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Development and quality assessment of *Lactobacillus paracasei* HII01 mediated fermented *Kaempferia parviflora wall. Ex. Baker juice*

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Abstract

Objective: To develop and evaluate *Lactobacillus paracasei* HII01 mediated fermented *Kaempferia parviflora* wall. Ex. Baker rhizome juice.
Methods: The changes in pH, acidity, ethanol, and reducing sugar content were measured by pH meter, titration, gas chromatography, and Dinitrosalicylic acid method, respectively. The total polyphenol content was measured by colorimetric method. ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)), and FRAP (ferric reducing antioxidant power) assays were performed to calculate the total antioxidant capacity, and reducing power, respectively.
Results: The pH and acidity of fermented *K. parviflora* juice (FKJ) were gradually reduced, and increased, respectively. The high total phenolic content was observed in F1, and F3 after 15 days of fermentation (1.14, and 1.02 mg GAE per ml sample, respectively). The total antioxidant capacity of F1 and F3 were 1.07, and 1. 2 mg TEAC per ml sample, respectively, after 20 days of fermentation. The sugar content was reduced during fermentation. The ethanol content of samples was ranging from 0.05-2.26 % (V/V). The microbial load was reduced during fermentation, and no pathogenic microbes were detected. The results suggested that about -20 days of fermentation period is sufficient to produce high-quality FKJ.
Conclusion: The first *L. paracasei* mediated fermented *K. parviflora* juice was developed with a high content of phenolic compounds, and antioxidants. FKJ was microbiologically safe, and it contains an acceptable level of ethanol and acidity. FKJ can be a potent functional food supplement if further characterization and pharmacological evaluation are made.

Keywords: Fermented plant juice, *Kaempferia parviflora* wall. Ex. Baker, Lactobacillus, Antioxidant, Phenolic compound, Ethanol.

INTRODUCTION

Kaempferia parviflora Wall. Ex. Baker (commonly called as Krachai-dam in Thai or Thai ginsengs or black ginsengs or black galingale or black ginger) belongs to Zingiberaceae family. *K. parviflora* has been used in Thai folk medicine for the treatment of stomach problems, inflammatory diseases, free radical damages, and to improve the male sexual activity [1-3].

Several bioactive principles have been identified in *K. parviflora* majorly polymethoxyflavonone such as 5-hydroxy-7-methoxyflavone, 5-hydroxy-7,4′-dimethoxyflavone, 5-hydroxy-3,7-dimethoxyflavone, 5-hydroxy-3,7,4′-trimethoxyflavone, 3,5,7-trimethoxyflavone, 5-hydroxy-3,7,3′,4′-tetramethoxyflavone, 5,7,4′-trimethoxyflavone, and flavonoids, chalcone and its derivatives [3]. Reports suggested that *K. parviflora* reduce the obesity in mice [4], induce apoptosis in several human cancer cells [5-9], inhibits the melanogenesis [10], reduce oxidant stress, and maintains the endothelium-dependent relaxation [11], and anti-inflammatory [12] property.

Fermentation is one of the strategies to preserve, and improve the quality of foods. Fermentation process simplifies and facilitates the absorption of active compounds in the food materials. A controlled fermentation process with strong starter culture enhanced the functional properties of fermented food. Lactobacillus strains are known as probiotic bacteria with antimicrobial activity [13-15], and lactic acid bacteria (LAB) based feremnted plant juices are considered as unctional food. We have developed and reported the fermented mushroom juice rich in γ-aminobutyric acid using food isolated of lactic acid bacteria (LAB) [16-18], and quality enhancement of *Phyllanthus emblica* fruit juice via LAB mediated fermentation [19].

As per our information, there is no report on development and assessment of phytochemical changes of fermented *K. parviflora* using the LAB as a starter culture. In the present day, we have developed *Lactobacillus paracasei* HII mediated fermented *K. parviflora* juice (FKJ), and studied the changes in pH, acidity, total phenolic content, total antioxidant capacity, reducing power, reducing sugar, ethanol content and microbiological safety of the FKJ.

MATERIALS AND METHODS

Raw materials, Strain, and investigational setup

Kaempferia parviflora Wall. Ex. Baker rhizome and cane sugar were bought from local market of Chiang Mai province, Chiang Mai, Thailand. Honey was acquired from Agricultural extension and development center, Chiang Mai. Health Innovation Institute, Chiang Mai kindly provided *Lactobacillus paracasei* HII01 strain. The fermentation of *K. parviflora* rhizome was carried out with cane sugar, or honey as carbon source using 10% of *L. paracasei* as starter culture.

The following are the details of fermentation setup: Formula 1 (F1): Cane sugar: *K. parviflora*: Water (1:3:10 ratio) +*L. paracasei* (10%); Formula 2 (F2): Honey: *K. parviflora*: Water (1:3:10 ratio) +10% *L. paracasei* (10%); Formula 3 (F3): Cane sugar: *K. parviflora*: Water (1:3:10 ratio); Formula 4 (F4): Honey: *K. parviflora*: Water (1:3:10 ratio); Control 1 (C1): Cane sugar: Water (1:10 ratio) +10% *L. paracasei* (10%); Control 2 (C2): Cane sugar: Water (1:10 ratio); Control 3 (C3): Honey: Water (1:10 ratio) +10% *L. paracasei* (10%); Control 4 (C4): Honey: Water (1:10 ratio).

