

THE OPTIMIZATION OF PINEAPPLE-BEYH DRINK FORMULATION AND ITS EFFICACY EFFECT AS ANTI-INFLAMMATORY USING SPRAGUE-DAWLEY RATS

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ABSTRACT

This study explores the multifaceted health-promoting properties of Beyh drink (BD) and pineapple-Beyh drink (PBD). The antioxidant capacity was assessed using DPPH and FRAP assays while anti-inflammatory potential was gauged using an egg albumin assay (EAA) and rat's paw oedema method (carrageenan-induced). The total phenolic content (TPC) was carried out using Folin-Ciocalteu's method. Results indicated substantial antioxidant activities for both samples, with BD displaying 83.49% inhibition in the DPPH assay and 443.23 mg FeSO₄/100g in the FRAP assay. While, PBD exhibited comparable antioxidant potential with 80.52% inhibition and 246.14 mg FeSO₄/100g in the respective assays. In nutritional analysis, PBD showed higher TPC content (364.37 mg GA Eq/100g) compared to BD (52.26 mg GA Eq/100g). Remarkably, both samples demonstrated anti-inflammatory effects, with BD and PBD displaying 66.77% and 80.45% inhibition in the EAA assay, respectively. The positive control, Ibuprofen (50mg/kg), showed 75.64% inhibition. These findings underscore the comprehensive health benefits of BD and PBD drinks, encompassing antioxidant, nutritional, and anti-inflammatory attributes. This research contributes valuable insights for the development of functional food products with integrated health benefits.

Keywords: Antioxidant, anti-inflammatory, paw oedema, egg albumin assay

INTRODUCTION

Beyh is a Royale Johore drink which specially made from combination of various spices. It is served as welcoming drink to guest at the court of the Johore Royalty since 1833. Beyh is prepared by brewing the aromatic spices such as cinnamon, cardamon, cloves, star anise, kaffir lime leaves and pandan leaves. All these spices have their own aroma and taste. These combinations give a very strong flavour and make it a refreshing drink. Besides their aroma and flavour, these spices also can protect from acute and chronic disease. It has been proved that spices possess antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, and glucose- and cholesterol-lowering activities as well as properties that affect cognition and mood (Jiang, 2019). Dried spices contain high levels of phenolics and other active phytochemicals. Most of the dominant class of polyphenols found in spices are phenolic acids and flavonoids (Varquez-Fresno et al. 2019).

In this study, Beyh drink's formulation was upgraded with addition of pineapple juice to become pineapple-Beyh drink. This new formulation can enhance the health benefits of the drink. The crude extract of pineapple contain bromelain, a group of protein digesting enzymes. It exhibits various fibrinolytic, antioedematous, antithrombotic, and anti-inflammatory activities. Bromelain is easily absorbed by the body without losing its proteolytic activity and with no major side effects. Other than that, bromelain have many therapeutic benefits like the treatment of angina pectoris, bronchitis, sinusitis, surgical trauma, and thrombophlebitis, debridement of wounds, and enhanced absorption of drugs, particularly antibiotics. It also has potential in relieving osteoarthritis, diarrhoea, and various cardiovascular disorders. In vivo studies showed that bromelain possesses some anticancerous activities and promotes apoptotic cell death (Pavan et al. 2012). Therefore, the new formulation of pineapple-Beyh drink was tested for its ability as anti-inflammatory health drink.

MATERIALS AND METHODS

Optimization of pineapple-Beyh formulation

The new formulation of pineapple-Beyh were determined using Screening Design to study the ratio effect of pineapple juice and Beyh on antioxidant and anti-inflammatory. All ten formulas were derived (Table 1) and all samples were prepared and kept for further analysis.

Table 1: Ratio of pineapple and Beyh in formulation

SAMPLE CODE	NB010	NB19	NB28	NB37	NB46	NB55	NB64	NB73	NB82	NB91	NB100
PINEAPPLE JUICE (%)	0	10	20	30	40	50	60	70	80	90	100
BEYH SYRUP (%)	100	90	80	70	60	50	40	30	20	10	0

Total phenolic content

TPC will be evaluated using a Folin–Ciocalteu (F–C) reagent as previously performed by Lim et al. (2007). About 0.3 ml samples in triplicate were added with 1.5 ml of Folin-Ciocalteu's reagent (diluted 10 times with water) and 1.2 ml of sodium carbonate (7.5% w/v). The mixture were stored in dark and allowed to stand for 30 min. Absorption at 765 nm was measured. Total phenol contents were expressed in gallic acid equivalents (mg per 100 g fresh fruit).

