Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Determination and Quantification of *p*-Coumaric Acid in Pineapples (*Ananas comosus*) Extracts using Gradient Mode RP-HPLC

Wan Mazlina Md Saad[1], Rasdin Ridwan[1], Nur Syamimi Md Lasim[1], Nur Liyana Mohd Rapi[1], Fatimah Salim[2,3]

[1]Centre of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA Selangor, Puncak Alam Campus, [2]Department of Chemistry, Centre of Foundation Studies, Universiti Teknologi MARA, Dengkil Campus, Dengkil, [3]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA Selangor, Puncak Alam Campus, Selangor, Malaysia

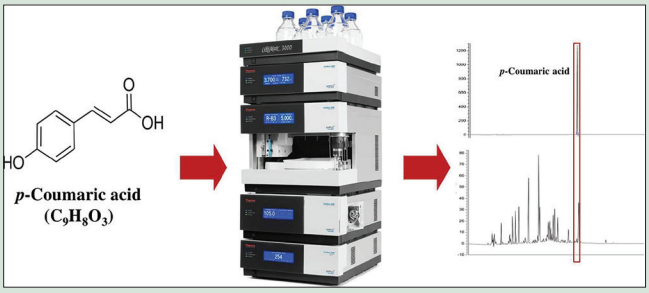
ABSTRACT

Background: Pineapple (*Ananas comosus*) is a good source of phenolic compounds such as *p*-coumaric acid. *P*-Coumaric acid is a hydroxycinnamic acid known to possess antioxidant, antimicrobial, antimutagenic, and immunoregulatory activities. **Objective:** The present study aims to determine and quantify the *p*-coumaric acid in the juice extract and methanol extract of unripe and ripe pineapples by gradient mode reverse-phase high-performance liquid chromatography (RP-HPLC). **Materials and Methods:** The analysis was carried out using 0.5% phosphoric acid and 100% acetonitrile (can) as mobile phase A and B at gradient elution conditions of 0–30 min from 5% B to 80% B, 30–33 min at 80% B, 33–35 min from 80% B to 5% B, and 35–40 min at 5% B. The chromatography separation was performed using Gemini C_{18} column at flow rate of 0.8 mL/min and detection was made at 280 nm. **Results:** *P*-Coumaric acid was determined at retention time of 16.16 min. Standard curves were linear at R^2 = 0.9973 with low limit of detection and limit of quantification being 0.0208 and 0.0694, respectively. *P*-Coumaric acid showed the highest concentration in juice extract of ripe pineapple, 11.76 µg/mL compared to the methanol extract, 0.03 µg/mL, respectively. *P*-Coumaric acid concentration in juice extract of unripe pineapple was 0.41 µg/mL. **Conclusion:** The gradient mode RP-HPLC proved to be efficient for determination and quantification of *p*-coumaric acid in pineapples extracts.

Key words: *Ananas comosus*, gradient mode, *p*-coumaric acid, pineapple, reverse-phase high-performance liquid chromatography

SUMMARY

- *P*-Coumaric acid was successfully determined and quantified in unripe and ripe pineapple extracts using gradient-mode reverse-phase high-performance liquid chromatography. *P*-Coumaric acid showed the highest concentration in juice extract of ripe pineapple compared to methanol extract.



Abbreviations Used: RP-HPLC: Reverse-phase high-performance liquid chromatography; ACN: Acetonitrile; H_3PO_4: Phosphoric acid; PLPs: Pineapple leaf phenols; HepG2: Hepatoma G2; PLEs: Pineapple leaf extracts; TC: Total cholesterol; TG: Triglyceride; DAD: Diode array detector; UV-Vis: Ultraviolet-visible; dH_2O: Distilled water; LOD: Limit of detection; LOQ: Limit of quantification.

