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Research Article

Preliminary Identification and Quantification of Quercetin Concentration and Its Comparison in Psidium Guajava L. (Guava) Fruit Ethanol Extract 50% and 70%

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ABSTRACT

In 80 % of developing countries as stated by official fact sheets and report a large proportion of the society still relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. The study on *Psidium Guajava* L. fruit ethanol extract from Dukuhwaluh village, Purwokerto, Central Java, Indonesia showed its potential in increasing the number of megakaryocytes, followed by the rise of thrombocyte values. The study's objective was to compare the quantity of the quercetin content in 50% and 70% ethanol. This study was of experimental design and began by determining the effective concentration of two groups of guava fruits ethanol extract. The percentage of quercetin content dissolved within 50% ethanol was 54.7344 mg/kg, and 70% ethanol was 28.8420 mg/kg respectively. Quercetin content of *Psidium Guajava* L. fruit ethanol extract fruits in 50% hydroethanolic was higher than that of 70% hydroethanolic extract.

Key words: *Psidium Guajava* L., Quercetin, Ethanol extract.

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INTRODUCTION

A large proportion of the society in Indonesia still relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Herbal medicines have often maintained their popularity for historical and cultural reasons. In this modern setting, the pharmaceutical industry has come to consider herbal medicine as a source for the identification of bioactive agents that can be produced in simpler forms and commercially distributed. However, usually, they are not looking to study rare plant species; they want to test the most commonly-used species.[1,2] Herbal medicines used as dietary supplements came in form of tablets, capsules, powders, teas, extracts, and fresh or dried plants.[3] Psidium *guajava* L. (Guava) (Figure 1) is found in countries with hot climates in areas such as South America, Europe, Africa, and Asia.[4] All parts of Guava have an old history of both medicinal and non-medicinal value. Indonesia as part of Asia is a tropical developing country with rich biodiversity and has been acknowledged by the world, confirmed that this plant is available in varieties and belongs to the *Myrtaceae* family, grows to 35 feet, and is recognizable by its brown and peeled off the bark.[5] The chemical composition of the fruits would be dependent on factors such as variety, maturity, and the environmental conditions within which they are grown.[6,7]

Taxonomy

Kingdom	:	Plantae
Order	:	Myrtales
Family	:	Myrtaceae
Subfamily	:	Myrtoideae
Genus	:	Psidium
Species	:	Guajava
Binomial name	:	*Psidium guajava* L.

A large number of antioxidants and phytochemicals including essential oils, polysaccharides, minerals, vitamins, enzymes, and triterpenoid acid alkaloids, steroids, glycosides, tannins, flavonoids, and saponins were found on Guava fruits, leaves, and plant as well as a higher content of vitamin C and vitamin A.[8] The fruit contains saponin, oleanolic acid, lyxopyranoside, arabopyranoside, guaijavarin, quercetin, and flavonoids.[9,10] Guava fruit, especially the pink one, contains antioxidants in high amounts and anti-providing nutrients which are essential for life and help control free radical activities. It contains a variety of phytochemicals that are beneficial for human health like diabetes, obesity, and high blood pressure, and also helpful in decreasing the incidences of degenerative diseases, inflammation, heart disease, cancer, arteriosclerosis, and arthritis.[11]

In fruits, the most abundant oxidants are polyphenols and ascorbic acid. Polyphenols are mostly flavonoids and are mainly present in glycoside and ester forms.[12] So far, quercetin is the most extensively studied flavonoid that has been shown to exhibit antioxidant, antiviral, antibacterial, anti-inflammatory, and anticarcinogenic properties.[13]

Quercetin is categorized as a flavonol, one of the six subclasses of flavonoid compounds. Quercetin contains three rings and five hydroxyl groups. Quercetin mainly occurs as glycosides, ethers, and, to a lesser extent, sulfates, and glycosylation of quercetin increases its hydrophilicity, stability, and bioavailability.[14-16] The International Union of Pure and Applied Chemistry (IUPAC) nomenclature for

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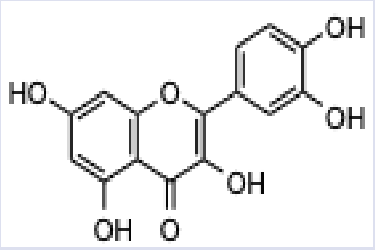

Figure 1: Chemical structure of quercetin

Figure 2: Dukuhwaluh village, Purwokerto

quercetin is 3, 31, 41, 5, 7-pentahydroxyflvanone (or its synonym 3, 31, 41, 5, 7-pentahydroxy-2-phenylchromen-4-one), has molecular weight 302,23 Dalton (Figure 2).