Fermentation

The fermentation setup, preparation of starter culture, and sample collection were described previously [19]. The fermentation was performed at room temperature (30 ± 2 °C) for 180 days, and samples were collected during the fermentation process and stored at -70 °C after the filtration (Whatman no. 42 filter paper) to determine the parameters kinetically.

Determination of acidity, pH, and total polyphenolic content

The pH, acidity, and total polyphenolic content of fermented juice at the various time point of fermentation was assessed as detailed previously [19-21].

Determination of ethanol, and reducing sugar content

The ethanol contents of fermented *K. parviflora* was determined by gas chromatography (GC-14B, Shimadzu, Japan) as reported earlier [22]. The reducing sugar content of the samples was calculated by the dinitrosalicylic acid method and denoted as mg glucose per ml of sample [23, 24].

Total antioxidant capacity

Total antioxidant capacity (TAC) of fermented *K. parviflora* juice was calculated by ABTS (2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) assay as detailed previously [25, 26]. The results were represented as mg of trolox equivalent antioxidant capacity (TEAC) per ml of sample.

FRAP Assay

The FRAP (Ferric Reducing Antioxidant Power) value of the test and control samples were assessed as described previously [19]. The values of FRAP assay were denoted as mg Fe_2SO_4 equivalents per ml sample.

Microbiological assessment

The samples were microbiologically examined as explained previously to ensure the microbial safety of the fermented *K. parviflora* juice. The plate count method was employed for the bacterial count determination using the specific medium for *Lactobacillus* spp., coliforms, *Salmonella* spp. [27].

Statistical Analysis

All the experiments were performed in triplicate. The values were denoted as Mean. Duncan's new multiple range tests determined the significant differences, at the 95% confidential level ($p < 0.05$) by SPSS v.17 (Chicago, SPSS Inc, U.S.A).

RESULTS AND DISCUSSION

pH of the fermented medium was reduced gradually in all the formulations. Notably, the formulas containing cane sugar (F1, and F3) showed low pH and high acidity compared to other experimental samples. In the case of control samples, C1 showed high acidity which is attributed to the presence of cane sugar that facilitates the spontaneous microbial growth than honey (Fig.1).

The total polyphenol content (TPC) of the samples was changed during fermentation. The high TPC was observed in F1, and F3 after 15 days of fermentation (1.14, and 1.02 mg GAE per ml sample, respectively). The samples C1 and C2 showed maximum TPC among control samples at 15 days (0.65, and 0.60 mg GAE per ml sample, respectively). Even after 180 days of fermentation the sample F1, and F3 showed maximum TPC with slight reduction compared to 15 days of the process. The results suggested that 15 days of fermentation by *L. paracasei* produces the phenolic compound rich FKJ (Fig. 2).

The total antioxidant capacity (TAC) of experimental samples F1 and F3 were 1.07, and 1. 2 mg TEAC per ml sample, respectively, after 20 days of fermentation. TAC of the samples was steadily condensed during the extended fermentation process. (Fig. 3A). Likely, the FRAP values of F1 and F3 was found the maximum (1.93, and 2.16 mg Fe_2SO_4 equivalent per ml sample, respectively) after 20 days of the fermentation process. The FRAP value of F1 and F3 was remained maximum among the experimental samples after 180 days of process but reduced compared to 20-day sample (Fig. 3B). The results proved that about 20 days of fermentation was enough to achieve FKJ with high TAC and FRAP value, and prolonged fermentation process reduces the quality of the FKJ regarding TAC.

The reducing sugar content of the samples was reduced while fermentation period was increased. The samples F1, F2, F3, and F4, showed a reduction in sugar content from 87.32 to 0.86, 68.25 to 1.5, 70.12 to 0.93, and 63.21 to 2.2 mg glucose equivalent per ml sample, respectively. The presence of starter culture accelerates the decrease of sugar content in fermentation media since they utilize that for their growth (Fig. 4A).

The ethanol content of F1, F2, F3, and F4 were ranging from 0.05-2.52, 0.14-2.26, 0.25-.81, and 0.27 to 1.65 % (V/V), respectively. The maximum of 3.86 % of ethanol was detected in C1 sample after 180 days of fermentation. All the experimental samples showed less than 3% of ethanol (Fig. 4B). According to Thai community product standard (TCPS 481/2004), the permissible level of ethanol in fermented plant juices is 3% (v/v) [22]. The FKJ developed in the study was safe as per TCPS regulations regarding ethanol content.