Correspondence:
Dr. Wan Mazlina Md Saad,
Centre of Medical Laboratory Technology,
Faculty of Health Sciences, Universiti Teknologi MARA Selangor, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia.
E-mail: wanmaz755@salam.edu.uitm.my

DOI: 10.4103/pr.pr_154_18

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Pineapple (*Ananas comosus*) belongs to *Bromeliaceae* family and one of the popular tropical and subtropical fruits.[1] Pineapple is a native to South America and is a nonseasonal fruit which available throughout the year. Pineapple has sweet taste, acidic flavor, and consumed around the world as juice, jelly, jam, and dried product. Good quality of pineapple fruit is indicated by the dense and juicy yellow-colored pulp with medium acidity and high sugar content.[2]

Pineapple has high content of phytonutrients and dietary antioxidants such as phenolics, flavonoids, minerals, and vitamins.[3] *P*-Coumaric acid, $C_9H_8O_3$ [Figure 1], is one of the phenolic compounds present in flesh and leaves of pineapple.[4] *P*-Coumaric acid is a natural hydroxycinnamic acid that exists in an esterified form with tartaric acid, the tartaric *p*-coumaroyl ester.[5] This compound is known to possess antioxidant, antimicrobial, antimutagenic, analgesic, sedative, and immunoregulatory activities.[6,7] The previous study found that *p*-coumaric acid from pineapple leaf phenols significantly prevent fat accumulation in oleic acid-induced hepatoma (HepG2) cells.[7] Cho *et al*.[8] and Chai *et al*.[9] demonstrated that pineapple leaf extracts significantly reduce plasma total cholesterol and triglyceride in obese rats owing to high content of phenolic acids including *p*-coumaric acid.

Several studies have demonstrated the biological effect of pineapple (*A. comosus*).[10-12] According to Romano *et al*.,[10] pineapple exhibits antiproliferative and chemopreventive effects *in vitro* and *in vivo* study. The study demonstrated that 1 µg/mL of bromelain derived from pineapple stem reduced cell proliferation and promoted apoptosis through caspase 3/7 activation in human colorectal carcinoma (Caco-2) cells. However, an optimal dose of 1–3 mg/kg prevent preneoplastic lesions, polyps, and tumor formation induced in mouse colon administered with carcinogenic agent, azoxymethane.[10] In another study, Yantih *et al*.[11] demonstrated

Cite this article as: Md Saad WM, Ridwan R, Lasim NS, Rapi NL, Salim F. Determination and quantification of *p*-coumaric acid in pineapples (*Ananas comosus*) extracts using gradient mode RP-HPLC. Phcog Res 2019;11:67-71.

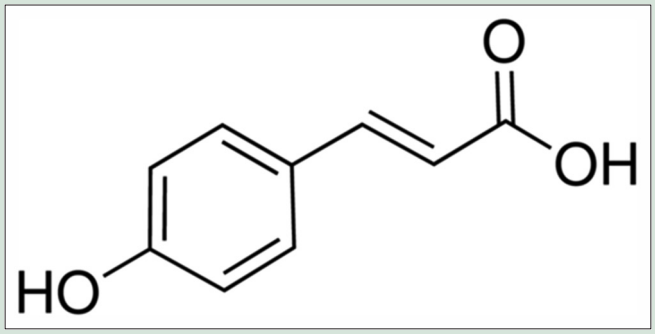


Figure 1: Molecular structure of *p*-coumaric acid (164.16 g/mol)

hepatoprotective effect of pineapple juice in rats. Supplementation of 2 mL/kg body weight of pineapple juice for 4 weeks decreased ALT and AST levels in rats induced with 27 mg/kg body weight of isoniazid. The authors suggested that the hepatoprotective effect may be due to antioxidant activity of phenolic compounds in the juice.[11] However, recent study by El-Shazly *et al.*[12] demonstrated antiobesity effect of pineapple juice in rats supplemented with high-fat diet (HFD). The results of the study showed that pineapple juice suppressed HFD-induced obesity in rats through reduction of body weight gain, serum lipid, hepatic lipid accumulation, and decreased the number and size of adipocytes after 8 weeks juice supplementation. The study suggested that consumption of pineapple juice that contains a whole synergistic interaction of phytonutrients, particularly phenolic compounds, may contribute to the antiobesity effect.[12] Overall, the findings from these previous studies suggest that pineapple (*A. comosus*) may provide beneficial effects for treatment and prevention of health-related diseases.[10-12]

