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Anti-hypertensive effect of pink guava (*Psidium guajava*) puree on spontaneous hypertensive rats

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Abstract: The objective of this study was to determine the physicochemical properties of pink guava (Psidium guajava) puree and its anti-hypertensive effect on Spontaneous Hypertensive Rats (SHR). Antioxidant activities of pink guava puree in water and ethanol extracts, based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, were 1.43±0.04 mg/gfm and 0.28±0.01 mg/gfm, respectively. A total of 24 male SHRs were divided into a control group, CG, and 3 treatment dosage groups [low dose group, LDG (0.5 g/kg body weight/day), medium dose group, MDG (1.0 g/kg body weight/day), and high dose group, HDG (2.0 g/kg body weight/day)]. Final body weights for treatment dosage groups were lower [MDG (313.01±31.25 g), HDG (318.56±17.96 g), LDG (320.01±22.70 g)] compared to CG (331.08±41.29 g). Final systolic blood pressure values from the beginning and the end of the experiment in MDG and HDG were 231-179 mmHg and 246-169mm Hg, respectively. These results were significantly lower when compared with CG (241-223 mmHg) from the beginning until the end of the experiment. As a conclusion, these results showed that pink guava puree has anti-hypertensive properties.

Keywords: physicochemical, pink guava, antioxidant, spontaneous hypertensive rats, hypertension

Introduction

Malaysia is one of the countries in Asia that is endowed with highly diverse biological resources. Natural phytochemical antioxidants, particularly in local fruits, have gained increasing interest among consumers and the scientific community (Rosa *et al.*, 2008). This is because epidemiological studies have reported that frequent consumption of fruits is associated with a healthy lifestyle (Mezadri *et al*., 2008).

Free radicals generated *in-vivo,* including reactive oxygen species, are responsible for the oxidative damage to lipids, proteins, deoxyribonucleic acid (DNA), and small molecules (Ajila and Prasada, 2008). As a result, they have been implicated in a number of multifactor degenerative diseases and aging processes, such as diabetes, cancer, and cardiovascular diseases, as well as the initiation and maintenance of hypertension (Manso *et al*., 2008). Recent evidence also indicates oxidative stress as the main mechanism responsible for cardiovascular complications observed in patients with metabolic syndrome, e.g., initiation or progression of the atherosclerotic process and alteration in lipid metabolism (Palmieri *et al*., 2006). Antioxidants thus play an important role in protecting the human body against damage caused by reactive oxygen species (Harold *et al*., 2007).

Hypertension affects nearly one in three American adults and as many as one billion individuals worldwide (Thom *et al*., 2006). The prevalence of hypertension increases with advancing age, with more than half of people aged 60 to 69 years affected (Burt *et al*., 1995). The prevalence of hypertension in Malaysian adult population found through screening ranged from 15% to 25% (Ministry of Health Malaysia, 1998). A recent survey by The National Health and Morbidity Survey II indicated a prevalence of 24% patients with hypertension, making them at risk for cardiovascular diseases (Ministry of Health Malaysia, 1998). This prevalence is higher than that reported by Vincent *et al*. (1997), which ranged from 11% to 15% for the

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Swiss region.

Control of elevated blood pressure has contributed to reduction in morbidity and mortality from stroke and coronary heart disease. The prevention and treatment of hypertension therefore becomes an important public health challenge (Ministry of Health Malaysia, 1998). Epidemiological studies demonstrate a protective effect of fruits against cardiovascular diseases, which may be related to phytochemical in these fruits (Winston and Craig, 1997). Although the majority of the evidence emphasizes the roles of vitamin E, vitamin C, and β-carotene, the presence of the phenolic antioxidants may also play a contributory role (Jimaima *et al*., 2007).

