RESEARCH ARTICLE

Unveiling phytochemicals in *P. guajava* leaves: Thin layer chromatography (TLC) investigation using methanol, chloroform and hexane

Wan Zahirul Syahmi Wan Bakree, Hannis Fadzillah Mohsin*, Ibtisam Abdul Wahab

Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia

***Corresponding Author**

Hannis Fadzillah Mohsin
Email:
hannis@uitm.edu.my

Abstract:

Guava (*Psidium guajava* L.), a member of the Myrtaceae family, is a widespread tropical tree, grown in a wide variety of tropical and subtropical regions for its medicinal and nutritional properties. The fruit, bark, root, and leaves of the guava plant have all been used to treat various diseases and ailments. Guava leaves are the most often and extensively utilised part of the guava plant for traditional purposes, worldwide. Due to their diverse phytochemical composition, guava leaf extracts have been studied for their biological activities, including anticancer, antidiabetic, antioxidant, antidiarrheal, and antibacterial properties. In this study, *P. guajava* leaves were extracted by using organic solvents of different polarities. The groups of compounds in the extract were identified via thin-layer chromatography (TLC) and preliminary qualitative analysis. The leaves powder was macerated with methanol, chloroform, and hexane, in parallel. The TLC analysis was carried out by mixing hexane: ethyl acetate (7:3) and hexane: ethyl acetate (3:7) to identify polar and non-polar compounds in the guava leaves extracts. The preliminary qualitative analysis is performed to detect the presence of alkaloid and phenolics by using the Dragendorff's reagent and 5% ferric chloride solution. Results from the phytochemical screening shows the presence of flavonoids, terpene, alkaloids, carbohydrates, and phenolics. In summary, this project could provide valuable insights and supplementary details for the plant's monograph.

Keywords: chromatography, guava, monograph, *Psidium*, sustainability

1. INTRODUCTION

Psidium guajava (P. guajava) plant, also known as guava (Figure 1; Globinmed, 2022), belongs to the Myrtoideae subfamily of Myrtaceae family (Gill, 2016). It was reported to have medicinal, nutritional, and industrial qualities. It is also a popular local crop because of its accessibility, low cost, transport and handling durability, and consumer preference (Medina & Herrero, 2016). The guava fruit has been produced and used in tropical places such as India, Bangladesh, Indonesia, Pakistan, and South America. It is originated in tropical America, but because of its adaptability, it is grown worldwide in tropical and subtropical regions (Díaz-de-Cerio et al., 2017). Guava has a lot of seeds that are quite viable, which contributes to its adaptability. The Spaniards are responsible for spreading the guava crop over the Pacific and the rest of the world. Unfavourable weather patterns have little impact on guava cultivation. However, the crop must be carefully managed to produce a high-quality harvest. Currently, India is the world's largest guava producer, followed by China, Thailand, and Pakistan (Gill, 2016). The guava's flavour, appearance, and the functional nutrients, are among the characteristics for its success in the agricultural & business (Uchôa-Thomaz et al., 2014).

The guava tree is a short tree that grows to a height of 7-10 metres. The plant forms a symmetrical dome and is distinguished by its thin, smooth, greenish-brown scaly bark that reveals a greenish layer underneath (Gill, 2016). The plant's extensive branching network results in various canopy shapes (Medina & Herrero, 2016). White, incurved-petalled flowers cover the plant, and the plant itself has a slight aroma. Guava leaves are smooth and have a light green hue (Figure 1). The leaves are coarsely hairy underneath, with prominent venation on the top surface (Gill, 2016; Kumar et al., 2021). The leaf form ranges from elliptic to oblong-lanceolate, rarely rounded, 4–10 cm long and 2.5–6 cm wide, and with short petioles 2–7 mm long (Medina & Herrero, 2016).