Analyses of phenolic compounds were commonly conducted using Folin–Ciocalteu;[13] however, the method is qualitative and lack of sensitivity.[14] Karthikeyan *et al.*[15] suggested that the efficient technique for qualitative and quantitative analysis of *p*-coumaric acid is by using chromatographic method owing to high selectivity and less of background interferences. To date, study on phenolic content particularly *p*-coumaric acid in Malaysia pineapple is still lacking. Thus, the present study aims for determination and quantification of *p*-coumaric acid in unripe and ripe of pineapples extracts using gradient mode reverse-phase high-performance liquid chromatography (RP-HPLC).

MATERIALS AND METHODS

Chemical and Reagents

P-Coumaric acid standard (purity ≥99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) and methanol of HPLC grade were purchased from Merck (Germany). Phosphoric acid (H_3PO_4) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was prepared using ultrapure water purifier system (Elgastat, Bucks, UK).

Instrument

The gradient-mode RP-HPLC was carried out using Thermo Scientific™ Dionex-UltiMate™ 3000 HPLC system equipped with solvent reservoirs, pump, autosampler injector, column oven, and ultraviolet-visible diode array detector module operated at four wavelengths per analysis. Chromeleon data software (Version 7, Thermo Fisher Scientific Inc., MA, USA) was used for data analysis.

Samples preparation

Unripe and ripe pineapples (*A. comosus*) were purchased from local market, Meru, Selangor. The pineapples peels were removed. Edible part of the flesh pineapples were cut into cubes, juiced, and filtered using sieve. The juices were filtered again using cotton wool placed into neck of the funnel to remove any debris or unnecessary matter. The juice extract was directly analyzed. For methanol extract, the unripe and ripe pineapple juices were frozen at −80°C. The frozen juices were the put in freeze-drier (Labconco, USA) for 4 days until completely dried. A known quantity of dried juice powders was extracted using methanol. The methanol extracts then evaporated using rotary evaporator and stored at 4°C until analysis.

Reverse-phase high-performance liquid chromatography analysis for *p*-coumaric acid

Standard preparation procedure

Stock solution of *p*-coumaric acid was prepared in methanol at 0.5 mg/mL and filtered through a 0.45-μm syringe filter. A series of working standard solution was prepared by diluting stock solution with methanol in the range of 0.1–400 μg/mL.

Sample preparation procedure

500 μL was taken directly from unripe juice extract and diluted with 500 μL distilled water (dH_2O). For methanol extract, freeze-dried unripe pineapple was dissolved in methanol at 5 mg/mL. For ripe pineapple juice extract, 300 μL of juice was diluted with 700 μL dH_2O while methanol extract was dissolved in methanol at 5 mg/mL. Samples were filtered through 0.45-μm filter prior injected to RP-HPLC.

Gradient mode reverse-phase high-performance liquid chromatography analysis

Determination and quantification of *p*-coumaric acid were performed according to the study conducted by Jakobek *et al.*[16] and method by Ridwan *et al.*,[17] with slightly modification. The RP-HPLC analysis was performed using Gemini C_{18} column, 250 mm × 4.6 mm, 110 Å, 3 μm (Phenomenex, Torrance, CA, USA) with a flow rate of 0.8 mL/min. Column temperature was maintained at 20°C while detection was performed at 280 nm. 10 μL of sample was injected into Gemini C_{18} column by using gradient elution program. Two solvents used were 0.5% H_3PO_4 (solvent A) and 100% ACN (solvent B). The elution conditions were as follows: 0–30 min from 5% B to 80% B, 30–33 min 80% B, 33–35 min from 80% B to 5% B, and 35–40 min 5% B. The concentrations of *p*-coumaric acid in samples were quantified based on calibration curve of standard. Concentration of *p*-coumaric acid in pineapple extracts was calculated using the formula:

Concentration of analyte = A/S

Where A is the peak area of analyte and S is the slope of calibration curve.