The total bacterial count of the samples was altered during the fermentation process. The samples with inoculum showed a gradual reduction since the substrate for the microbial growth was depleted after 30 days of fermentation. Whereas, the bacterial load was slowly increased in uninoculated samples. The same scenario was observed in control samples. After 180 days of fermentation, the samples with LAB starter showed a reduction in microbial load while other samples exhibited high microbial content (Fig. 5A). *Lactobacillus* spp. load in FKJ has also reduced in a time-dependent manner. A gradual reduction in lactobacillus content was observed even after 30 days of fermentation (Fig. 5B). The samples F1, F3, F4, and C2, showed bacillus load at the first day of fermentation, whereas there was no bacillus were observed after the first day (Fig. 5C). The representative pathogenic bacterial strains (*E. coli*, and *Salmonella* spp.), yeast, and molds were not found in the samples at any time point of the fermentation process (Table 1). The results suggested that the developed FKJ was microbiologically safe.

Several pharmacological properties were reported about the compounds of *K. parviflora*, and different solvent extracts of *K. parviflora* rhizomes. The phytochemical content of methanolic extract of *K. parviflora* was assessed, and about sixteen compounds were identified based on the reported literature. The active compounds of *K. parviflora*, particularly flavonoid derivatives exhibited high soluble epoxide hydrolase inhibitor activity compared to other compounds [28]. Methoxyflavones derived from *K. parviflora* was tested for anti-inflammatory activity, and results suggested that 5-hydroxy-3,7,3′,4′-tetramethoxyflavone can effectively suppress the lipopolysaccharide induced nitric oxide release, and prostaglandin E2 in RAW264.7 cells, while inactive on tumor necrosis factor-alpha (TNF-γ) release [29]. The ethanolic extract of *K. parviflora* has been studied for the improvement of sexual activities in a rodent model. The results suggested that the supplementation of about 240 mg/kg BW of *K. parviflora* extract decreased the time of rat courtship behavior, and the study warned that the consumption of a high dose of *K. parviflora* is not advisable [30]. The ethyl acetate extract of *K. parviflora* showed anti-obesity like activity such as reduce the body mass and accumulation of visceral fat, consequences of diabetic conditions, in obese type II diabetes mice model [31].

The scientific reports about the properties of FKJ were limited. The present study explained the changes in TPC, and TAC of *K. parviflora* during fermentation, and also reported the alteration in pH, acidity, reducing sugar, and ethanol content of FKJ.

Table 1: The load of pathogenic microbes in fermented FKJ.

Parameter assessed	Samples	
	F1-F4	C1-C4
E. coli	Not detected	Not detected
Salmonella spp.	Not detected	Not detected
Yeast	Not detected	Not detected
Mold	Not detected	Not detected