For decades efforts have been undertaken to isolate, characterize, and extract bioactive components of natural plant sources. Extraction plays an important role as the primary step. The aim of this process is to obtain the maximum concentration of target compounds and of the highest activity of the extracts.[17] The extraction and purification of phytochemical and antioxidant substances from the plant material are generally affected by various factors including time, temperature, solvent concentration, and solvent polarity. A chemical test for the screening of bioactive chemical constituents in the guava was carried out with extracts using a guide of phytochemical methods as described by Harborne.[18] The extract was chemically tested for the presence of flavonoids, quinone, triterpenoid/steroid, alkaloids, tannins, and saponins. Depending on the chemical nature, various phytochemicals are extracted in solvents of different polarities as no single solvent may be reliable to extract all the phytochemical and antioxidant compounds present in the plant material.[19,20] The serial exhaustive extraction method involves the successive extraction with solvents of increasing polarity from non-polar (n-hexane) to more polar solvent (water) to ensure the extraction of a wide range of compounds with different polarity.[21-23] Studies have reported that solvent polarity significantly affects the extract yield and antioxidant activity of phenolic compounds in plant material.[24,25] However, there is a paucity of research investigating the most effective solvent for guava fruit. Therefore, in this study, to extract secondary metabolite content in guava fruit with ethanol 50 % and 70 % were analyzed and evaluated with regard to quercetin concentration.

MATERIALS AND METHODS

Guava sample collection

Fresh guava fruits were collected from farmland located in Dukuh Waluh village, Purwokerto, Central Java, Indonesia (Figure 2) Random ripe fruit samples were collected into plastic bags with appropriate labeling and were stored in an ice cooler box to be transported to the laboratory for extraction. The fruit samples were substantiated by Central Laboratory, Jatinangor.

Reagents and solvent preparation

For HPLC grade Water (Milli-Q, Acetonitrile, Methanol (Merck), Quercetin standard was produced by Sigma Aldrich. The solvents use ethanol, methanol, and water in combination ethanol: water (1:1, 1:5, 1:7 v/v), isocratic mobile phases its composition of mixture: 0.1% acetic acid, acetonitrile, and methanol (40:50:10) respectively, at temperature 30^{0}C.

Instrument

The equipment used to examine the extract was *Waters Alliance e2695*HPLC. Column LiChroCART 250-4,6 RP 18E: UV 254 nm. The proposed HPLC method designed for the analysis of guava products is accurate, precise, sensitive, simple, easy to perform, rapid, and versatile. It is easily applicable to a large number of samples in a short time and with a low cost in terms of reagents, solvents, and equipment. In addition, the sample treatment is very simple.[26]

Preparation of sample for HPLC analysis

Precisely 0.1 g of guava extract was weighed and dissolved in methanol to a volume of 10 mL. Filtered with a 0.45 µm size filter. From the above solution, 10 µL was taken and injected into the HPLC tool. The analysis time required is 35 minutes. For the reference standards procedure is gallic acid with the following concentration: 10, 20, 30, 40, and 50 mg. Then, the gallic acid compound was dissolved into 10 mL methanol and filtered with a 0.45 µm filter. 10 µL compounds were injected into the HPLC instrument.

Column LiChroCART 250-4,6 RP 18E with heating at 30 °C was used as a stationary phase and eluted with isocratic mobile phases consisting of 0.1% acetic acid in water: acetonitrile: methanol (40:50:10). Separation time was 30 minutes, the flow rate was 1 mL/minute and detection was at 254 nm. Condition of the sample by various concentrations: 5, 10, 15, 20, and 25 ppm. Meanwhile for the gallic acid in various concentrations 50, 100, 200, 400, and 800 ppm. Standard calibration curves were made by plotting the retention time against the peak area.

Preparation of standard stock solution

Accurately weighed 10 mg of standard reference quercetin was diluted in 10 ml of methanol. 1 ml of this solvent continued to be diluted until 10 ml total volume of methanol was reached. Thereafter the solution with a concentration of, 5,10,15,20, and 25 ppm were made by pipetting 0,5, 1, 1,5, and 2,5 ml respectively. Each solution was then diluted until the total volume was 10 ml.