Method validation

The validation of gradient-mode RP-HPLC method was achieved by determined the linearity of calibration curve, limit of detection (LOD) and limit of quantification (LOQ). The linearity of gradient-mode RP-HPLC method for quantification of compound was determined by injecting 5 μL of six different concentrations of *p*-coumaric acid standard at 0.1, 1, 100, 200, 300, and 400 μg/mL. Linear regression analysis was constructed by plotting a graph of peak area against *p*-coumaric acid standard concentrations. The regression equation was calculated in the form of y = mx, where y was the peak area of compound and x was concentration. Regression coefficient (R^2) was calculated from the calibration curve. The LOD is the lowest amount of analyte in the samples that can be detected but not necessarily

quantitated under the stated experimental conditions, while LOQ is the lowest amount of analyte in sample which can be quantitatively determined with suitable precision and accuracy.[18] LOD and LOQ can be expressed as LOD = $3S_a/b$ and LOQ = $10S_a/b$ where S_a is the standard deviation of the response which can be estimated by standard deviation of Y-intercept of regression line and b is the slope of calibration curve.

RESULTS AND DISCUSSION

Determination of *p*-coumaric acid in standard and pineapples extracts

The present gradient mode RP-HPLC for analysis of *p*-coumaric acid in unripe and ripe pineapples utilized 0.5% H_3PO_4 and 100% ACN as mobile phase A and B, respectively. 0.5% H_3PO_4 was used as mobile phase A to suppressed ionization of phenolic and carboxylic groups, leading to improvement of retention time and resolution of the target compound.[19] To produce optimum chromatographic separation, organic modifier (ACN) was chosen as mobile phase B to alter compound's retention time by lowering polarity of the mobile phase. Chen *et al.*[20] supported that mobile phase combinations containing modifier such as H_3PO_4 with ACN were efficient for phenolic compounds analysis. The chosen gradient elution (0–30 min from 5% B to 80% B; 30–33 min 80% B; 33–35 min from 80%, B to 5% B) at flow rate of 0.8 mL/min resulted that most compounds were eluted including *p*-coumaric acid. The used of reverse-phase column Gemini C_{18} as the stationary phase resulted in good separation of *p*-coumaric acid. Ferreira *et al.*[21] supported that C_{18} column demonstrated good retention time and peak symmetry of *p*-coumaric acid. The wavelength was set at 280 nm in accordance to the previous study conducted by Häkkinen[22] that showed 280 nm provide excellent detection of *p*-coumaric acid in fruit samples.

The peak of *p*-coumaric acid standard was detected at retention time of 16.16 min. The retention time of *p*-coumaric acid in samples was compared to the standard peak [Figures 2 and 3]. The peak of *p*-coumaric acid in juice extract of unripe and ripe pineapple and methanol extract of ripe pineapple was detected at 16.16 min. However, *p*-coumaric acid was not detected in methanol extract of unripe pineapple. The retention time (min), height, and peak area of standard and samples are tabulated in Table 1. Longer retention time for *p*-coumaric acid in the present study may be due to higher affinity of the compound towards the stationary phase. Xu and Howard[23] supported that *p*-coumaric acid is a less polar compound compared to other phenolic acids such as gallic acid, chlorogenic acid, and caffeic acid, thus leading to increased time for the compound to be eluted.