Sarah *et al.* (2008) reported that a high fruit diet proved more effective in improving systolic blood pressure and diet quality in adolescents with elevated blood pressure. Ample intakes of fruits have been linked epidemiologically with reduced risk for hypertension. Carotenoids also have the potential to prevent peroxynitrite-mediated damage, although, as contrasted with flavonoids, a recent meta-analysis of epidemiological studies suggests that high lutein intakes may modestly reduce coronary risk (Sarah *et al.*, 2008). Sherma *et al*. (2007) investigated the potential of the purple passion fruit peel extract, a mixture of bioflavonoids, phenolic acids, and anthocyanins, in Spontaneous Hypertension Rats (SHR) and humans. The results suggest that the extract mediated nitric oxide modulation and could be offered as a safe alternative treatment to hypertensive patients. Fatehi *et al*. (2005) indicated that the aqueous extract of barberry has beneficial effects on the cardiovascular system, suggesting a potential use for treatment of hypertension. Naama *et al.* (2005) reported that high-flavonoid sweetie juice (a hybrid between grapefruit and pummelo) was shown to have a significant beneficial effect in reducing diastolic blood pressure, compared with the effect observed with low-flavonoid sweetie juice, in patients with stage I hypertension.

Fruits from the tropical and subtropical climates are known to be associated with many medicinal properties (Yurena *et al*., 2006). Guava (*Psidium guajava*) fruit is considered to be highly nutritious because it contains a high level of ascorbic acid (50–300 mg/100 g fresh weight), which is three to six times higher than oranges (Mercadante *et al*., 1999). Red-fleshed Brazilian guava has several carotenoids such as phytofluene, ß-carotene, ß-cryptoxanthin, lycopene, rubixanthin, and lutein (Thaipong *et al.*, 2006). Setiawan *et al.* (2001) reported that Indonesian guava is an excellent source of provitamin A carotenoids. Phenolic compounds such as myricetin and apigenin are also at high levels in guava fruits (Miean and Mohamed, 2001). The trend of going back to nature is gaining popularity globally; therefore, growing interest in fruit products has fostered research into their sources (Chen and Yen, 2007). The objective of this study was to determine the physicochemical properties of pink guava (*Psidium guajava*) puree and its anti-hypertensive effect on SHRs.

Materials and Methods

Fruits

Pink guava (*Psidium guajava*) puree and fruits from *Beaumont Sungkai* variety obtained directly from Golden Hope Food & Beverages farm located in the State of Perak, Malaysia. The main criteria of selection were that the fruits are available throughout the year, non-seasonal, consumable by all population, and expected to have high antioxidative properties.

Physicochemical property analysis

Proximate analysis for protein, fat, carbohydrate, ash, moisture, energy, niacin, vitamin A and vitamin C were done according to the methods of the Association of Official Analytical Chemists; AOAC (2000). Vitamins B1 and B2 were measured based on the method described by Esteve *et al.* (2001) using High Performance Liquid Chromatography (HPLC); meanwhile, calcium, iron, potassium, and sodium were determined using an Atomic Absorption Spectrophotometer (Demirbas, 2005). Phosphorus value was determined using the direct competitive ELISA method. Total soluble solids and pH value were determined with a hand refractometer (Atago, Model HSR-500, Tokyo, Japan) and a Hanna pH meter (pH 210, Bedford, UK), respectively. The color was determined using Lovibond Tintometer.

Pink guava extract

Fresh pink guava fruits were selected according to the uniformity of shape and colour. The extract preparation was done according to Lee and Wicker (1991) with some modifications. The whole pink guava fruit (500 g) was homogenized and extracted in 100 ml of 70% ethanol for 4-hours at room temperature. The extract was then filtered through Whatman No. 41 paper and rinsed with 50 ml of ethanol. The extract was immediately used for 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was done according to the

method of Brand-Williams *et al*. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10 ml stock solution with 45 ml methanol to obtain an absorbance of 1.1±0.02 units at 515 nm using the spectrophotometer. Pink guava puree extracts (150 µl) were allowed to react with 2850 µl of the DPPH solution for 24 hours in the dark. Then the absorbance was measured at 515 nm. The standard curve was linear between 25 and 800 µM Trolox. Results were expressed in mg/g fresh mass. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