Guava fruit extraction procedure and quercetin measurement

The steps of quercetin isolation were as followed, the fruit was washed in tap water and cut into small pieces placed into a blender to be grounded. Ethanol solvent, 50%, and 70% were used for the maceration extraction procedure. Then, the filtering was conducted using a funnel buncher. The filtrate produced from the filtration was concentrated using a rotary evaporator at 40°C to obtain the result of concentrated extract and was suspended using distilled water as needed. The extract was afterward collected and stored at 4°C until use. The sample treatment procedure then followed the HPLC protocol (Figure 3).

RESULTS AND DISCUSSION

Herbal plants have a wide range of phytochemicals that are directly dependent on the plant developmental stage, plant parts, and the solvents used to extract and isolate these phytochemicals.[27-30] This study

a b

Figure 3: *Psidium guajava* L. extract (a) 50% and (b) 70%

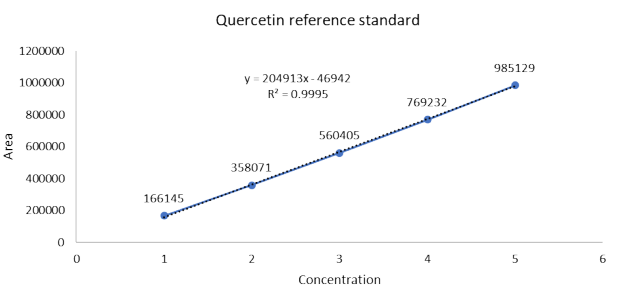

Figure 4: The standard curve of the quercetin reference standard in concentrations of 5, 10, 15, 20, and 25 ppm

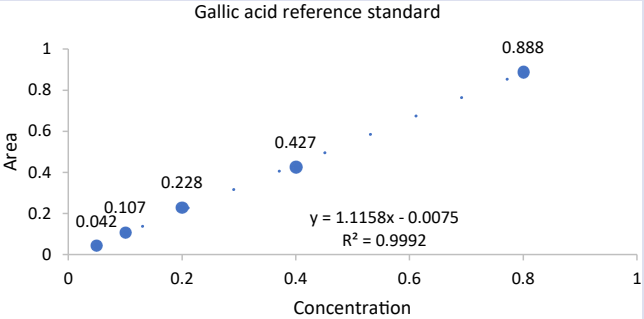

Figure 5: The standard curve of the gallic acid reference standard in various concentrations of 50, 100, 200, 400, and 800 ppm

showed the presence of quercetin in *Psidium guajava* L. fruit extract at different values at different concentrations. Quercetin is one of the flavonols, these meet the fact that plants are an excellent source of secondary metabolites such as phenolics, flavonoids, alkaloids, lignans, and terpenoids. Secondary metabolites have been extensively used since ancient times and are still very popular in the treatment of various diseases and disorders.[31,32] Primarily, plants produce these secondary metabolites (phenols, flavonoids, and tannins) for their defense system which in turn can be used for the treatment of other living organisms facing ROS-mediated chromosomal, ultrastructural, DNA damages, and protein denaturation and deactivation at both translational and post-translational levels.[33-35]

Quercetin ($(C_{15}H_{10})_7$) is an aglycone, lacking an attached sugar. It is a brilliant citron yellow needle crystal and entirely insoluble in cold water, poorly soluble in hot water, but quite soluble in alcohol and lipids. A quercetin glycoside is formed by attaching a glycosyl group (a sugar such as glucose, rhamnose, or routines) as a replacement for one of the OH groups (commonly at position 3). The attached glycosyl group can change the solubility, absorption, and *in vivo* effects. To be noted, the presence of a glycosyl group (quercetin glycoside) results in increased water solubility compared to quercetin aglycone.[36,37] This glycoside is unique in the attachment of the glycosyl group. Broadly speaking the term quercetin should be used to describe the aglycone only and this is used in the research and supplement industry. For further studies, the determination of quercetin in *Psidium Guajava* L. may give different outcomes (Figure 6).