Guava leaves are the most often and extensively utilised part of the guava plant for medicinal purposes, worldwide. Numerous Latin American and Caribbean countries, as well as India, Bangladesh, Pakistan, Kenya, Nigeria, Madagascar, Malaysia, Namibia, South Africa, Papua New Guinea, Thailand, and Indonesia, have traditionally used guava-derived products to treat a variety of communicable and non-communicable diseases, including gastrointestinal disease, hepatic damage, bacterial and fungal infection, fever, rheumatism, respiratory illness, cough, diabetes, pain, wounds, mouth ulcers, uterine bleeding, blennorrhagia and menstrual

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disorders. Due to their diverse phytochemical composition, guava leaf extracts have been studied for their biological activities, including anticancer, antidiabetic, antioxidant, antidiarrheal, and antibacterial properties (Ugbogu et al., 2022; Lok et al., 2023). As such, this study aims to extract *P. guajava* leaves using organic solvent of different polarities and identify the group of compounds (Figure 2) in the extract of this research sample, via thin-layer chromatography (TLC) and preliminary qualitative analysis.

Figure 1. A fruiting *Psidium* plant (left) and a mature *Psidium* fruit with seeds (right).

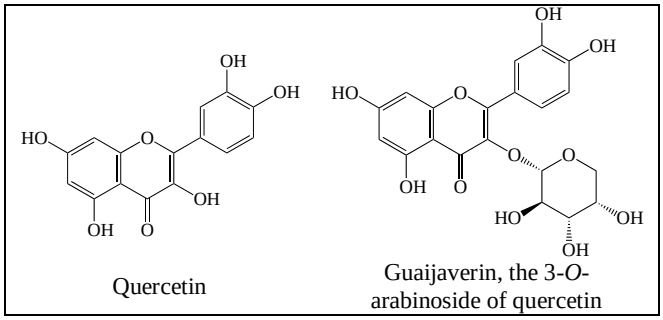

Figure 2. The structures of natural molecules from *P. guajava* (Prabu et al., 2014).

2. MATERIALS AND METHODS

2.1. Equipment and materials

The equipment includes pre-coated silica gel 60 F254 sheets (Merck, Darmstadt, Germany), TLC development glass chambers, Whatman filter paper number 1, Cole-Parmer ultraviolet (UV) viewing cabinet, and Bosch GHG 630 DCE heat gun. The plant specimen was *P. guajava* leaves. They were collected from a guava tree in Terengganu, Malaysia, in March 2022. The raw materials were authenticated by one of the authors (Mohsin, H. F.) by using macroscopic evidences. The chemicals were reagent grade, which include ethyl acetate, methanol, hexane, Dragendorff's reagent, anisaldehyde–sulfuric acid reagent, 5% ferric chloride solution, and standard quercetin (Sigma). Distilled water was also utilised to compose the TLC mobile phase.

2.2. Preparation of extracts

The extraction of *P. guajava* leaves was prepared according to procedures described by Biswas et al., (2013) and Seo et al., (2014). Prior to the extraction, the leaves were cut in small pieces and stored in drying rack for 3 days. After the guava leaves have dried, they were put into a blender and ground into a powder. The sample is weighted prior to the extraction. The solvent extraction were methanol, chloroform, and hexane. 100 ml solvents were added to the 10 g of powdered leaf samples in the separate flask, covered with an aluminium foil, to prevent evaporation and light. The samples were agitated and the extracts were filtered after a week by using Whatman filter paper No. 1. Subsequently, they were dried at 40°C using a rotary evaporator. The weight of each extract was then measured and recorded.

2.3. Thin layer chromatography (TLC)

The procedures for TLC screening were modified from Moura et al., (2012) and ALhaidari et al., (2019). The TLC plate was prepared using a pre-coated silica gel 60 F_{254} sheets (Merck, Darmstadt, Germany) as a stationary phase with a length of 10 cm and 5 cm in width. Using a pencil, one centimetre was drawn from each baseline to denote the solvent front and sample. The mobile phase consisting of hexane: ethyl acetate (7:3) was prepared and transferred into TLC tank. Using a capillary tube, three drops of each extract and a quercetin standard were put at the bottom of the TLC plate. The plate was then put for separation in a saturated TLC tank. When the mobile phase reached two-thirds the length of the plate, it was removed from the tank and left to dry at room temperature until the solvent has been completely removed. The silica plate was examined using UV light with a wavelength of 254 nm from a UVP portable lamp in a Cole-Parmer UV viewing cabinet, to enhance the compound's visibility. The spots were circled with a pencil, and the Rf value was calculated. The procedure was repeated with different mobile phase which were hexane: ethyl acetate (3:7). A glass sprayer containing an anisaldehyde–sulfuric acid reagent was used to perform the chemical derivatization. The plate was dried at 100°C using a Bosch heat gun until the visible compound could be detected.