Method validation

Linear regression was obtained from the calibration curve of *p*-coumaric acid [Table 2], and good correlation was achieved between the concentration of *p*-coumaric acid standard and peak area. The linear regression equation was y = 0.8643x with regression coefficient, R^2 = 0.9973. The result of regression coefficient value in the present study was comparable with Bataglion *et al.*[24] study with R^2 = 0.998 for determination of phenolic compounds including *p*-coumaric acid in fruit samples. The LOD and LOQ were conducted for sensitivity assessment of the gradient-mode RP-HPLC. LOD is the lowest amount of analyte in sample that can be detected but not necessarily quantitated under stated experimental conditions. LOQ is the lowest amount of analyte in sample that can be quantitatively determined with suitable precision and accuracy.[25] The LOD and LOQ were 0.0208 and 0.0694 µg/mL, respectively. The linearity, LOD, and LOQ showed that the gradient mode RP-HPLC method was accurate and sensitive for quantification of *p*-coumaric acid.

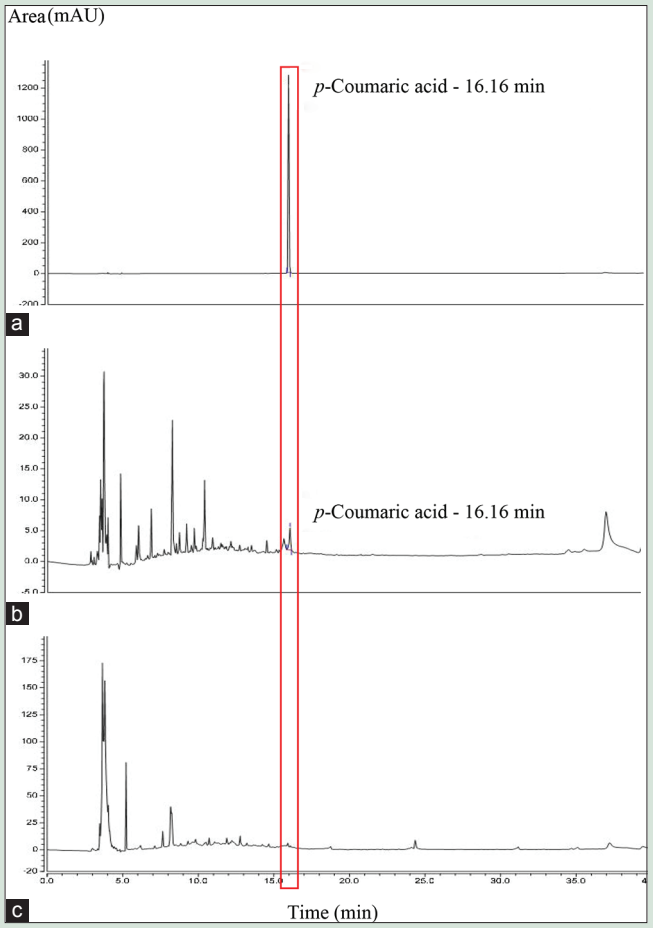


Figure 2: Comparative chromatograms of *p*–Coumaric acid. (A) Standard of *p*-Coumaric acid, (B) Juice extract of unripe pineapple and (B) Methanol extract of unripe pineapple. *p*-Coumaric acid was detected at 16.16 min in standard and juice extract of unripe pineapple under 280 nm wavelength using gradient elution method of 0.5% H_3PO_4 and 100% ACN as mobile phases and flow rate at 0.8 min/mL

Table 1: Retention time (min), height (mAU), and peak area (mAU*s) of samples and standard

Compound	Samples	Extracts	Retention time (min)	Height (mAU)	Area (mAU*s)
P-Coumaric acid	Standard	N/A	16.16	1241.803	95.931
	Unripe	Juice extract	16.16	3.567	0.357
	Ripe		16.16	35.561	3.051
	Unripe	Methanol extract	N/A	N/A	N/A
	Ripe		16.16	2.712	0.011

N/A: Not available

Table 2: Calibration data form gradient mode reverse-phase high-performance liquid chromatography