Antioxidant analysis

There are three assays involved in this study namely free radical scavenging activity (DPPH), ferric reducing power (FRAP) and ferrous ion chelating activity (FIC) following method by Lim et al (2007). The DPPH was measured using methanolic DPPH solution at 517 nm in the presence of the extract. The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for 30 min in dark place. The antioxidant activity was expressed as:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] * 100$$

The ferric reducing power of the fruit extracts was determined by using potassium ferricyanide–ferric chloride method (Lim et al, 2007). An extract amounting to 1 ml were added to 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 min, after which 2.5 ml trichloroacetic acid (10%) was added. About 2.5 ml of the mixture was taken and mixed with 2.5 ml water and 0.5 ml 1% FeCl₃. The absorbance at 700 nm was measured after allowing the solution to stand for 30 min.

The ferrous ion chelating (FIC) activity will be measured by the decrease in the absorbance at 562 nm of the iron (II)-ferrozine complex (Lim et al. 2007). One milliliter 0.125 mM FeSO₄, and 1.0 ml 0.3125 mM ferrozine will be mixed with 1.0 ml sample (with different dilutions). The mixture will be allowed to equilibrate for 10 min before measuring the absorbance. Sample solutions with appropriate dilutions will be used as blanks as the sample extract may also absorb at this wavelength. The ability of the sample to chelate ferrous ion will be calculated relative to the control (consisting of iron and ferrozine only) using the formula

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] * 100$$

Egg albumin denaturation

The invitro study for anti-inflammatory activity were conducted according to egg albumin denaturation method by Vanlalhrui et al. (2019).

The 5 ml reaction mixture which consist of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml phosphate buffered saline (pH 6.4) and 2 ml of of plant extracts. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2°C in an incubator for 15 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac was used as reference drug and treated similarly for determination of absorbance. The Percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = [(100 - A_{\text{control}} - A_{\text{sample}}) \times 100] / A_{\text{control}}$$

Sensory analysis

Sensory analysis was carried out using seventh scale hedonic method involving 60 untrained panellists.

Carrageenan induced rat paw oedema

Anti-inflammatory activities were determined by carrageenan induced edema test in the hind paws of rats according to Shankar et al. (2012) with some modification. Animals were fasted for 24 hours before starting the experiment with free access to water. Rats were allocated randomly into 4 groups, each one contains six animals; (a) control normal (distilled water) (b) control negative (distilled water) (c) control positive (Voveran 20 mg/kg BW) and (d) pineapple-Beyh (3 ml/kg BW). Approximately 0.1 ml of a 1% suspension of Carrageenan (Sigma Aldrich) in normal saline was freshly prepared before experiment and was injected into the

plantar side of right hind paws of the rats. The extracts were given to the rats 1hr before the carrageenan injection. The paw thickness was measured before the experiment begin (0hr) and every 1hr interval until 3hrs.

RESULTS AND DISCUSSION

Optimization of pineapple-Beyh formulation

All pineapple-Beyh formulation were screened for its phenolic content (TPC), antioxidant (DPPH, FRAP and FIC) and anti-inflammatory (EAA) activity. Table 2 shows TPC and antioxidant activity of ten formulations. There are two formulations (NB73 and NB82) showing a high content of TPC (364.37 mg GA Eq/100g, 490 mg GA Eq/100g) and high activity of DPPH (80.52 % inhibition, 82.08 % inhibition) and FRAP (246.14 mg FeSO₄ Eq/100g, 276.97 mg FeSO₄ Eq/100g), respectively. For FIC, there is no chelating activity from the sample. The more pineapple juice being added into the formulation the higher DPPH and FRAP activity shown. As for FIC, the chelating ability was decreased with increasing pineapple juice in the formulation. According to Yuris (2014), the chelating ability of three different pineapple varieties (Josephine, Morris, and Sarawak) were low as compared to other fruits such as banana and star fruit. Low of chelating power in antioxidant assay showed that pineapple-Beyh drink was categorized as a weak secondary antioxidant. Secondary antioxidants are those antioxidants that able to chelate metal especially in Fenton reaction, generating hydroxyl radicals which can cause peroxidation of biological molecules especially lipid (Moure et al.2009). The good ability of pineapple-Beyh drink in donating electron (DPPH) or hydrogen (FRAP) to the free radical, forming a stable compound plays a good primary antioxidant (Lim et al 2007). Primary antioxidant can terminate chain reaction, and stop free radical formation.