Animal studies

Twenty-four male SHRs with an average weight of 200–250 g were purchased from University of Malaya animal house. The rats were divided into a control group, CG, and three treatment dosage groups [low dose group, LDG (0.5 g/kg body weight/day), medium dose group, MDG (1.0 g/kg body weight/day), and high dose group, HDG (2.0 g/kg body weight/day)]. The rats were acclimatized for one week in an air-conditioned room 25±2°C to a 12:12-hour light (07:30 am - 07:30 pm)/dark cycle and were given free access to standard rat chow and reversed osmosis water *ad libitum*. The dosage groups were given pink guava puree via force-feeding for 28 days in individual metabolic cages. The control group was given reverse osmosis water. Each group consisted of six animals, and body weight was measured weekly. The systolic blood pressure value was measured weekly under a waking condition by a tail-cuff method. At the end of experiment, the rats were fasted overnight (12-14 hours) and euthanized under an anesthetic condition with ether, and blood was collected from the portal vein or posterior vena cava for biochemical measurements. Organs were collected and frozen in liquid nitrogen by at -70°C. Animals were treated following the guidelines and approval established by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC).

Systolic blood pressure (SBP)

Systolic blood pressure was measured by using the tail-cuff method (IITC Life Science, Model 179 Non-Invasive Blood Pressure (NIBP) Multi Channel Blood Pressure System, USA). Blood pressure was taken with the rats under conscious conditions at the beginning of the experiment and at every week of the experiment. Systolic blood pressure values are the means of three measurements per rat, and were measured weekly. During acclimatization, the rats were put in the restrainers for 10 to 15 minutes at 30°C every day to make the rats comfortable with the restrainers, tail-cuff detector and warming chamber.

Blood collection

Animals were fasted overnight prior to blood collection. Blood for clinical chemistry studies was collected into tubes containing ethylenediaminetetraaceticacid(EDTA)anticoagulant and centrifuged at 3500 g for 20 minutes to obtain the plasma. The plasma samples were kept frozen at -80°C until being used for antioxidant capacity and oxidative status assays. An ADVIA 120 Hematology Analyzer (Bayer Diagnostics) was used to measure the following parameters: total antioxidant status, urea, total bilirubin, γ-glutamyl transpeptidase, alkaline phosphate, total protein, albumin and creatinine. Globulin (total protein minus albumin) and albumin/globulin ratio values were calculated.

Statistical analysis

Results are expressed as mean values ± standard deviation. Comparison of means of three measurements, using a significance level of $P<0.05$, was performed by one-way analysis of variance (ANOVA) using the SAS System for Windows, version 6.12 software.

Results and Discussion

Physicochemical properties of pink guava puree

The values of pH, total soluble solids and moisture content were not significantly different among pink guava parts: seeds, skin and flesh, whole fruit, and puree (Table 1). pH ranged from 4.44 to 4.90; meanwhile, total soluble solids value was 8.6°Brix in pink guava puree and ranged from 6.8 to 11.0°Brix in the other parts. Cheng *et al.* (2007) also observed no significant differences for pH and total soluble solids among the guava samples studied. The pH values were slightly lower from this study, ranging from 3.90 to 3.93, which was consistent with the values reported by Yusof (2003), i.e., 3.2 to 4.1. Sugars are the major soluble solids in fruit. The data of total soluble solids measurements do not differ significantly from one another because samples were freshly prepared, and it is believed that onset of microbial fermentation had not taken place in the samples prior to analysis. Moisture content was 90.8% in puree and ranged from 64.1 to 79.4% in the

Table 1. Physicochemical properties of pink guava puree

Parts of pink guava	pH	Total soluble solid (°Brix)	Moisture content (%)
Seed	4.90[a]	11.0[a]	64.1[a]
Skin & Flesh	4.47[a]	10.0[a]	79.4[a]
Whole	4.44[a]	6.8[a]	78.0[a]
Puree	4.44[a]	8.6[a]	90.8[a]

Superscripts with different letters are significantly different at P<0.05 within the same column; n=6

Table 2. Nutrient and color analysis of pink guava puree

Proximate analysis		Value
Macronutrients	Energy	147 kJ
	Carbohydrate	7.00%
	Protein	1.70%
	Fat	0.0%
	Ash	0.50%
Micronutrients	Vitamin A	108.50 mg/100 g
	Vitamin B1	BDL
	Vitamin B2	BDL
	Vitamin C	73.10 mg/100 g
	Niacin	13.30 mg/100 g
	Phosphorus	0.77 mg/100 g
	Calcium	131.66 ppm
	Iron	20.44 ppm
	Potassium	149.80 ppm
	Sodium	139.95 ppm
Color ratio	7.2 red/ 6.0 yellow/ 1.0 blue	

BDL: Below detection limit; n=6

other parts of pink guava.