2.4. Preliminary qualitative analysis

The qualitative analysis (Chakraborty et al., 2010); Shaikh & Patil, 2020) was carried out to detect the presence of alkaloids and phenolics in each extract of guava leaves. For the test for alkaloid, two ml of Dragendorff's reagent was added to a few ml of filtrate by the side of the test tube. A prominent yellow participate could indicate a positive result. Meanwhile, the extract (50 mg) was dissolved in 5 ml of distilled water, to detect the presence of phenolic compounds. Then, a few drops of 5% ferric chloride solution were added to the solution. A dark green or bluish black colour would indicate the presence of phenolic compounds.

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3. RESULTS AND DISCUSSION

3.1. Percentage of yield extract

The yield of extracts was shown in Table 1. The percentage yield was calculated based on 10 g of the sample powder used in the extraction and the mass of crude extracts. The amount obtained from methanol, chloroform and hexane extracts were 3.75 g, 2.75 g, and 4.08 g respectively, in which hexane provided the highest yield.

Table 1. The percentage yield of *P. guajava* leaves extracts.

Solvent	Weight of the extract (g)	Percentage yield (% w/w)
Methanol	1.53	15.30
Chloroform	0.98	9.80
Hexane	1.35	13.50

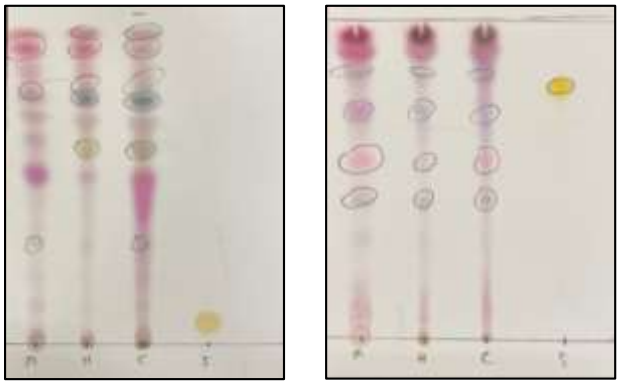
3.2. Thin Layer Chromatography (TLC) screening of *P. guajava leaves*

It is noted that the TLC data on the *Psidium* extract was not available in the national online monograph (Globinmed, 2022). Nevertheless, Metwally et al., (2011) provided a guideline on a typical chromatographic pattern of the ethyl acetate extract of the guava leaf. The compound separation could be achieved by utilising quercetin (labelled as S) Figure 3) in the TLC examination. The silica gel plate was subjected to a middle polar system with ethyl acetate : methanol : water : acetic acid (100:2:1:4 drops) as the developing solvent. Meanwhile, the ammonia was used as the revealing reagent. In this experiment, five mobile phases were tested to achieve a good resolution in moving the compounds in the extract via capillary action. The use of hazardous benzene as the TLC solvent could be avoided (Sarkar et al., 2011). The results of each mobile phases tested were shown in Table 2.

Finally, hexane: ethyl acetate (7:3) and hexane: ethyl acetate (3:7) were used as the mobile phases as they showed better results for polar and non-polar compounds, as compared to the rest of the mobile phases. Quaternary acidified solvent system for the TLC (Shirke et al., 2023) was not adapted in this study. Figure 4 showed that the derivatization of the TLC plate by using anisaldehyde–sulfuric acid reagent provided the fingerprints of the investigated samples, that were not seen under UV light (254 nm). This reagent produced a result in which the compound is more pronounced. There were six to seven identified spots in methanol, hexane, and chloroform extracts, on the TLC plate of hexane: ethyl acetate (7:3), after spraying with anisaldehyde–sulfuric acid. On the other hand, for the TLC plate of hexane: ethyl acetate (3:7), four to five spots were identified in methanol, chloroform, and hexane extracts, after the TLC plate was introduced to the sulfuric anisaldehyde. The Rf value for standard quercetin in hexane: ethyl acetate (7:3) and (3:7) are 0.07 and 0.80, respectively.

