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Effects of *Etlingera elatior* inflorescence extract on beneficial bacteria of the healthy human gut

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ABSTRACT

Background

Etlingera species is an enduring plant in the Zingiberaceae family, with more than 100 species local to many countries. It has been utilized commonly as a culinary spice or eaten crude for its therapeutic impacts. This research is intended to study the effects of *E. elatior* inflorescence, which is commonly known as bunga kantan in Peninsular Malaysia, upon the microbiota of healthy human gut.

Material and methods

The closed bud *E. elatior* inflorescence was cleaned and dried in the hot air oven and grounded into fine powder. The extract from the *E. elatior* inflorescence was obtained using hot water extraction method. The effects of the inflorescence extract on *L. rhamnosus* and *L. acidophilus* were studied through micro-broth dilution process where different concentration of sample was incorporated into a broth medium followed by the application of a standardized volume of Lactobacillus sp. into the medium of the 96 well plate.

Results

Growth was seen in both *L. rhamnosus* and *L. acidophilus*, indicating that *E. elatior* inflorescence acted like a prebiotic towards *L. acidophilus* and *L. rhamnosus*

Conclusion

E. elatior inflorescence concentrate acts like a prebiotic towards *L. acidophilus* incomparable to L. rhamnosus

Keywords

Etlingera elatior, extraction, inflorescence, micro-broth dilution, prebiotics

Background

Etlingera species is a clan of Indo-Pacific earthbound and enduring plants in the Zingiberaceae family, with more than 100 species local to Indonesia, Vietnam, Thailand, and Malaysia, just as being regularly developed and utilized across Southeast Asia. Etlingera is an edible plant that is utilized as a culinary spice or eaten crude for its therapeutic impacts. It is likewise used as a part in neighborhood items like cleansers, shampoos, and perfumery. Peninsular Malaysia is home to an aggregate of 15 Etlingera species [1].

The inflorescence of the *E. elatior* is known as Bunga Kantan in Peninsular Malaysia, Bunga Kecombrang or Honje in Indonesia, and Kaalaa in Thailand. In Malaysia, *E. elatior* is frequently utilized as fixings in dinners, for example, laksa asam, nasi kerabu, and nasi ulam. The blossom buds are utilized in a dish called arisk ikan mas in North Sumatra. It's known as asam cekala in Karo, and the bloom buds are a critical component in the Karo variant of sayur asam, and they're excellent for cooking new fish [2]. In Malaysia, the product of *E. elatior* is utilized to ease ear infections, while the leaves are utilized to clean injuries [3]. To kill personal stench, the leaves of *E. elatior* are once in a while mixed with other fragrant spices and water [4].

Notwithstanding Etlingera elatior (Torch ginger) being a fixing agreeable to the Malaysians, there is relatively less research on the outcomes of its utilization on the normal flora of the healthy human gut. Earlier research have reported that the extract of *E. elatior* from Kelantan, Malaysia contained bioactive phenolic and flavonoid compounds which was associated with high antioxidant properties [5]. Phenolic compounds or polyphenols are phytochemicals, and previous studies have documented polyphenols from blueberry are indicated as potential prebiotics and have a strong potential to combat with obesity by improving the gut microbiota [6].

Consuming Polyphenol improves gut health and lowering the risk of coronary heart disease [7]. Dietary polyphenols have a key role in the alteration and activity of colonic microbiota [8]; and [9] posited that dietary polyphenols have the potential to restore the gut microbiota balance.

This study aimed to contemplate and to quantify the impacts of the hot water extract's bioactive compounds of *E. elatior* inflorescence on the selected normal flora, *L. rhamnosus* and *L. acidophilus*, in the healthy human gut.

Material and methods

Sample collection and preparation

Twenty closed bud *E. elatior* inflorescence were taken and the calyx portion of those inflorescence were cut off, allowing only the drying of petal, lip, anther and stigma part of the inflorescence. The inflorescence was sliced into small pieces, weighed and left drying in a hot air oven for three days at 40ºC. After the drying process, the dried inflorescence weighed again to get the dried weight and ground to form powdered dried E. elatior inflorescence. The powder form was stored in a 200mL glass reagent bottle at room temperature.