Pink guava puree (Table 2) provided 147 kJ energy, 7.0% carbohydrate, 1.7% protein, 0% fat, 0.5% ash, vitamin A (108.50 mg/100 g), vitamin C (73.10 mg/100 g), calcium (131.66 ppm), iron (20.44 ppm), potassium (149.80 ppm), sodium (139.95 ppm), niacin (13.30 mg/100 g), and phosphorus (0.77 mg/100 g). Vitamin B1 and B2 values were below detection limits. The color was with the ratio of 7.2 red/ 6.0 yellow/ 1.0 blue by using Lovibond Tintometer. The vitamin C contents in pink guava puree were higher compared to other fruit crops. The ranges of vitamin C contents (mg/100 g) were 4.8–13.2 in nectarines, 3.6–12.6 in peaches, 2.5–10.2 in plums (Gil *et al.*, 2002), 19.0 in star fruit, 27.5 in pineapple, 60.5 in mango, and 13.8 in lychee (Luximon-Ramma *et al.*, 2003).

Table 3. Antioxidant activity of pink guava puree based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Parts of pink guava	Water extract (mg/gfm)	Ethanol extract (mg/gfm)
Seed	0.63 ± 0.03[d]	0.12 ± 0.01[d]
Skin & Flesh	1.58 ± 0.03[a]	0.41 ± 0.01[a]
Whole	1.55 ± 0.02[b]	0.40 ± 0.0[b]
Puree	1.43 ± 0.04[c]	0.28 ± 0.01[c]

Superscripts with different letters are significantly different at P<0.05 within the same column; n=6

Antioxidant activity of pink guava in seeds, skin and flesh, whole fruit, and puree

Antioxidant activities were slightly different in water and ethanol pink guava extracts based on DPPH assay (Table 3). Skin and flesh had highest antioxidant activity values in water and ethanol extracts (1.58 mg/gfm; 0.41 mg/gfm), followed by whole fruit (1.55 mg/gfm; 0.40 mg/gfm), puree (1.43 mg/gfm; 0.28 mg/gfm), and seeds (0.63 mg/gfm; 0.12 mg/gfm). Therefore, guava can be considered as one of the fruits that have an exceptionally high antioxidant activity. The antioxidant activities obtained in the present study were higher than other fruit crops. Alothman *et al.* (2009) reported that the antioxidant activity of Thai seedless guava was higher, ranged from 1.23-1.91 mg/gfm followed by pisang mas (0.24-0.72 mg/gfm) and honey pineapple (0.35-0.55 mg/gfm) respectively.

Body weights

As shown in Figure 1, mean body weights were similar in all groups at the start of the study. At the time of killing at day 29, mean body weight was lowest in MDG (313.01±31.25 g), followed by HDG (318.56±17.96 g) and LDG (320.01±22.70 g) compared to CG (331.08±41.29 g); however, no significant differences were found among the groups. Pink guava puree intake had no effect on bodyweight gain. Administration of pink guava (*Psidium guajava*) puree produced no clinical and toxic signs such as vomiting, red eye, fur loss, adverse effects or deaths in the SHRs. This finding is similar to the Konan *et al.* (2007) report.