Compound	Linear equation	Regression coefficient (R^2)	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
P-Coumaric acid	y=0.8643x	0.9973	0.1–400	0.0208	0.0694

LOD: Limit of detection; LOQ: Limit of quantification

Quantification of *p*-coumaric acid in pineapple extracts

Consumption of pineapples extracts rich in *p*-coumaric acid is beneficial for diseases prevention. Thus, an efficient gradient-mode RP-HPLC is essential for quantification of *p*-coumaric acid in juice extracts and methanol extracts. The concentration of *p*-coumaric acid was calculated based on calibration curve achieved with good correlation coefficients and linear regression equations, $R^2 = 0.9973$, y = 0.8643x. The results are tabulated in Table 3.

Ripe pineapple juice extract showed the highest concentration of *p*-coumaric acid, 11.75 µg/mL when compared to methanol extract, 0.03 µg/mL. The concentration of *p*-coumaric acid in ripe pineapple juice extract was in the range of Wen and Wrolstad's[3] study reported that *p*-coumaric acid content range from 1 to 21 µg/mL in pineapple juice samples. The concentration of *p*-coumaric acid in juice extract of unripe pineapple was 0.41 µg/mL while no *p*-coumaric acid detected in the methanol extract. Shofian *et al.*[26] stated that extraction method is highly influence the concentration of compound quantified in certain fruits. Ridwan *et al.*[27] supported that methanol extract may diminish the solubility of bioactive compounds in fruit result in lower yield compared to fruit juice extract. The present result demonstrated juice extract of ripe pineapple most effectively quantified higher yield of *p*-coumaric acid, thus suggesting that consumption of pineapple directly from juice is essential for the prevention of health-related diseases.

Table 3: *P*-coumaric acid concentration (µg/mL) in juices extract and methanol extract of unripe and ripe of pineapples

Compound	Pineapple types	Juice extract (µg/mL)	Methanol extract (µg/mL)
P-Coumaric acid	Unripe	0.41	N/A
	Ripe	11.75	0.03