Table 2: Antioxidant activity and total phenolic content in ten formulations of pineapple-Beyh

SAMPLE	Antioxidant capacity			Total phenolic content
	DPPH (% absorption)	FRAP (mg FESO ₄ Eq/100 g)	FIC (% absorption)	TPC (mg GA Eq/100 g)
NB19	53.55 ±0.35 ⁱ	101.26 ±1.56 ^j	27.68 ±0.50 ^b	161.47 ±1.64 ^g
NB28	59.14±0.34 ^h	232.92±0.99 ^e	17.71±0.50 ^c	190.37±0.33 ^f
NB37	63.05±0.50 ^g	144.16±3.97 ⁱ	10.07±0.19 ^d	214.17±2.10 ^{ef}
NB46	70.09±0.57 ^f	162.22±3.60 ^h	6.64±5.75 ^e	231.23±2.11 ^e
NB55	73.48±0.064 ^e	190.41±0.67 ^g	-ND-	1395.87±14.98 ^a
NB64	78.47±0.17 ^c	212.23±18.41 ^f	-ND-	357.86±0.68 ^c
NB73	80.52±0.23 ^b	246.14±2.21 ^d	-ND-	364.37±9.53 ^c
NB82	82.08±0.72 ^a	276.97±2.51 ^c	-ND-	490.47±5.72 ^b
NB91	80.74±0.25 ^b	411.16±2.97 ^a	-ND-	329.81±49.33 ^d
NB100	76.27±0.23 ^d	351.70±6.07 ^b	-ND-	494.01±9.57 ^b
NB010	37.16±0.57 ^j	65.77±2.49 ^k	34.66±0.19 ^a	112.19±1.70 ^h

Notes: ^{abcdehgh} different alphabet in the same column shows significantly different, ND – Not detected

The egg albumin denaturation assay (EAA) determines the ability of pineapple-Beyh formulation as anti-inflammatory. Table 3 shows the EAA value of pineapple-Beyh formulation. The highest EAA value was in formulation NB28 (84.15 % inhibition), followed by NB19, NB73 and NB82 (81.75%, 80.45% and 79.74% inhibition, respectively). Beside bromelain in pineapple, spices also possess anti-inflammatory activity (Jiang et al. 2019). Combination of pineapple and spices enhanced the anti-inflammatory ability in the formulation.

Table 3: Egg albumin assay (EAA) in ten formulations of pineapple-Beyh

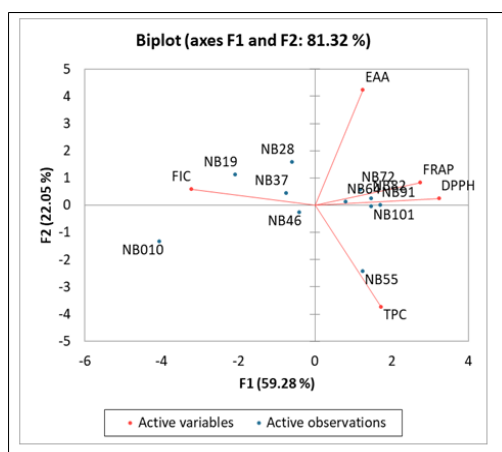
SAMPEL	EAA (% inhibition)
NB19	81.75±3.52 ^{ab}
NB28	84.15±3.40 ^a
NB37	78.72±3.22 ^{ab}
NB46	74.50±1.40 ^b
NB55	75.03±1.55 ^b
NB64	78.12±2.61 ^{ab}
NB73	80.45±1.91 ^{ab}
NB82	79.74±2.55 ^{ab}
NB91	75.24±4.27 ^b
NB100	77.42±1.83 ^{ab}
NB010	66.77±4.80 ^c
Ibuprofen (50mg)	75.64±9.02 ^b