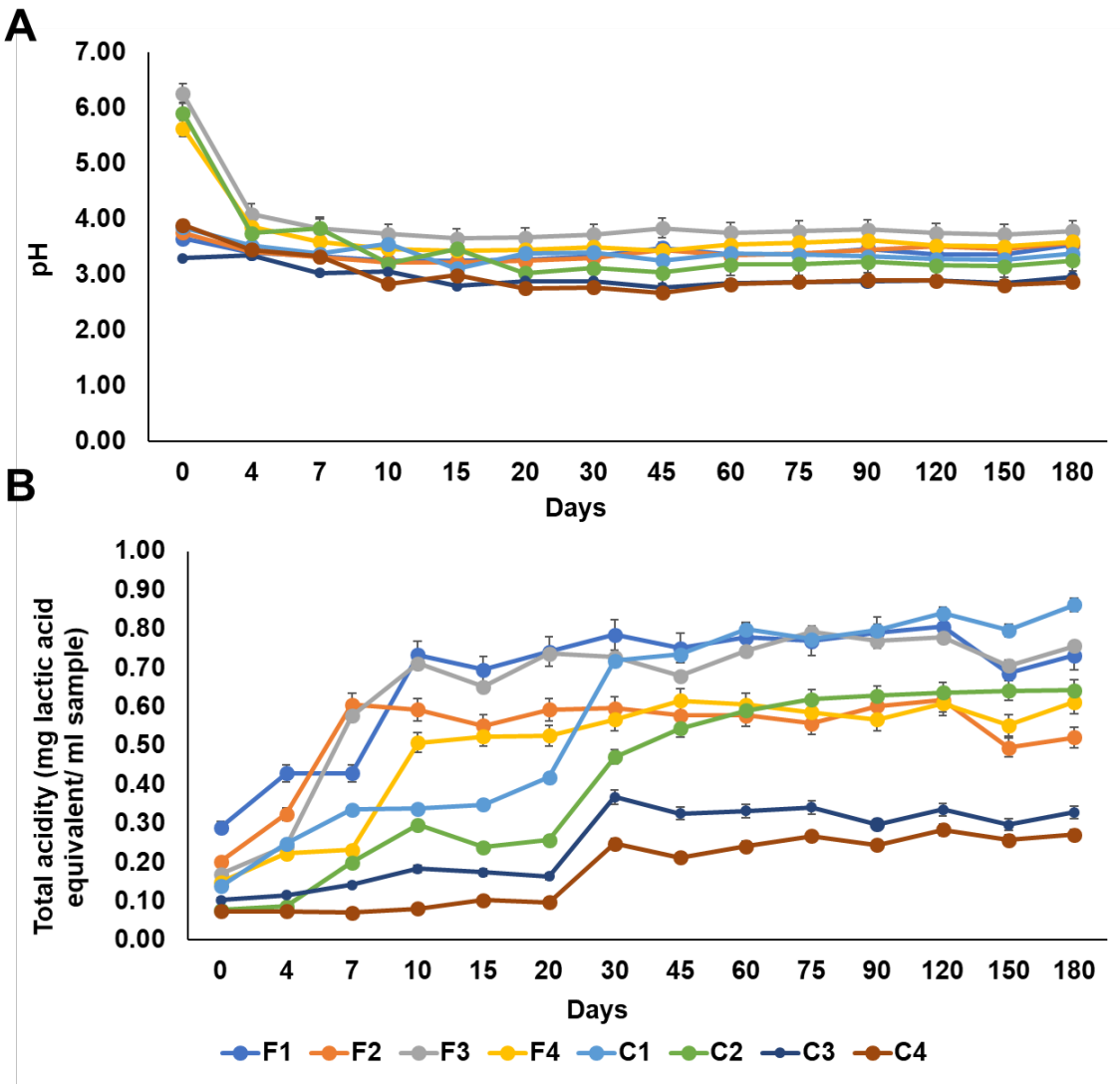

Fig 1: pH (A) and acidity (B) profile of fermented *K. parviflora* juice.

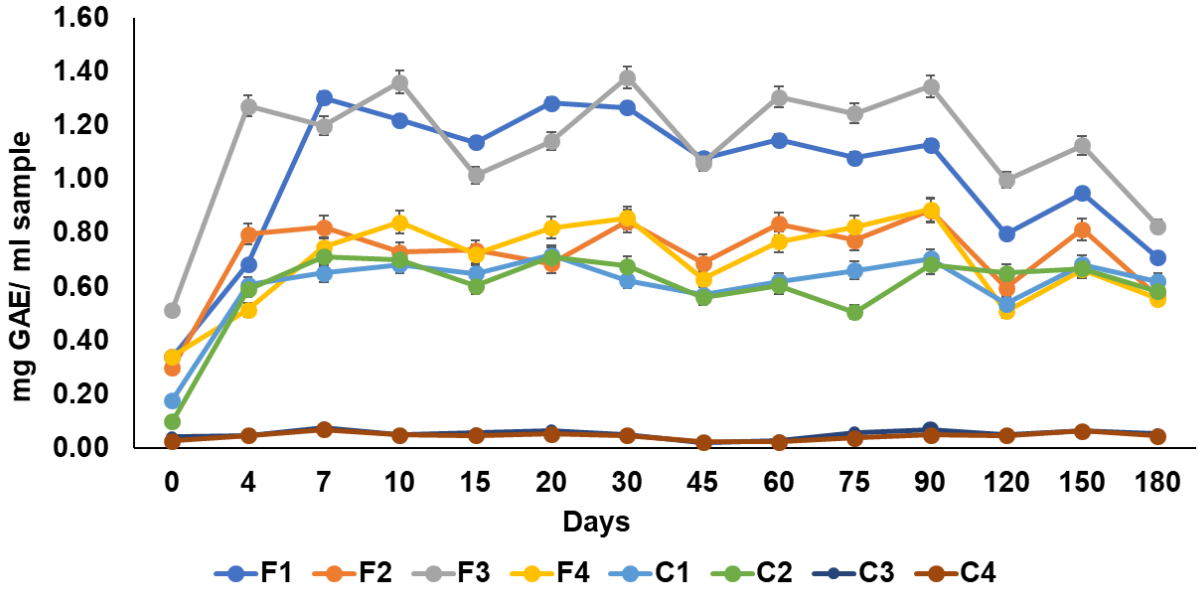

Fig 2: Total phenolic acid content of fermented *K. parviflora* juice. The results were represented as mg Gallic acid equivalent per ml of sample.