Extraction of E. elatior inflorescence

E. elatior inflorescence extract was extracted using vacuum and filter paper extraction method. About 20g of dried *E. elatior* inflorescence powder was added into 200 mL of distilled water, boiled at 49ºC for 15 minutes, and left undistributed for 24 hours. The sample was then filtered using vacuum filtration technique using absorbent cotton wool roll and gravity filtration technique using Whatman filter paper. The extract was moved into 15 mL falcon tubes and centrifuged at 3,500rpm for 30 minutes. The extract was then put away into 50 mL falcon tubes and stored at 40ºC until additional utilization.

Cultivation of Lactobacillus sp.

L. rhamnosus, ATCC 7469 (L. 7469) and *L. acidophilus*, ATCC 4356 (L. 4356) were purchased from the American Type Culture Collection (ATCC). Both the *L. rhamnosus* and *L. acidophilus* arrived in freeze dried form and were reconstituted upon arrival. The Lactobacillus sp. were initially cultured in a MRS broth media and MRS agar, respectively, in order to have a master medium that could be recultured when in use. This was done weekly in order to have sufficient amount of Lactobacillus in use during the micro-broth dilution study.

Stock solution of *E. elatior* inflorescence extract was prepared and diluted with different volume of Mueller Hinton broth to achieve master concentration. The master concentration was further diluted with 1 mL of sterile distilled water to produce a two-fold serial dilution. 50 µL of MRS broth, 50 µL of master concentrations, 50 µL of respective Lactobacillus sp. and 50 µL of the two-fold diluents were added into each respective wells, and incubated for 24 hours in an anaerobic condition at 37oC. After 24 hours incubation, 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT reagent) was distributed in each wells in use, and incubated for 2 hours. After the 2 hours incubation, 100 µL of dimethyl sulfoxide (DMSO) was distributed in each wells in use, and incubated for 1 hour. After the incubation, the absorbance value was measured at 546 nm using ELISA reader in order to quantify the effect of the extract towards the bacteria in a qualitative manner.

Data management and statistical analysis

The absorbance value obtained from the ELISA reader was entered into WPS Excel and presented as the mean standard deviation of the replicates. SPSS version 11.5 (SPSS INC., Chicago, IL, USA) was used to run one-way analysis of variance (ANOVA) and Turkey tests to assess significant group differences. Means were considered statistically significant if p value <0.05

Ethical committee approval

Ethical approval was obtained from the University Research Ethics Committee, QIU, Perak, Malaysia.

Results

Table 1: Result of two-fold serial dilution using *E. elatior* extract against *L. acidophilus* and *L. rhamnosus* in 96 well plate

Repetitive	Extraction	Concentration	*L. acidophilus*					*L. rhamnosus*				
			Positive control	Negative control	Dilution			Positive control	Negative control	Dilution		
					1	2	3			1	2	3
1	1st batch	2mg/ml	0.084	0.057	0.060	0.061	0.072	0.093	0.057	0.060	0.060	0.072
		4mg/ml	0.069	0.055	0.056	0.055	0.066	0.076	0.055	0.058	0.060	0.067
		6mg/ml	0.059	0.051	0.052	0.055	0.053	0.055	0.056	0.058	0.061	0.064
	2nd batch	2mg/ml	0.062	0.065	0.064	0.066	0.063	0.062	0.065	0.064	0.071	0.070
		4mg/ml	0.055	0.060	0.060	0.060	0.061	0.052	0.064	0.064	0.064	0.065
		6mg/ml	0.050	0.063	0.059	0.060	0.063	0.051	0.064	0.060	0.066	0.067
2	1st batch	2mg/ml	0.228	0.055	0.303	0.306	0.338	0.138	0.267	0.303	0.306	0.313
		4mg/ml	0.295	0.056	0.185	0.146	0.116	0.197	0.285	0.303	0.312	0.311
		6mg/ml	0.326	0.059	0.173	0.148	0.126	0.185	0.279	0.318	0.302	0.307
	2nd batch	2mg/ml	0.114	0.054	0.304	0.202	0.199	0.099	0.374	0.404	0.404	0.433
		4mg/ml	0.075	0.056	0.149	0.146	0.101	0.075	0.371	0.447	0.473	0.471
		6mg/ml	0.080	0.056	0.188	0.157	0.152	0.089	0.376	0.608	0.402	0.400