The solvent extraction in this study was chosen to extract bioactive components from fruit extracts. Solvent extraction is a process designed to separate soluble antioxidant compounds by diffusion from a solid matrix such as plant tissue using a liquid matrix or solvent. It is noted that a solvent system for extraction is selected according to the purpose of extraction such as preparation or analysis, the nature of interesting components, the physicochemical properties of the matrix, the availability of reagents and equipment, cost, and safety concerns.[38] From other studies also total secondary metabolites and their antioxidant capacity greatly depend on the solvent and plant part used for extraction.[39] In the present study, organic solvents and their ratio combination (hydroethanolic extract) were employed based on the polarity index and solvent miscibility according to HPLC Solvent Guide, Solvent Miscibility and Viscosity Chart adapted from Paul Sadek, 2002, which was previously adapted by various researchers.[29,40,41]

The crude extract quantity, purity, and quality greatly depend on the plant part used and the solvent used for the extraction.[29,42] Solvents with different concentrations can be affected by the level of active compounds produced in the extract. Methanol, ethanol, and acetone were the most common solvent either singly or in combination with aqueous. From the result, guava fruits extract that has been diluted in ethanol at 50 % yielded a higher concentration than 70% this is consistent with previous reports showing that polar compounds, such as phenolic compounds and flavonoids, were more soluble than 50% hydroethanolic extract, therefore, was higher than in the water extract of guava leaves.[43]

These data imply a 50% ethanol extract yield of 65216 while 70% ethanol is 12159 (Table 1; Table 2). From the figure below (Figure 4; Figure 5), formulated the equation y = 204913x – 46942 (standard curve), thus the difference amount of quercetin concentration for 50% ethanol was 54.7344 mg/kg while 70% ethanol was 28.8420 mg/kg.

Another study found that the order of increasing phenolic compound content of Hieracium Pilosella was 50% hydroethanolic extract > 80% hydromethanolic extract > water extract.[44] According to another research study, the phenolic compound content was highest in 40% hydroethanolic extract.[45,46] Based on the polarity solvent arranged shown in the following table below.[47] (Table 3)

Previous research informed that methanol and ethanol can dissolve polar compounds, such as sugar, amino acid, glycoside compounds, phenolic compounds with low and medium molecular weights and medium polarity, aglycon flavonoid, anthocyanin, terpenoid, saponin,

Table 1: Standard quercetin concentration per area.

Concentration (ppm)	Area
5	166145
10	358071
15	560405
20	769232
25	985129

Table 2: Result of quercetin concentration.

Guava Sample Extract	Area
50%	65216
70%	12159

Table 3: Solvent polarity.

Solvent	formula	boiling point (°C)	melting point (°C)	density (g/mL)	solubility in H_2O (g/100g)	relative polarity	eluant strength	threshold limits (ppm)	vapor pressure 20°C (hPa)
Ethanol	C_2H_6O	78.5	-114.1	0.789	M	0.654	0.88	100	59
Methanol	CH_4O	64.6	-98	0.791	M	0.762	0.95	200	128
Water	H_2O	100	0	0.998	M	1	>>1		