Systolic blood pressure (SBP)

The present study was designed to evaluate the effects of pink guava (*Psidium guajava*) puree on SBP of SHRs. This study has demonstrated that pink guava puree has an anti-hypertensive effect and was useful

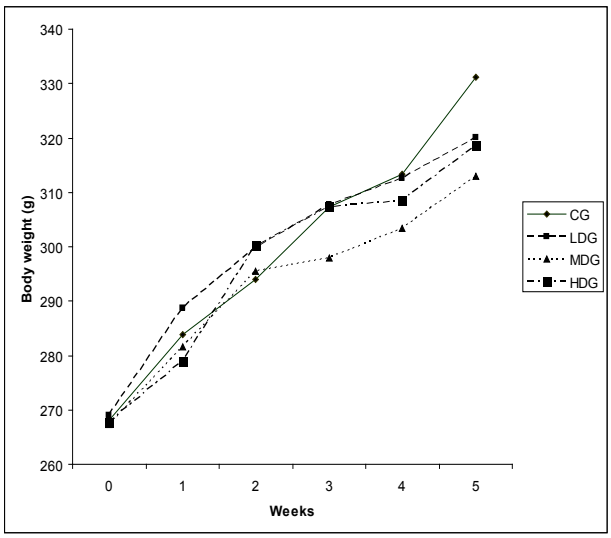

Figure 1. Mean body weight of Spontaneous Hypertensive Rats supplemented with pink guava puree
Not significantly different (P<0.05); n=6
CG : Control Group (distilled water)
LDG : Low Dosage Group (0.5 g/kg body weight)
MDG : Medium Dosage Group (1.0 g/kg body weight)
HDG : High Dosage Group (2.0 g/kg body weight)

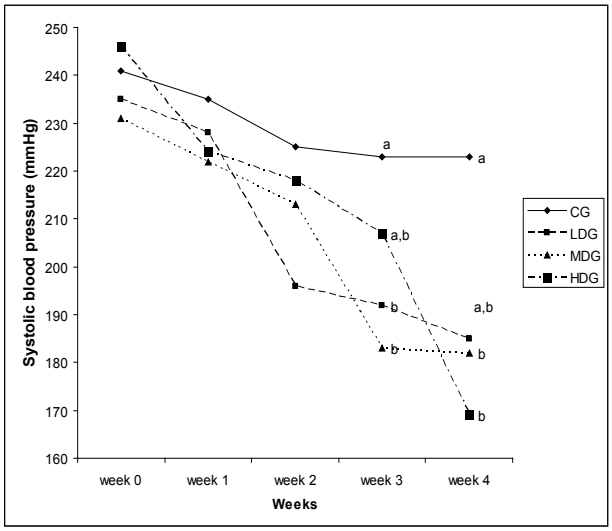

Figure 2. Systolic blood pressure value of Spontaneous Hypertensive Rats supplemented with pink guava puree
Superscripts with different letters are significantly different at P<0.05 within the same week; n=6
CG : Control Group (distilled water)
LDG : Low Dosage Group (0.5 g/kg body weight)
MDG : Medium Dosage Group (1.0 g/kg body weight)
HDG : High Dosage Group (2.0 g/kg body weight)

in reducing SBP. Figure 2 shows the SBP values for all groups. Compared with their corresponding controls, SBP for SHRs in the MDG was significantly lower (P<0.05) than for those in the CG for weeks 3 and 4 (183±43 mmHg and 179±25 mmHg, respectively). SBP values before the treatment for CG were 241±16 mmHg, for LDG, 235±9 mmHg, for MDG, 231±13 mmHg, and for HDG, 246±14 mmHg. For week 3, LDG rats also showed significantly decreased (P<0.05) SBP at 192±3 mmHg compared to CG rats at 223±10 mmHg; meanwhile, for the week 4, HDG rats demonstrated a significantly lower (P<0.05) SBP value at 169±32 mmHg compared to CG rats at 223±43 mmHg. Treatment groups showed a significant decreased (P<0.05) SBP value at third and fourth weeks.

Al-Awwadi *et al.* (2004), de Moura *et al.* (2004) and Pechnova *et al.* (2004) reported that polyphenols are able to prevent cardiac hypertrophy and the production of reactive oxygen species, as well as improving vascular function. The vasodilator and antioxidant actions exerted by guava extract have been reported (Jim *et al.*, 2001). Ram *et al.* (1992) did a study on patients with essential hypertension. In their study, the guava extracts were administered before meals in a randomized and single blind fashion for twelve weeks. There was a significant net decrease (P<0.05) in systolic blood pressures values with a significant net increase (P<0.05) in high-density lipoprotein cholesterol after twelve weeks of guava fruit substitution in patient groups with essential hypertension. All the currently available evidence favors the hypothesis of the beneficial effects of pink guava (*Psidium guajava*) puree intake in the reduction of hypertension.