Table 1 shows the average of quadruple absorbance level of *E. elatior* inflorescence in three different master concentrations, MC 1, MC 2 and MC 3, under three dilution level, 0.5, 0.25 and 0.125, against *L. acidophilus* and *L. rhamnosus* retrieved from the ELISA reader at a single wavelength of 546 nm in comparison to positive control consisting of *E. elatior* inflorescence extract in MRS medium, and negative control consisting of *L. acidophilus* and *L. rhamnosus* in MRS medium. The study was performed in replication in order to show the consistency of the extract's effect towards the bacteria, *L. acidophilus* and *L. rhamnosus*. The extract used in repetitive study 1 was an extract obtained using the vacuum filtration method, whereas for the extract used in repetitive study 2, was an extract obtained using filter paper. The repetitive 1 extract was obtained two weeks beforehand, prior to the micro-broth dilution study whereas the repetitive 2 extract was freshly obtained and used the following day for the micro-broth dilution study

Table 2: Mean difference of *L. rhamnosus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3.

Variable	Mean	(SD)	F	P value
Positive control	0.125	0.099		
Negative control	0.057	0.004		
MC 1	0.185	0.133	1.524	0.208
MC 2	0.100	0.047		
MC 3	0.107	0.054		

The alpha was set to 0.05. If the P value was lesser than 0.05, there is no significant mean difference of *L. rhamnosus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3. Based on the data obtained, the P value is 0.208, which is more than the cutoff point 0.05. In conclusion, there is no significant mean difference of *L. rhamnosus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3, $F(4, 55) = 1.52$, $p = 0.208$.

Table 3: Mean difference of *L. acidophilus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3

Variable	Mean±SD	F	P value
Positive control	0.125± 0.099		
Negative control	0.057±0.004		
MC 1	0.185±0.133	3.962	0.007
MC 2	0.100±0.047		
MC 3	0.107±0.054		

The alpha was set to 0.05. If the P<0.05, there is no significant mean difference of *L. acidophilus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3. In conclusion, there is a significant mean difference of *L. acidophilus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3, $F(4, 55) = 3.96$, $p = 0.007$.

Discussion

L. rhamnosus and *L. acidophilus* were one of a probiotic strain chosen in this. Both have a significant impact in the typical working of the human digestive system. One of the most widely used probiotic strains is *L. rhamnosus*. It is utilized in the prevention and treatment of gastrointestinal infection such as gastroenteritis and diarrhea, as well as the stimulation of immune responses that improve immunization and even avoid specific hypersensitivity indications [10]. Consuming probiotics containing *L. acidophilus* and *L. rhamnosus*, can forestall or manage

intestinal diseases, further develop lactose assimilation in lactose maldigestors, diminish serum cholesterol levels, and apply anticarcinogenic movement. [11].

Notwithstanding of micro-broth dilution method being a qualitative approach, it shows that there is a significant mean difference of *L. acidophilus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3, whereas for L.rhamnosus, there is no significant mean difference between the variables, indicating that the concentrate acts more like a prebiotic towards *L. acidophilus* incomparable to *L. rhamnosus*. This findings are similar with Phuriyakorn et al., reasoning that the total phenolic content (TPC) of Etlingera elatior (*E. elatior*) showed a prebiotic-like activity by radically diminishing the populaces of pathogenic microorganisms by delivering short chain unsaturated fats [12].