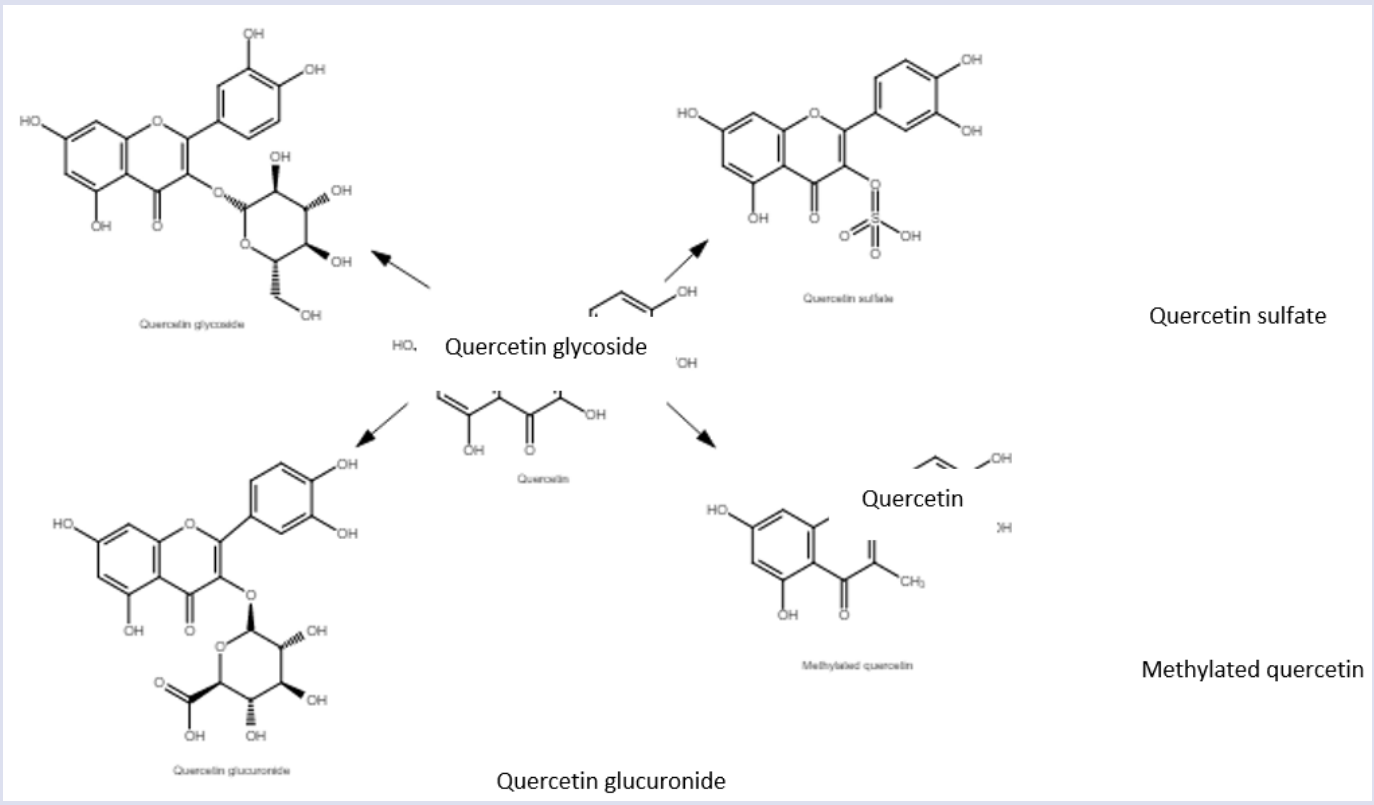

Figure 6: Molecular structure of quercetins

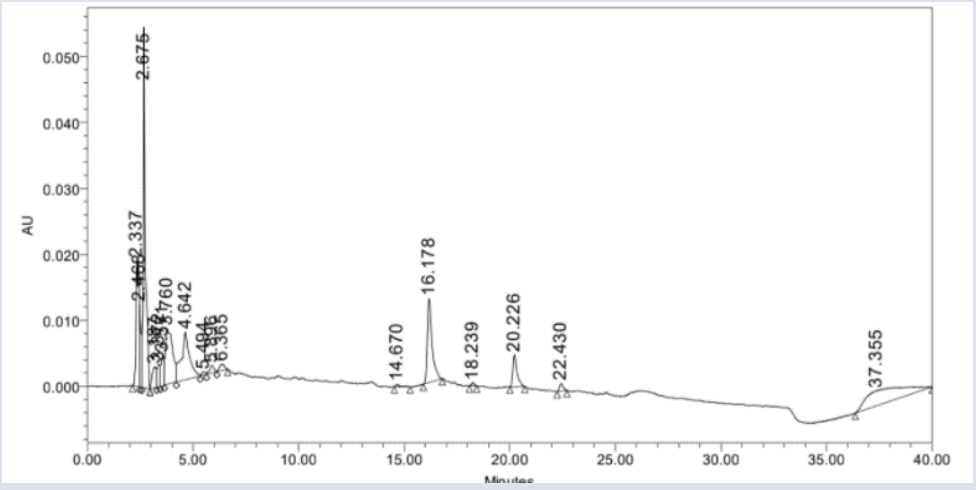

Figure 7: Chromatogram of quercetin content in the extract of guava fruit by HPLC analysis; 50% solvent: a mixture of water-methanol