Blood chemistry profile

Blood chemistry analysis showed no significant difference in total antioxidant status, urea, alkaline phosphate and globulin between the treatment groups, as shown in Table 4. Total antioxidant status ranged between 1.63 mmol/l and 1.68 mmol/l; meanwhile, urea ranged from 8.73 mmol to 9.86 mmol/l. Alkaline phosphate ranged from 140.33 U/l to 160.67 U/l and globulin ranged from 24.06 g/l to 25.72 g/l. There was a significant difference (P<0.05) in total bilirubin, γ-glutamyl transpeptidase, total protein, albumin, A/G ratio and creatinine. LDG (3.51 μmol/l) had significantly lower (P<0.05) total bilirubin amounts, compared to CG (4.25 μmol/l). γ-glutamyl transpeptidase levels were significantly higher (P<0.05) in HDG (8.67 U/l), compared to CG (7.00 U/l), LDG (7.67 U/l) and MDG (7.00 U/l). LDG (63.09 g/l) had significantly higher (P<0.05) total protein amounts, compared to CG (60.19 g/l), MDG (60.43 g/l) and HDG (59.30 g/l). The albumin level was also significantly higher (P<0.05) in LDG (37.37 g/l) than in the CG (36.13 g/l), MDG (34.77 g/l) and HDG (34.30 g/l). Globulin values were calculated

Table 4. Blood chemistry of Spontaneous Hypertensive Rats supplemented with pink guava puree

Dosage	CG	LDG	MDG	HDG
Total antioxidant status (mmol/l)	1.68±0.04[a]	1.68±0.02[a]	1.68±0.04[a]	1.63±0.04[a]
Urea (mmol/l)	9.86±0.63[a]	8.97±1.36[a]	9.04±0.38[a]	8.73±1.06[a]
Total bilirubin (µmol/l)	4.25±0.15[a]	3.51±0.41[b]	3.85±0.20[a,b]	3.54±1.00[a,b]
γ-glutamyl transpeptidase (U/l)	7.00±0.77[b]	7.67±0.52[a,b]	7.00±0.71[b]	8.67±1.86[a]
Alkaline phosphate (U/l)	160.67±3.61[a]	148.00±14.39[a]	147.67±5.96[a]	140.33±27.57[a]
Total protein (g/l)	60.19±2.91[b]	63.09±2.89[a]	60.43±1.27[b]	59.30±0.73[b]
Albumin (g/l)	36.13±0.68[b]	37.37±1.00[a]	34.77±0.76[c]	34.30±0.73[c]
Globulin (g/l)	24.06±2.37[a]	25.72±2.25[a]	25.66±0.52[a]	25.00±2.14[a]
A/G ratio	1.50±0.11[a]	1.45±0.17[a,b]	1.36±0.10[b]	1.37±0.13[b]
Creatinine (µmol/l)	52.47±0.58[a,b]	54.40±0.53[a]	53.85±0.56[a]	50.74±0.57[b]

Superscripts with different letters are significantly different at $P<0.05$ within the same row; n=6
CG : Control Group (distilled water)
LDG : Low Dosage Group (0.5 g/kg body weight)
MDG : Medium Dosage Group (1.0 g/kg body weight)
HDG : High Dosage Group (2.0 g/kg body weight)

by total protein minus albumin, and no significant difference can be seen. Albumin: globulin ratio values were significantly different ($P<0.05$) in MDG (1.36) and HDG (1.37), compared to CG (1.50). Creatinine values were significantly different ($P<0.05$) in LDG (54.40 µmol/l) and MDG (53.85 µmol/l), compared to HDG (50.74 µmol/l). Konan *et al*. (2007) found that the fruit extract/ juices were generally tolerated by rats based on biochemical analyses of renal and hepato-biliary functions, such as the level of urea, creatinine and alkaline phosphate value. These findings were similar with this study.

Conclusion

In conclusion, these results show that pink guava (*Psidium guajava*) puree has anti-hypertensive properties and high nutritional value.

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