Table 2. The mobiles phases for TLC screening.

Solvent mixtures	Ratio (v/v)	Observation
Hexane: ethyl acetate	8:2	Good separation but it is less distinctive.
Chloroform: methanol	7:3	This mobile phase causes streaking on TLC plate which may be due to the concentrated sample.
Methanol: hexane	1:1	This mobile phase cause smearing upward.
Hexane: ethyl acetate	7:3	Excellent and more distinctive separation for non-polar compounds.
Hexane: ethyl acetate	3:7	It is more polar, and it shows good separation for polar compounds.



(i) (ii)

Figure 3: The TLC profile of *P. guajava* leaves extract, using sulfuric anisaldehyde as the staining reagent. Two mobile phases were used, (i) hexane: ethyl acetate (7:3) and (ii) hexane: ethyl acetate (3:7).

Based on Figure 4, the TLC plate using hexane: ethyl acetate (7:3), which was sprayed with anisaldehyde–sulfuric acid, showed the presence of carbohydrate and polyols, as the spots appeared as pink/red for all extracts. Furthermore, there were blue spots on the TLC plate for all extracts, which indicated the presence of monoterpene (Gerlach et al., 2018). Spots with Rf value of 0.91, 0.89 and 0.89 in methanol, hexane and chloroform extracts, respectively indicated the presence of phytosterols. Reference marker of 0.88 has been used to conclude phytosterols on the TLC plate (Chakraborty et al., 2010). There is a presence of flavonoid, specifically quercetin, for all extracts in hexane: ethyl acetate (3:7). The spots with Rf value of 0.84, 0.84 and 0.82 in methanol, hexane

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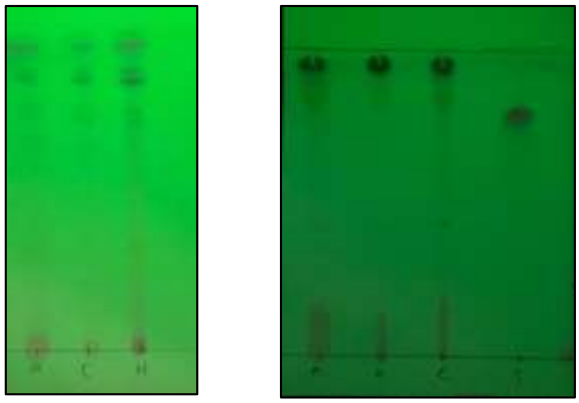
and chloroform extracts, respectively shared comparatively similar Rf value with standard quercetin, which was 0.80.



(i) (ii)

Figure 4: The chromatographic profile of *P. guajava* leaves extract, visualized under UV (254 nm). Two mobile phases were used, (i) hexane: ethyl acetate (7:3) and (ii) hexane: ethyl acetate (3:7).

Astuti et al., (2017) conducted the stability tests of the *Psidium* compounds on the High Performance Thin Layer Chromatography (HPTLC) plate and in solution. Based on the HPTLC, the guava leaves extract is stable on the plate and in methanolic solution because there were no differences in the zone intensity, no disappearing zones and no new zones could be observed. Tiwari et al. (2021) was also in agreement that HPLTC method is reproducible and selective for the estimation of four bioactive compounds, including quercetin (Figure 2), gallic acid, eugenol and β-sitosterol in *P. guajava* extracts. Quercetin-3-*O*-sulfate is the major compound from the *P. guajava* ethanol extract and was isolated from this plant for the first time (Nguyen et al., 2023), under neutral condition. However, the isolation of quercetin-3-*O*-sulfate could not be summarised. The TLC eluting mixtures in this study was not parallel to that quintet solvent system of ethyl acetate – formic acid – acetic acid – water – methanol (50: 2: 2: 5: 2), to order to detect quercetin-3-*O*-sulfate (Rf = 0.53) (Nguyen et al., 2023). The polarity of quercetin-3-*O*-sulfate may not suit the nonpolar condition of the mobile TLC environment in here. These work could be another guide in experimenting LC of the *Psidium* components in the future.