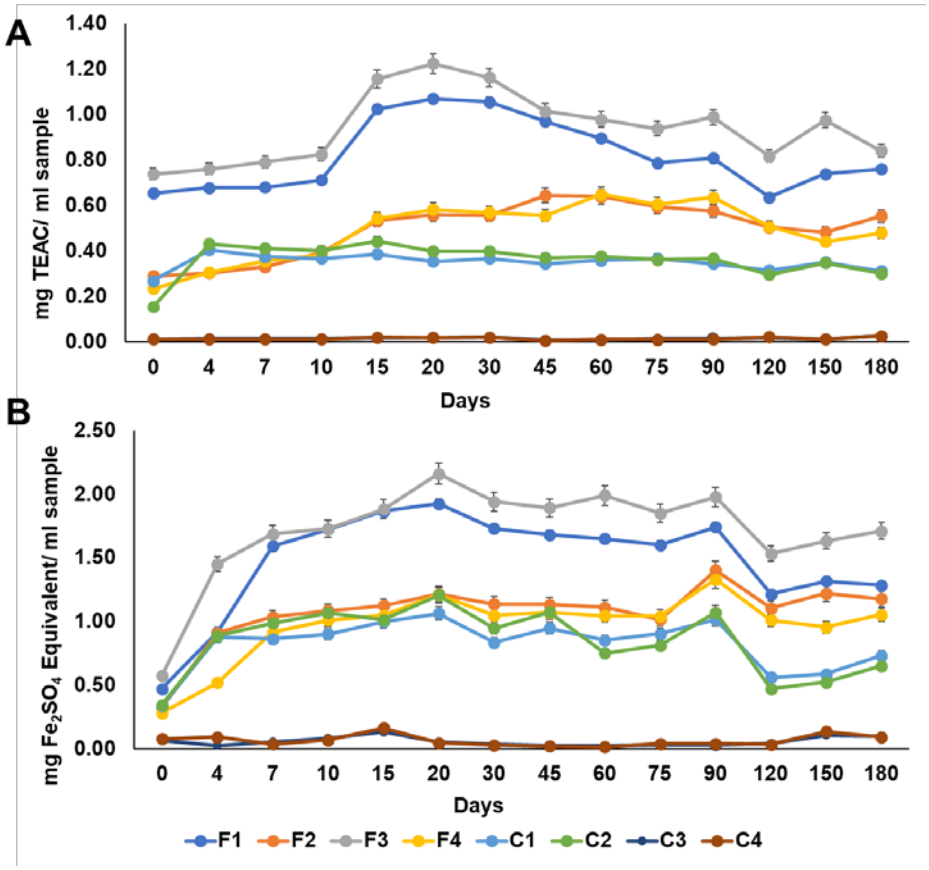

Fig 3: Total antioxidant capacity (A), represented as mg TEAC per ml of sample, and reducing power (B) of fermented *K. parviflora* juice.

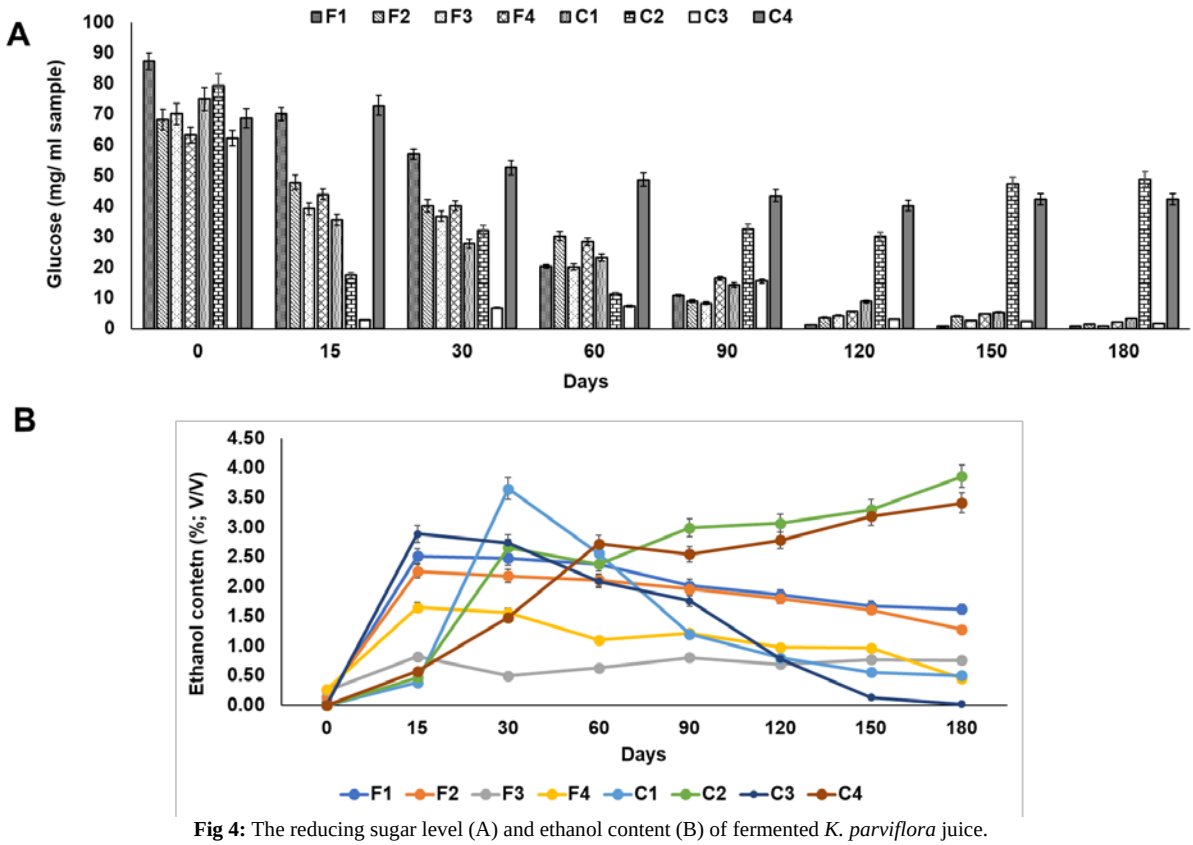

Fig 4: The reducing sugar level (A) and ethanol content (B) of fermented *K. parviflora* juice.