N/A: Not available

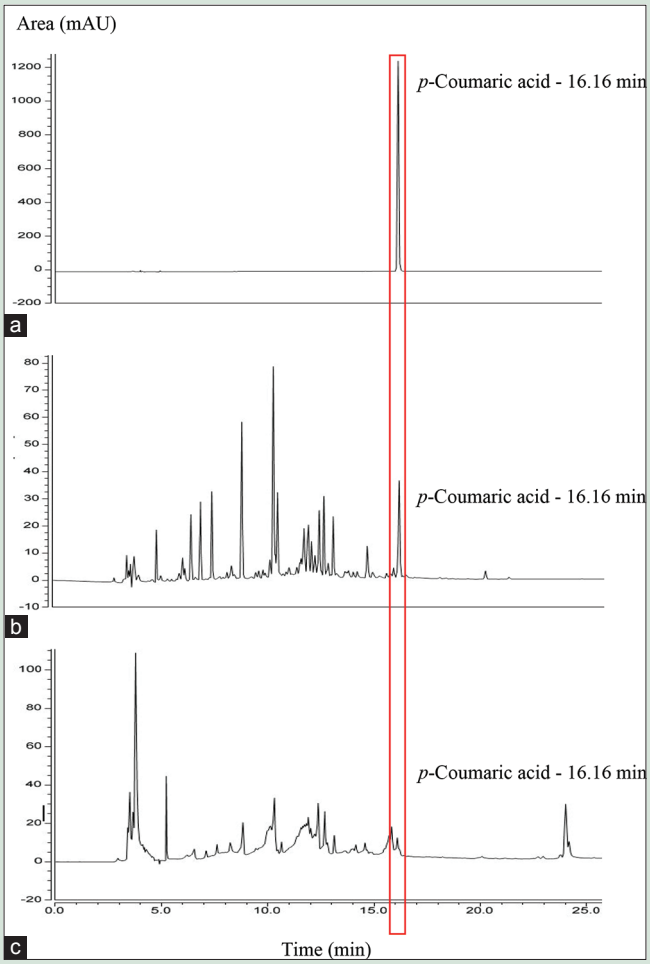


Figure 3: Comparative chromatograms of *p*–Coumaric acid. (a) Standard of *p*-Coumaric acid, (b) Juice extract of ripe pineapple and (c) Methanol extract of ripe pineapple. *p*-coumaric acid was detected at 16.16 min in standard and juice extract of ripe pineapple under 280 nm wavelength using gradient elution method of 0.5% H3PO4 and 100% ACN as mobile phases and flow rate at 0.8 min/mL

CONCLUSION

The gradient-mode RP-HPLC has been successfully determined and quantified of *p*-coumaric acid concentration in both extracts of unripe and ripe pineapples. The validated method is accurate and sensitive with good linearity (≥0.99), low values of LOD and LOQ. Juice extract effectively yielded higher *p*-coumaric acid, thus potentially used for quantitative routine analysis in fruit juices. The high content of *p*-coumaric acid in juice extract of ripe pineapple suggested pineapple as a good source of nutraceutical and health benefit ingredients. However, further researches are necessary to explore the biological activities of *p*-coumaric acid in pineapple to support its potential for disease prevention.

Acknowledgements

We acknowledge the Ministry of Higher Education, Malaysia, through Fundamental Research Grant Scheme (FRGS Grant No: FRGS/1/2016/WAB01/UITM/02/3) and Institute of Research Management and Innovation (IRMI), Universiti Teknologi MARA (UiTM) for funding the study and Atta-ur-Rahman Institute, UiTM Puncak Alam, Centre of Medical Laboratory Technology, and Centre of Postgraduate Study, Faculty of Health Sciences, UiTM Selangor, Puncak Alam Campus, for providing facilities throughout this study.

Financial support and sponsorship

The study is funded by the Ministry of Higher Education, Malaysia, through Fundamental Research Grant Scheme (FRGS Grant No: FRGS/1/2016/WAB01/UITM/02/3) and IRMI, UiTM.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Adhikarimayum H, Kshetrimayum G, Maibam D. Evaluation of antioxidant properties of phenolics extracted from *Ananas comosus* L. Not Sci Biol 2010;2:68-71.
2. Kuan C, Lee T. A new pineapple cultivar *Ananas comosus* (L.) merr. ("Tainung No. 22"). HortScience 2018;53:578-81.
3. Wen L, Wrolstad RE. Phenolic composition of authentic pineapple juice. J Food Sci 2002;67:155-61.

