture of Mangosteen peel, Roselle and Noni (625mg/ml)	Commercial brand
1	*Staphylococcus aureus*	10 mm	10 mm	16 mm	12 mm	8 mm
2	*Bacillus cereus*	8 mm	9 mm	11 mm	11 mm	9 mm
3	*Escherichia coli*	9 mm	12 mm	18 mm	11 mm	9 mm
4	*Psedomanasaruginosa*	9 mm	8 mm	7 mm	10 mm	8 mm
5	*Canidida albicans*	13 mm	9 mm	9 mm	9 mm	8 mm

The results for the sensory evaluation of the bio-enzymes prepared from local plant parts (mixture of Mangosteen peel, Roselle and Noni) and that of a selected commercial brand bio-enzyme were analyzed using paired t-test using software "Statistical Package for Social Science" (SPSS). The results of the sensory evaluation test results are displayed in Table 2 and Table 3.

Table 2. T-test Results of Hedonic Test between Commercial Brand and Bio-enzyme Product

Attribute	Sample	Mean & Standard Deviation	Significant
Taste	Commercial Brand	4.87 ±1.592	0.752
	Bio-enzyme extract	5.00 ±1.800	
Colour	Commercial Brand	4.80 ±1.400	0.008
	Bio-enzyme extract	5.60 ±1.499	
Aroma	Commercial Brand	4.37 ±1.790	0.789
	Bio-enzyme extract	4.47 ±1.995	
Overall acceptance	Commercial Brand	5.03 ±1.450	0.271
	Bio-enzyme extract	5.40 ±1.567	

Table 3. T-test Results of Scoring Test between Commercial Brand and Bio-enzyme Product

Attribute	Sample	Mean & Standard Deviation	Significant
Taste	Commercial Brand	4.417±1.9787	0.076
	Bio-enzyme extract	5.383±2.5787	
Colour	Commercial Brand	4.917±1.7718	0.001
	Bio-enzyme extract	6.950±1.6833	
Aroma	Commercial Brand	6.233±1.9016	0.723
	Bio-enzyme extract	6.400±2.2105	

For hedonic test, there were no significant differences in the attributes of taste, color and overall acceptance between the commercial brand bio-enzyme and prepared bio-enzyme extract. Panelists slightly like both products in taste, color and overall acceptance. However, there was a significant difference in the attribute of color of between the commercial brand bio-enzyme and the prepared bio-enzyme extract. Panelists prefer the colour of the bio-enzyme extract prepared from local plant parts.

The scoring test found that there is no significant difference in the sweetness of two samples and no significant difference in the strength of the aroma of two samples. However, there was a significant difference in the colour of the commercial brand bio-enzyme and prepared bio-enzyme extract. The panelists found the colour of the prepared bio-enzyme extracts to be darker than the commercial product. It can be deduced based on the sensory evaluation that the panelists prefer darker coloured bio-enzyme extracts.

4. Conclusion

The findings of this study suggested that locally sourced plant parts such as mangosteen peel, noni fruit and roselle petals have the potential to replace exotic and not easily available plant parts such as goji berries, maqui berries, acai berries and gac fruits in the production of bio-enzyme extracts with comparable, if not more superior, antimicrobial and sensory attributes.

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