3.3. Preliminary qualitative analysis

All extracts were tested with a Dragendorff's reagent and 5% ferric chloride solution, to detect the presence of alkaloid and phenolic compounds. Based on Table 3, only methanol extract showed positive results for alkaloid and phenolics. In test for alkaloid, methanol extracts showed positive result due to its colour changes from greenish black to prominent yellow. This indicated the presence of alkaloid. Meanwhile, in test for flavonoid, the addition of 5% ferric chloride solution showed positive results in methanol extracts by the appearance of bluish black colour, which indicated the presence of phenolic compounds. However, chloroform and hexane extracts showed negative results for both alkaloid and phenolics.

Table 3. The phytochemical screening of guava extracts.

Extracts	Alkaloid	Phenolic compound
Methanol	+	+
Chloroform	-	-
Hexane	-	-

+ = present; - = absent

4. CONCLUSION

P. guajava leaves were successfully extracted through maceration by using methanol, chloroform, and hexane. The TLC solvent systems consisted of hexane: ethyl acetate (7:3) and (3:7), which manifested the best separation of compounds. The phytochemical screening of methanol, chloroform, and hexane extracts revealed the presence flavonoids, terpene, alkaloids, carbohydrates, and phenolic compounds by positive reaction with the respective test reagents. The analysis showed the presence of phytoconstituents in methanol and chloroform. The result of the study could be useful for the description and additional information for the plant's monograph.

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REFERENCES

ALhaidari, S. A., Al-Deen, A. M., Al-Kaf, A. G., Al-Hadi, F. A., Abdullah, Q., Mahbashi, A. A. & Al-Dubai, F. A. (2019). Antimicrobial And Antioxidant Activity Of *Psidium Guajava* (Guava) Medicinal Plant Leaves Used In Folk Medicine For Treatment Of Wounds And Burns In Hufash District Al Mahweet Governorate–Yemen. *Journal of Pharmaceutical Research*, *4*(2), 6-13.

Astuti, M. Darusman, L. K. & Rafi, M. (2017). High performance thin layer chromatography fingerprint analysis of guava (*Psidium guajava*) leaves. IOP Conf. Series: *Journal of Physics: Conf. Series*, 835, 012018

Biswas, B., Rogers, K., McLaughlin, F., Daniels, D. & Yadav, A. (2013). Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. *International Journal Of Microbiology*, Article ID 746165, 7 pages, 2013. https://doi.org/10.1155/2013/746165.

Chakraborty, D. D., Ravi, V. & Chakraborty, P. (2010). Phytochemical evaluation and TLC protocol of various extracts of *Bombax ceiba* Linn. *International Journal of Pharmaceutical Sciences and Research*, *1*(8), 66-73.

Díaz-de-Cerio, E., Verardo, V., Gómez-Caravaca, A. M., Fernández-Gutiérrez, A. & Segura-Carretero, A. (2017). Health effects of *Psidium guajava* L. Leaves: An overview of the last decade. *International Journal Of Molecular Sciences*, *18*(4), 897.

Gerlach, A. D., Gadea, A., da Silveira, R. M., Clerc, P., Lohézic-le Dévéhat, F. (2018). The use of anisaldehyde sulfuric acid as an

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alternative spray reagent in TLC analysis reveals three classes of compounds in the genus *Usnea Adans* (Parmeliaceae, lichenized Ascomycota). doi:10.20944/preprints201802.0151.v1.

Gill, K. S. (2016). Guavas in *Encyclopedia of Food and Health*, Caballero B, Finglas, PM, Toldrá, F (Eds.), Academic Press, 270–277. https://doi.org/10.1016/B978-0-12-384947-2.00363-9.

Globinmed (2022). Malaysian Herbal Monograph, *Psidium guajava* L., viewed on 9th August 2023, https://globinmed.com/medicinal_herbs/psidium-guajava-l-r/.