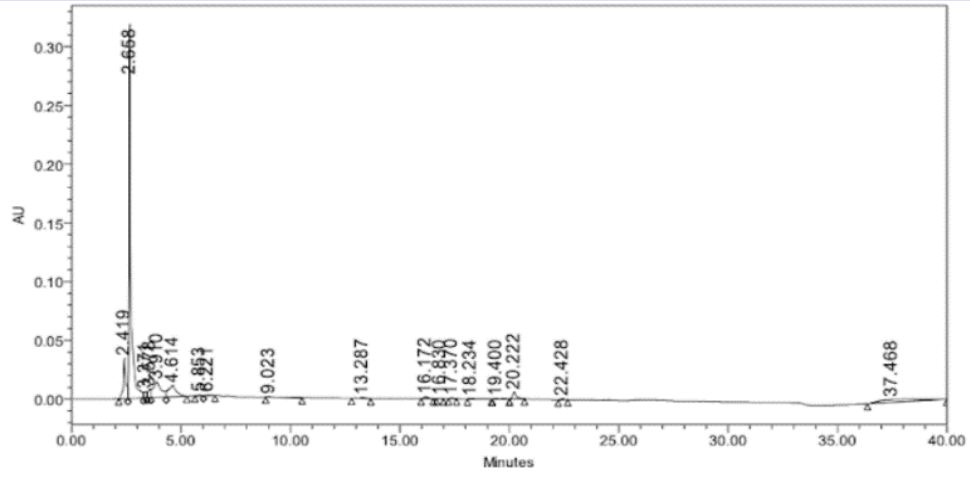

Figure 8: Chromatogram of quercetin content in the extract of guava fruit by HPLC analysis; 70% solvent: a mixture of water-methanol

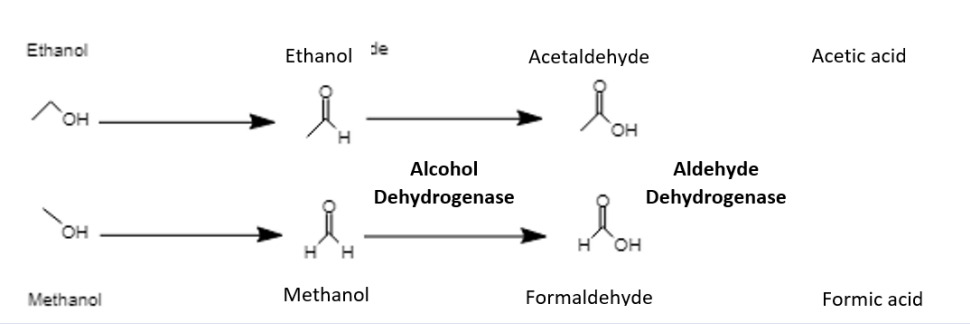

Figure 9: Metabolism of ethanol and methanol

tannin, xantoxilin, totarol, quacinoid, lacton, flavone, phenone, and polyphenol.[48,49] However, ethanol is the primary choice due to its universal, safety and cost, methanol is poisonous and not suitable if administered to the biological system, its effect would be irreversible.

From this figure, ethanol is converted by aldehyde dehydrogenase to acetaldehyde then by aldehyde dehydrogenase into the less toxic acetic acid, or vinegar. In methanol the end product is formic acid (also known as formate) is formed, this form is not well tolerated as acetic acid. Accumulation of this chemical in the blood deprives cells of oxygen by inhibiting the enzyme cytochrome c oxidase in their mitochondria, a key element of the respiratory electron transport chain. Formic acid, together with formaldehyde, are responsible for nerve damage, blindness, and other unpleasant effects associated with methanol poisoning.[50,51] (Figure 9)

Psidium Guajava L. has many varieties according to Eddy Jusuf[14] the quercetin content of each plant number was found to be varied in both, red fruit and white fruit varieties. The highest, >6.0% quercetin content was obtained in two numbers of red fruit and one from white fruit, and the lowest, <0.6% obtained in two numbers of white fruit. Proteomic profile analyzed from cut and blended fresh leaves after extraction using antiproteolytic buffer in coldness brings us to make a phenogram giving the variability of genetic kinship, that 35 varieties were divided into three groups of kinship; first, all cultivars of red fruit, secondly sixteen cultivars of white fruit, and third one cultivar of white fruit.[14]

CONCLUSION

Psidium guajava L. has a long history of traditional use for a wide range of ailments. The fruit as well as its juice is freely consumed for its great taste and nutritional benefits. This study intended to find the best extraction solvent for quercetin in guava fruits using various solvents concentration. The quercetin content in 50% hydroethanolic extracts was higher than that of 70% hydroethanolic extract.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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