Cardona et al., have reported that when polyphenols is changed to bioactive synthetics by the colonic microbiota, it effects on the intestinal biology and host wellbeing. [8] Fluctuating concentration of certain polyphenols may influence the gut microbial composition, as indicated by proof from in vitro creature and human examination and keeping in mind that specific bacterial species might be repressed, others can flourish in the accessible specialty of the environment [13]. The impact of polyphenols on bacterial development and digestion is reliant upon the construction of the polyphenol, the measurements or dosage utilized, and the microorganism strain [14]. Late examination recommends that polyphenols may work in an assortment of ways on bacterial cells. Polyphenols, for instance, can connect to the membranes of bacterial cells in a dose-dependent way, disrupting membrane function thus suppressing cell development [15]. Bacteria exposed to antioxidants up - regulation proteins involved in defensive systems that offer protection to the cells, while physiological and biochemical proteins involved in amino acid and protein synthesis, as well as phospholipid, carbon, and energy metabolism, are downregulated [16]. Under the guideline of chemical signal molecules, most bacteria may change phenotypic features, including virulence factors, as an element of cell density.

According to the findings of Ghasemzadeh et al., aqueous solvents, rather than ethanol, are recommended for extracting polyphenolic compounds, flavonoids, and tannins from *E. elatior* inflorescence. Authors have studied on the *E. elatior* inflorescence from three distinct areas of Malaysia which were Kelantan, Pahang and Johor, where they studied on the total phenolics content (TPC), total flavonoids content (TFC), total tannin content (TTC), as well as the antioxidant, anticancer and antibacterial properties the inflorescence itself. Authors found aqueous and ethanol, inflorescence from the various areas yielded various outcomes TPC, TFC, TTC and antioxidant activity (AA) [5]. Based on the result obtained through ELISA reader and statistical analysis in this study, there are fluctuations in the absorbance values indicating that there are no significant mean difference of *L. rhamnosus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3, as well as the mean difference of the master concentration: 1, 2 and 3, of *L. rhamnosus* and *L. acidophilus* in micro-broth dilution absorbance between the variables; positive control, negative control and two-fold serial dilution: 0.5, 0.25 and 0.125, but in comparison to the *L. acidophilus*, there is a significant mean difference of *L. acidophilus* micro-broth dilution absorbance between the variables; positive control, negative control and master concentration: 1, 2 and 3 which may be due to systemic errors or random errors.

Conclusion

L. rhamnosus and *L. acidophilus* were one of a probiotic strain selected for the study. Both have a significant impact in the typical working of the human digestive system. Consuming probiotics containing *L. acidophilus* and *L. rhamnosus*, then again, can forestall or manage intestinal diseases, further develop lactose assimilation in lactose maldigestors, diminish serum cholesterol levels, and apply anticarcinogenic movement.

Limitation and future scope of the study

There are few limitations for this study, as listed below.

- The effects of *Etlingera elatior* (*E. elatior*) inflorescence towards other beneficial bacteria
- Effect does *E. elatior* inflorescence towards the beneficial bacteria
- Effect does of the *E. elatior* inflorescence extract collected from different extraction methods towards the beneficial bacteria

Relevance of the study

Studies have been conducted in relation to the anti-microbial effect of *E. elatior* inflorescence but thus far no studies have been conducted on the prebiotic effect of the inflorescence. With this study, the beneficial effect of *E. elatior* can be brought to light.

Abbreviations

American Type Culture Collection (ATCC), antioxidant activity (AA), dimethyl sulfoxide (DMSO), total phenolic content (TPC), total tannin content (TTC)

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Authors' contribution

Study planning, data collection, data analysis/ interpretation, manuscript writing, manuscript revision, final approval and agreement to be accountable for all aspects of the work.

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Availability of data and materials
All data underlying the results are available as part of the article, and no additional source data are required separately as additional material for this research.

Competing interests
None declared.

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