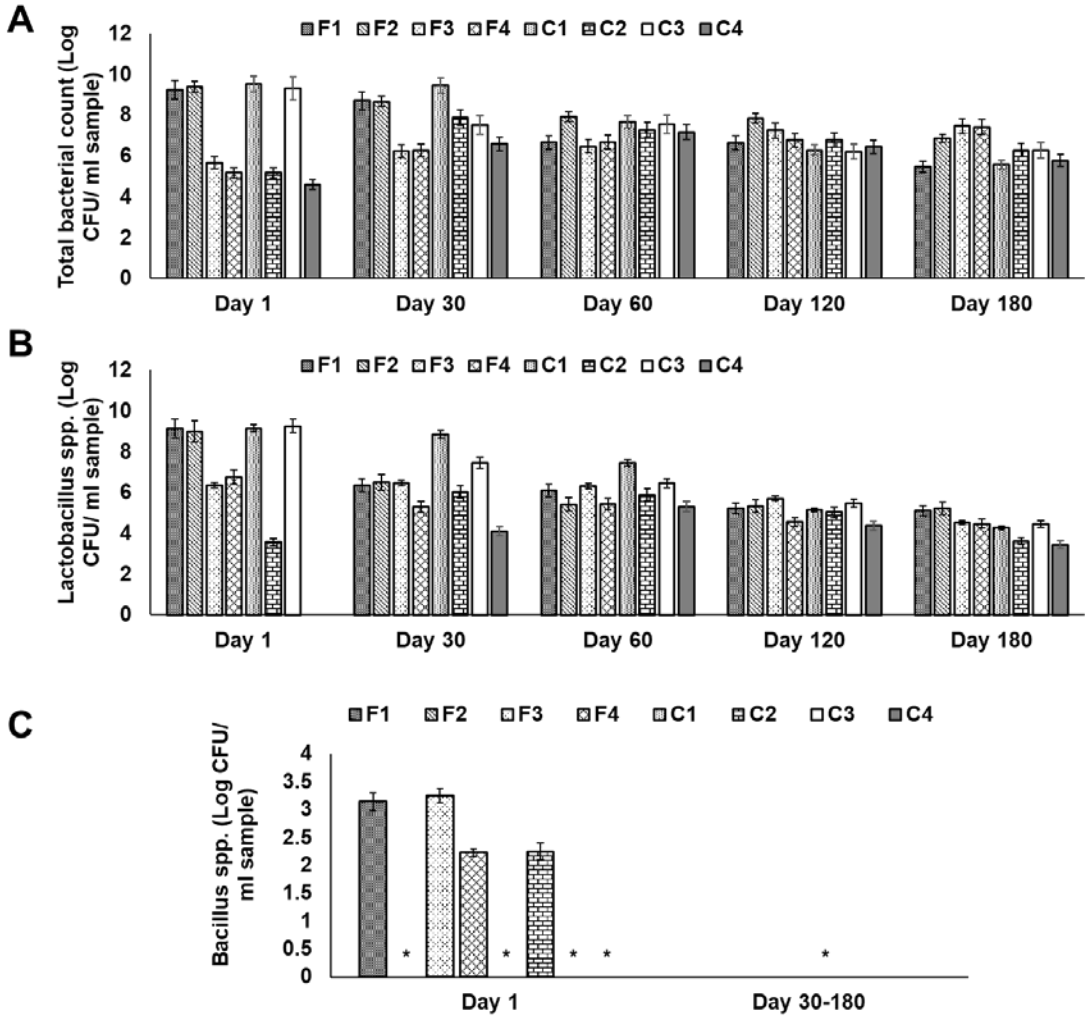

Fig 5: The total bacterial (A), Lactobacillus app. (B), and Bacillus spp. (C) load in fermented *K. parviflora* juice. * indicates no microbes were detected.

CONCLUSION

Lactic acid bacteria (*L. paracasei* HII) fermented *K. parviflora* juice has been developed and studied the fluctuations in bioactive compounds (total phenolic compounds), and bioactivity (antioxidant capacity) during the fermentation process was evaluated. The results revealed that about 15-20 days of fermentation period is sufficient to produce high-quality FKJ regarding phytochemical enrichment and activity. The safer, in terms of quantity, consumption of FKJ can act as a food supplement to manage metabolic disorders like diabetes and obesity and to treat inflammation and carcinogenesis. The present study was a primary attempt to develop FKJ, and further *in vivo* and clinical studies may prove the pharmacological application of FKJ.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTIONS

CC involved in the study design, review, and finalization of the manuscript. BSS and PK contributed to data analysis, manuscript preparation and critical revision of the manuscript. SS, SP, YT, KC is responsible for wet lab experiments, data collection, and analysis. All the authors agree with the content of the manuscript.