Note: ^{abc} different alphabet in the same column shows significantly different

The best formulation among tenth formulation derived from Screening Design were analyse using Principal Component Analysis (PCA) dan Agglomerative Hierarchical Clustering (AHC) software. The PCA biplot (Figure 1) shows the comparison of the pineapple-Beyh formulation and antioxidant (DPPH, FRAP, FIC), TPC as well as anti-inflammatory (EAA) parameters with a

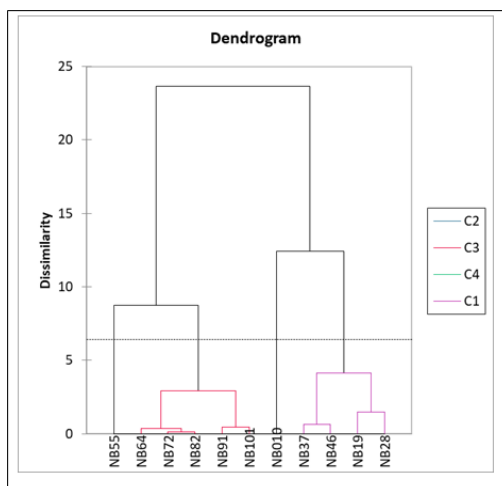
cumulative variance of 81.32%, with F1 loading approaching 60% of the variance. Pearson correlation indicates a significant positive correlation (0.761) between the antioxidant parameters DPPH and FRAP, while FIC shows a significant negative correlation with DPPH and FRAP (-0.971 and -0.722). F1 loading suggests that beverages with a higher Beyh ratio result in a higher FIC value, while beverages with a higher pineapple juice ratio led to higher DPPH and FRAP values. The plot also indicates that higher Beyh syrup ratios correspond to lower EAA values. Three samples with high DPPH and FRAP values are NB101, NB91, and NB82. This suggests that beverages with a high pineapple ratio can effectively reduce DPPH radical levels and ferric ion reduction. Additionally, high Beyh syrup ratios can effectively chelate Fe²⁺ ions. The plot also suggests that the addition of Beyh syrup imparts anti-inflammatory characteristics to this mixed beverage and synergizes with pineapple juice. Mixing equal ratios of both demonstrates synergy in terms of significant TPC values.

Figure 1: Principal component analysis (PCA) for optimization



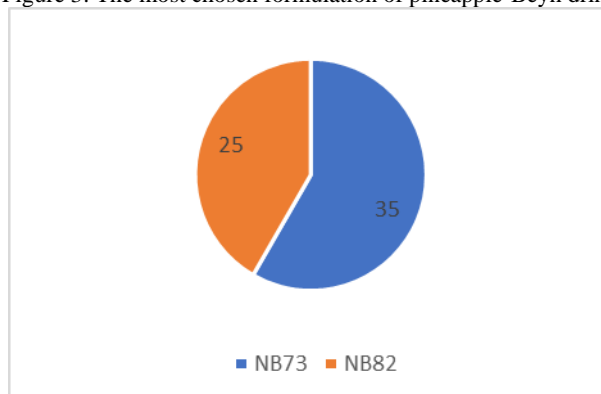
The AHC dendrogram (Figure 2) indicates clustering into 4 clusters. Samples within the same cluster share similar characteristics, while different clusters signify dissimilar features. Samples NB55 and NB010 exhibit clear dissimilarities. From cluster 3 (C3), two subclusters emerge: C3-A (samples NB64, NB73, and NB82) and C3-B (samples NB91 and NB101). Within subcluster C3-A, further clustering reveals two smaller groups, C3-A-i (NB64) and C3-A-ii (NB73 and NB82). Based on this clustering method, the formulation of samples NB73 and NB82 is suggested as a mixture with high antioxidant and anti-inflammatory properties.

Figure 2: Agglomerative hierarchical clustering (AHC) analysis for optimization



The final two formulations chosen by the software were undergone sensory analysis involving 60 untrained panellists. These samples will be evaluated using a hedonic scale with five attributes: colour, aroma, viscosity, taste, and overall acceptance. Panellists also need to evaluate samples using a paired preference test and choose their preferred formulation between two of them. Figure 3 shows that 35 of 60 panellists chose NB73 over NB82. Therefore, the pineapple-Beyh formulation NB73 was proceeded for in vivo anti-inflammatory study using carrageenan-induced method.