4. Milind P, Pooja G. Is pineapple a fine apple? Ann Pharm Pharm Sci 2010;1:134-41.
5. Salameh D, Brandam C, Medawar W, Lteif R, Strehaiano P. Highlight on the problems generated by p-coumaric acid analysis in wine fermentations. Food Chem 2008;107:1661-7.
6. Ou SY, Teng JW, Zhao YY, Zhao J. *P*-Coumaric acid production from lignocelluloses. In: Phenolic Acids: Composition, Applications and Health Benefits. New York: Nova Science Publishers; 2012. p. 63-71.
7. Xie W, Zhang S, Lei F, Ouyang X, Du L. *Ananas comosus* L. leaf phenols and p-coumaric acid regulate liver fat metabolism by upregulating CPT-1 expression. Evid Based Complement Alternat Med 2014;2014:903258.
8. Cho AS, Jeon SM, Kim MJ, Yeo J, Seo KI, Choi MS, *et al.* Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food Chem Toxicol 2010;48:937-43.
9. Chai Y, Lei F, Tian Y, Yuan Z, Lu X, Zhao S, *et al.* Comprehensive study of the intestinal absorption of four phenolic compounds after oral administration of *Ananas comosus* leaf extract *in vivo* and *in vitro*. Afr J Pharm 2013;7:1781-92.
10. Romano B, Fasolino I, Pagano E, Capasso R, Pace S, De Rosa G, *et al.* The chemopreventive action of bromelain, from pineapple stem (*Ananas comosus* L.), on colon carcinogenesis is related to antiproliferative and proapoptotic effects. Mol Nutr Food Res 2014;58:457-65.
11. Yantih N, Harahap Y, Sumaryono W, Setiabudy R, Rahayu L. Hepatoprotective activity of pineapple (*Ananas comosus*) juice on isoniazid-induced rats. J Biol Sci 2017;17:388-93.
12. El-Shazly SA, Ahmed MM, Al-Harbi MS, Alkafafy ME, El-Sawy HB, Amer SA. Physiological and molecular study on the anti-obesity effects of pineapple (*Ananas comosus*) juice in male Wistar rat. Food Sci Biotechnol 2018;27:1429-38.
13. Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW, Walker RB, *et al.* Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. J Agric Food Chem 2010;58:8139-44.
14. Hossain MA, Rahman SM. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. Food Res Int 2011;44:672-6.
15. Karthikeyan R, Devadasu C, Srinivasa Babu P. Isolation, characterization, and RP-HPLC estimation of *P*-Coumaric acid from methanolic extract of Durva grass (*Cynodon dactylon* Linn.) (Pers.). Int J Anal Chem 2015;2015:201386.
16. Jakobek L, Seruga M, Novak I, Medvidovic-Kosanovic M. Flavonols, phenolic acids and antioxidant activity of some red fruits. Deutsch Lebensmitt Rundsch 2007;103:369-78.
17. Ridwan R, Abduk Razak HR, Adenan MI, Md Saad WM. Separation of L-arginine and L-citrulline in red and yellow crimson watermelon (*Citrullus lanatus*) juices extract using HPLC gradient mode. Malays J Anal Sci 2018;22:785-93.
18. Ariese F, Beyer J, Jonsson G, Porte C, Krahn MM. Review of analytical methods for determining metabolites of polycyclic aromatic compounds (PACs) in fish bile. ICES Tech Mar Environ Sci 2005;39:1-49.
19. Haminiuk CW, Maciel GM, Plata-Oviedo MS, Peralta RM. Phenolic compounds in fruits – An overview. Int J Food Sci Tech 2012;47:2023-44.
20. Chen HJ, Inbaraj BS, Chen BH. Determination of phenolic acids and flavonoids in *Taraxacum formosanum* Kitam by liquid chromatography-tandem mass spectrometry coupled with a post-column derivatization technique. Int J Mol Sci 2012;13:260-85.
21. Ferreira PS, Victorelli FD, Fonseca-Santos B, Chorilli M. A review of analytical methods for *p*-coumaric acid in plant-based products, beverages, and biological matrices. Crit Rev Anal Chem 2018;48:1-11.
22. Häkkinen S. Flavonols and Phenolic Acids in Berries and Berry Products. PhD Thesis, University of Kuopio, Finland; 2000.
23. Xu Z, Howard LR. Analysis of antioxidant-rich phytochemicals. Analysis of Antioxidant-Rich Phytochemicals. New Jersey: John Wiley and Sons; 2012.
24. Bataglion GA, da Silva FM, Eberlin MN, Koolen HH. Determination of the phenolic composition from Brazilian tropical fruits by UHPLC-MS/MS. Food Chem 2015;180:280-7.
25. Shrivastava A, Gupta V. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron Young Sci 2011;2:21-5.
26. Shofian NM, Hamid AA, Osman A, Saari N, Anwar F, Dek MS, *et al.* Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. Int J Mol Sci 2011;12:4678-92.
27. Ridwan R, Abdul Razak HR, Adenan MI, Md Saad WM. Development of isocratic RP-HPLC method for separation and quantification of L-citrulline and L-arginine in watermelons. Int J Anal Chem 2018;2018:4798530.