REFERENCES

1. Chaturapanich, G., Chaiyakul, S., Verawatnapakul, V., Pholpramool, C. Effects of *Kaempferia parviflora* extracts on reproductive parameters and spermatic blood flow in male rats. *Reproduction*. 2008, *136*, 515 - 522.
2. Chaturapanich, G., Chaiyakul, S., Verawatnapakul, V., Yimlamai, T., Pholpramool, C. Enhancement of aphrodisiac activity in male rats by ethanol extract of *Kaempferia parviflora* and exercise training. *Andrologia.* 2011, *4*, 323 - 328.
3. Lert-Amornpat, T., Maketon, C., Fungfuang, W. Effect of *Kaempferia parviflora* on sexual performance in streptozotocin-induced diabetic male rats. *Andrologia*. 2017, *49*, e12770. https://doi.org.10.1111/and.12770.
4. Akase, T., Shimada, T., Terabayashi, S., Ikeya, Y., Sanada, H., Aburada, M. Antiobesity effects of *Kaempferia parviflora* in spontaneously obese type II diabetic mice. *J. Nat. Med*. 2011, *65*, 73 - 80.
5. Banjerdpongchai, R., Suwannachot, K., Rattanapanone, V., Sripanidkulchai, B. Ethanolic rhizome extract from *Kaempferia*