Kumar, M., Tomar, M., Amarowicz, R., Saurabh, V., Nair, M. S., Maheshwari, C., Sasi, M., Prajapati, U., Hasan, M., Singh, S., Changan, S., Prajapat, R. K., Berwal, M. K. & Satankar, V. (2021). Guava (*Psidium guajava* L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. *Foods*, *10*(4), 752. doi: 10.3390/foods10040752.

Lok, B., Babu, D., Tabana, Y., Dahham, S. S., Adam, M. A. A., Barakat, K. & Sandai, D. (2023). The Anticancer Potential of *Psidium guajava* (Guava) Extracts. *Life*., *13*(2), 346. https://doi.org/10.3390/life13020346

Medina, N. N. & Herrero, J. V. (2016). Guava (*Psidium guajava* L.) cultivars: an important source of nutrients for human health. In *Nutritional Composition Of Fruit Cultivars*, 287-315, Academic Press.

Metwally, A. M., Omar, A. A., Ghazy, N. M., Harraz, F. M. & El Sohafy, S. M. (2011). Monograph of *Psidium guajava* L. leaves. *Pharmacognosy Journal*, *3*(21), 89 – 104.

Moura, P. M., Prado, G. H., Meireles, M. A. & Pereira, C. G. (2012). Supercritical fluid extraction from guava (*Psidium guajava*) leaves: global yield, composition and kinetic data. *The Journal of Supercritical Fluids*, *62*, 116-22.

Nguyen, P.-D., Hérent, M.-F., Le, T.-B., Bui, T.-B.-H., Bui, T.-B.-H., Do, T.-T.-H., Nguyen, T.-P., Scippo, M.-L., Kestemont, P., Leclercq J. Q. (2023). Isolation of quercetin-3-O-sulfate and quantification of major compounds from *Psidium guajava* L. from Vietnam. *Journal of Food Composition and Analysis*, 115, 104928.

Sarkar, J., Pal, S., Bhattacharya, S. & Biswas, M. (2011). Thin layer chromatographic profiling and evaluation of analgesic activity of Psidium guajava leaf extracts in mice. *Journal of Advanced Pharmacy Education & Research*, 2, 177-183.

Shaikh, J. R. & Patil, M. K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603-608.

Shirke, K. S., Kamble, T. S., Uppar, K. B., Naware, N. S., Bangar, S. B., Ambatkar, S. S., Patil, M. & Jain, A. (2023). A mini review on the analytical method and its validation for *Psidium guajava*. *SciPhy*, *2*(2), 13-21.

Seo, J., Lee, S., Elam, M. L., Johnson, S. A., Kang, J. & Arjmandi, B. H. (2014). Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. *Food Science & Nutrition*, *2*(2), 174-80.

Tiwari, S., Shukla, P.K., Dwivedi, J. & Khatoon, S. (2021). Simultaneous quantification of four active metabolites in *Psidium guajava* L. by a validated high-performance thin-layer chromatography method. *JPC-J Planar Chromat*., *34*, 61–69. https://doi.org/10.1007/s00764-021-00083-y

Ugbogu, E. A., Emmanuel, O., Uche, M. E., Dike, E. D., Okoro, B. C., Ibe, C., Ude, V. C., Ekweogu, C. N. & Ugbogu, O. C. (2022). The ethnobotanical, phytochemistry and pharmacological activities of *Psidium guajava* L., *Arabian Journal of Chemistry*, *15*(5), 103759.

Prabu, G. & Gnanamani, A. & Sayeed, S. (2006). Guaijaverin - A plant flavonoid as potential antiplaque agent against *Streptococcus mutans*. *Journal Of Applied Microbiology*, *101*, 487-95. 10.1111/j.1365-2672.2006.02912.x.

Uchôa-Thomaz, A. M. A., Sousa, E. C., Carioca, J. O. B., de Morais, S. M., de Lima, A., Martins, C. G., Alexandrino, C. D., Ferreira, P. A. T., Rodrigues, A. L. M., Rodrigues, S. P., Thomaz, J. C. de A., Silva, J., do N. & Rodrigues, L. L. (2014). Chemical composition, fatty acid profile and bioactive compounds of guava seeds (*Psidium guajava* L.). *Food Sci. Technol*. (Campinas), *3*(1), 1-8.

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