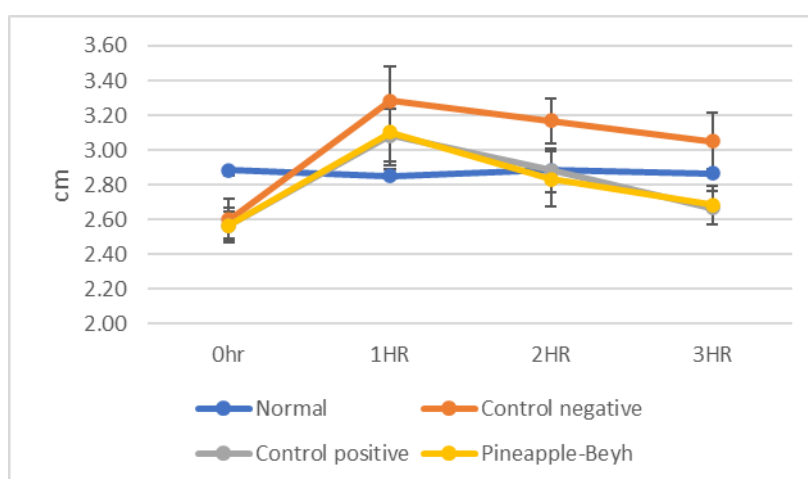
Figure 3: The most chosen formulation of pineapple-Beyh drink



Carrageenan induced rat paw oedema

The mechanism of carrageenan induced rat paw oedema is based on the production of free radical by neutrophil. According to Shankar et al. (2012), there two phases system during development of carrageenan induced oedema. The first phase which is during the first hour of carrageenan inflammation where cytoplasmic enzymes serotonin was released from the mast cells. The second phase happened during the second to third hour of inflammation when prostaglandin was released at the inflammation area. Prostaglandin was synthesis by cyclo-oxygenase enzyme (Singh et al. 2011). Figure 5 show that pineapple-Beyh drink was able to reduce inflammation after carrageenan induced. The inflammation started to reduce during the second hour and at the third hour. The paw volume of rats supplemented with pineapple-Beyh drinks were significantly low ($p < 0.05$). this can be inferred that pineapple-Beyh have the inhibitory effect on carrageenan-induced inflammation and it may be due to ability to inhibit cyclo-oxygenase enzyme to synthesis prostalglandin.

Figure 5: Volume of carrageenan-induced rat's paw



CONCLUSION

The addition of pineapple juice in Beyh drink has been proven to enhance the health benefit of the product as anti-inflammatory.

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REFERENCES

- Vanlalhruii, C., Malsawmtluangi, H. & Lahlhenmawia. (2019). Evaluation of in vitro anti-inflammatory activity of the spadix of *Colocasia affinis*. *Science Vision*. 19(2), 30–34 doi.org/10.33493/scivis.19.02.06
- Shankar, J., Vetriselvan, S., Gayathir, S., Ishwin, S., Shereenjeet, G., Hemah, D.G. & Yaashini, A. (2012) Comparative evaluation of anti-inflammatory activity of extract of curcuma longa and standard drug in carrageenan induced paw edema model using albino wistar rats. *International Journal of Biological & Pharmaceutical Research*. 3(4), 538-544
- Singh, M., Kumar, V., Singh, I., Gauttam, V., Kalia, A.N. (2010). Anti-inflammatory activity of aqueous extract of *Mirabilis jalapa* Linn. leaves. *Pharmacognosy Research*. 2(6), 364-367

- Moure, A., Cruz, J.M., France, D., Dominguez, J.M., Sineiro, J., Dominguez, H. et al. (2001). Natural Antioxidants from residual sources. *Food Chemistry*. 72, 145–171 [doi.org/10.1016/S0308-8146\(00\)00223-5](https://doi.org/10.1016/S0308-8146(00)00223-5)
- Jiang, T.A. (2019). Health Benefits of Culinary Herbs and Spices. *Journal of AOAC International*. 102(2), 395-412
- Yuris, A. (2014). A comparative study of the antioxidant properties of three pineapple (*ananas comosus l.*) Varieties. *Journal of Food Studies*. 31(1) 40-56
- Vazquez-Fresno, R., Rosana, A.R.R., Sajed, T., Onokome-Okome, T., Wishart, N.A. & Wishart, D.S. (2019). Herbs and spices – Biomarkers of intake based on human intervention studies – A systematic review. *Genes & Nutrition*. 14(18), 1-27