parviflora Wall. ex. Baker induces apoptosis in HL-60 cells. *Asian Pacific J. Cancer Prev*. 2008, *9*, 595 - 600.
6. Banjerdpongchai, R., Chanwikruy, Y., Rattanapanone, V., Sripanidkulchai, B. induction of apoptosis in the human leukemic U937 cell line by *Kaempferia parviflora* Wall.ex. Baker extract and effects of paclitaxel and camptothecin. *Asian Pacific J. Cancer Prev*. 2009, *10*, 1137 - 1140.
7. Leardkamolkarn, V., Tiamyuyen, S., Sripanidkulchai, B. Pharmacological activity of *Kaempferia parviflora* extract against human bile duct cancer cell lines. *Asian Pacific J. Cancer Prev*. 2009, *10*, 695 - 698.
8. Hossain, M.A., Wongsrikaew, N., Yoo, G., Han, J., Shin, C. Cytotoxic effects of polymethoxyflavones isolated from *Kaempferia parviflora*. *J. Korean Soc. Appl. Biol. Chem*. 2012, *55*, 471 - 476.
9. Potikanond, S., Sookkhee, S., Takuathung, M.N., Mungkornasawakul, P., Wikan, N., Smith, D.R., Nimlamool, W. *Kaempferia parviflora* extract exhibits anti-cancer activity against HeLa cervical cancer cells. *Front. Pharmacol*. 2017, *8*, 630. doi: 10.3389/fphar.2017.00630.
10. Ninomiya, K., Matsumoto, T., Chaipech, S., Miyake, S., Katsuyama, Y., Tsuboyama, A., Pongpiriyadacha, Y., Hayakawa, T., Muraoka, O., Morikawa, T. Simultaneous quantitative analysis of 12 methoxyflavones with melanogenesis inhibitory activity from the rhizomes of *Kaempferia parviflora*. *J. Nat. Med*. 2016, *70*, 179 - 189.
11. Malakul, W., Thirawarapan, S., Ingkaninan, K., Sawasdee, P. Effects of *Kaempferia parviflora* Wall. Ex Baker on endothelial dysfunction in streptozotocin-induced diabetic rats. *J. Ethnopharmacol*. 2011, *133*, 371 - 377.
12. Sae-wong, C., Tansakul, P., Tewtrakul, S. Anti-inflammatory mechanism of *Kaempferia parviflora* in murine macrophage cells (RAW264.7) and in experimental animals. *J. Ethnopharmacol*. 2009, *124*, 576 - 580.
13. Mohanty, D., Ray, P. Evaluation of probiotic and antimicrobial properties of *Lactobacillus* strains isolated from dairy products. *Int. J. Pharm. Pharm. Sci.* 2016, *8*(11), 230 - 234.
14. Prabhurajeshwar, C., Chandrakanth, R.K. Development of *in vitro* methodologies for inhibition of pathogenic bacteria by potential probiotic *Lactobacillus* sps; An evidence for production of antimicrobial substances. *Int. J. Pharm. Pharm. Sci.* 2016, *8*(12), 277 - 286.
15. Mohanty, D., Saini, M.R., Mohapatra, S. *In vitro* study on release of bioactive antimicrobial compounds from dairy products by certain promising probiotic *Lactobacillus* strains. *Int. J. Pharm. Pharm. Sci.* 2017, *9*(4), 27 - 31.
16. Woraharn, S., Lailerd, N., Sivamaruthi, B.S., Wangcharoen, W., Sirisattha, S., Chaiyasut, C. Screening and kinetics of glutaminase and glutamate decarboxylase producing lactic acid bacteria from fermented Thai foods. *Food Sci. Technol. (Campinas)* 2014, *34*(4), 793 - 799.
17. Woraharn, S., Lailerd, N., Sivamaruthi, B.S., Wangcharoen, W., Peerajan, S., Sirisattha, S., Chaiyasut, C. Development of fermented *Hericium erinaceus* juice with high content of L-glutamine and L-glutamic acid. *Int. J. Food Sci. Technol.* 2015, *50*, 2104 - 2112.
18. Woraharn, S., Lailerd, N., Sivamaruthi, B.S., Wangcharoen, W., Sirisattha, S., Peerajan, S., Chaiyasut, C. Evaluation of factors that influence the L-glutamic and γ-aminobutyric acid production during *Hericium erinaceus* fermentation by lactic acid bacteria. *Cyta-J. Food*. 2016, *14*(1), 47 - 54.
19. Peerajan, S., Chaiyasut, C., Sirilun, S., Chaiyasut, K., Kesika, P., Sivamaruthi, B.S. Enrichment of nutritional value of *Phyllanthus emblica* fruit juice using the probiotic bacterium, *Lactobacillus paracasei* HII01 mediated fermentation. *Food Sci. Technol. (Campinas)*. 2016, *36*(1), 116 - 123.
20. Chaiyasut, C., Makhamrueang, N., Peerajan, S., Sivamaruthi, B.S. Assessment of organic acid content, and brix value of representative indigenous fermented plant beverages of Thailand. *Asian J. Pharm. Clin. Res.* 2017, *10*(1), 350 - 354.
21. Chaiyasut, C., Kesika, P., Chaiyasut, K., Sittiyuno, P., Peerajan, S., Sivamaruthi, B.S. 2017. Total phenolic content and free radical scavenging activity of representative medicinal plants of Thailand. *Asian J. Pharm. Clin. Res.* 2017, *10*(11), 137 - 141.
22. Chaiyasut, C., Sivamaruthi, B.S., Peerajan, S., Sirilun, S., Chaiyasut, K., Kesika, P. 2017. Assessment of heavy metals, minerals, alcohol, and fusel oil content of selected fermented plant beverages of Thailand. *Int. Food Res. J.* 2017, *24*, 126 - 133.
23. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 1959, *31*(3), 426 - 428.
24. Sirilun, S., Sivamaruthi, B.S., Kesika, P., Makhamrueang, N., Chaiyasut, K., Peerajan, S., Chaiyasut, C. Development and evaluation of mustard green pickled liquid as starter for *Morinda citrifolia* Linn fermentation. *Int. Food Res. J.* 2017, *24*, 2170 - 2176.
25. Sivamaruthi, B.S., Pengkumsri, N., Saelee, M., Kesika, P., Sirilun, S., Peerajan, S., Chaiyasut, C. Impact of physical treatments on stability and radical scavenging capacity of anthocyanidins. *Int. J. Pharm. Pharm. Sci.* 2016, *8*(1), 162 - 167.
26. Chaiyasut, C., Sivamaruthi, B.S., Pengkumsri, N., Sirilun, S., Peerajan, S., Chaiyasut, K., Kesika, P. Anthocyanin profile and its antioxidant activity of widely used fruits, vegetables, and flowers in Thailand. *Asian J. Pharm. Clin. Res.* 2016, *9*(6), 218 - 224.
27. Pattananandecha, T., Sirilun, S., Duangjitcharoen, Y., Sivamaruthi, B.S., Suwannalert, P., Peerajan, S., Chaiyasut, C. Hydrolyzed inulin alleviates the azoxymethane-induced preneoplastic aberrant crypt foci by altering selected intestinal microbiota in Sprague-Dawley rats. *Pharm. Biol*. 2016, *54*, 1596 - 1605.
28. Thao, N.P., Luyen, B.T.T., Kim, J.H., Jo, A.R., Yang, S.Y., Dat, N.T., Minh, C.V., Kim, Y. H. Soluble epoxide hydrolase inhibitory activity by rhizomes of *Kaempferia parviflora* Wall. ex Baker. *Med. Chem. Res*. 2016, *25*, 704 - 711.
29. Tewtrakul, S., Subhadhirasakul, S. Effects of compounds from *Kaempferia parviflora* on nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha productions in RAW264.7 macrophage cells. *J. Ethnopharmacol*. 2008, *120*, 81 - 84.
30. Sudwan, P., Saenphet, K., Saenphet, S., Suwansirikul, S. Effect of *Kaempferia parviflora* Wall. ex. Baker on sexual activity of male rats and its toxicity. *Southeast Asian J. Trop. Med. Public Health*. 2006, *37*(Suppl 3), 210 - 215.
31. Shimada, T., Horikawa, T., Ikeya, Y., Matsuo, H., Kinoshita, K., Taguchi, T., Ichinose, K., Takahashi, K., Aburada, M. Preventive effect of *Kaempferia parviflora* ethyl acetate extract and its major components polymethoxyflavonoid on metabolic diseases. *Fitoterapia*. 2011, *82*, 1272 - 1278.