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Identifying Metabolites in Complex Extract of Sigararutang Coffee Beans with NMR Spectroscopy Method

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Abstract

The sigararutang is a superior variety of arabica coffee fruiting continuously and has an exquisite taste. However, the chemical information of sigararutang coffee is still limited in the literature. In this paper, metabolites of sigararutang coffee were analyzed with 1H NMR spectroscopy and then further verified by 2D NMR techniques, comprising TOCSY, COSY, and J-Resolved. The samples used in this study were green beans of sigararutang coffee obtained from Malabar Mountain, Bandung, West Java, Indonesia. Accumulatively, 17 metabolites were successfully recognized in the extracts of sigararutang coffee, including sucrose, caffeine, trigonelline, 3-CQA, 5-CQA, 4-CQA, quinic acid, malic acid, lactic acid, citric acid, asparagine, alanine, lipid, myo-inositol, GABA, formic acid, and choline in the single-step analysis. In addition, some metabolites of sigarangutang coffee were quantified relatively by the integration of ^{1}H NMR signals. Sucrose (6.62%, w/w) was the most rich component in the green coffee bean of the *C.arabica* var.sigararutang. The amounts of GABA, malic acid, and choline with concentrations below 0.1%, were also calculated successfully. This study demonstrated the ability of NMR techniques for identifying and quantifying metabolites in the extracts of sigararutang coffee without any separation.

Keywords: *Coffea arabica var. sigararutang; metabolite; ^{1}H NMR*

1. Introduction

The application of Nuclear Magnetic Resonance (NMR) spectroscopy increases extensively in various research fields. Currently, NMR is not only limited to determine the structure of pure compounds but also can be intended for the detection and measurement of level compounds in the multi-compound mixtures[1]. The development of NMR use is inseparable from the presence of metabolite databases for example the human metabolite database (www.hmdb.ca), urine metabolome database (www.urinematabolome.ca), and secondary metabolite bioinformatics portal (SMBP at http://www.secondarymetabolite.org) assisting in the interpretation of the signals existent in the NMR spectra. Furthermore, the presence of 2D NMR is able to confirm further the identification of the compounds by interpreting the overlapped signals commonly found in the extract samples [2].

In the natural product chemistry field, NMR has been used for identifying compounds without any separation in the organism extracts, including marine sponge [3], *Cannabis sativa* L. trichomes [4], *Amphoricarpus* [5] and *Curcuma* [6]. Likewise in the food sector, NMR techniques have been used in the detection and measurement of level of a series of metabolites in several foods without separation, such as in vinegar [7], soybeans [8], coffee [9], sweet cherries [10], hazelnuts [11], saffron [12], tomatoes [13] and wines [14].

Considering the variety of the large sample containing different metabolites profiles, it is still necessary to develop an NMR approach that is applied in various samples to enrich the metabolite database for further use. One of them is in coffee. Coffee is the most beverage after plain water [15]. Identification of coffee metabolites had been reported previously, either by using proximate analysis [16], [17] or by using instruments including GC-MS [18], ICP [19], and NMR [9]. NMR offers advantages in the terms of metabolite analysis, including simplicity of sample preparation and a wide range of analyzes (not only dependent on volatile metabolites). NMR has the disadvantage of low sensitivity. However, it can be solved by applying the ultrahigh magnetic fields NMR and the cryogenic NMR technique [20].

The sigararutang is a superior variety of arabica coffee beans. This variety has advantages in terms of flavor and coffee bean productivity. These coffee beans have an exquisite flavor and can bear fruit throughout the year, so that they are more profitable in terms of coffee production (Decree of the Minister of Agriculture No.205/Kpts/SR.120/4/2005). The chemical content of coffee possibly influences the superiority of sigararutang variety in the flavor and the productivity.

This work purposes to comprehensively analyze the metabolites contained in the green arabica coffee beans var. sigararutang using ^{1}H NMR combined with 2D NMR technique, namely TOCSY, COSY and J-resolved. The metabolites that were identified were further confirmed with the human metabolite database (www.hmdb.ca). Seventeen compounds from sigararutang coffee were identified, including the major and minor metabolites. It confirmed that NMR has a good ability in identifying coffee compounds in the extracts even without separation.

2. Experimental

2.1. Material

The coffee samples utilized in this study were grean bean of arabica coffee with sigararutang variety provided by Rahayu Farmer Group in Pangalengan, Bandung, West Java. The chemical used in this work were deuterium oxide (Germany, CID: 887), 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid sodium salt (Germany, CID: 23688921), disodium hydrogen phosphate (Germany, CID: 24203), and sodium dihydrogen phosphate (Germany, CID: 516949), purchased from Merck (Darmstadt, Germany).

2.2. Instrumentations

Coffee mill 600 N (Yang Chia Machine Work,Taiwan) was used to grind the green coffee beans. An ultrasonic cleaner bath (Mujigae, South Korea) was operated to sonicate the samples. Microcentrifuge (Benchmark Scientific, United States) was employed to centrifuge the samples. NMR Variant Unity INOVA-500 Spectrometer (Agilent

Technologies, Santa Clara, United States) was applied to record ^{1}H NMR and TOCSY, COSY and J-Resolved spectra of the *C.arabica* var. sigararutang.

2.3. Methods

2.3.1. Sample preparation

Coffee powder (200 mg) was extracted using 1 mL of deuterium oxide containing TSP (1.0 mM) in phosphate buffer (pH = 6.0). Afterward, it was mixed using a vortex and then sonicated for 10 minutes. The sample was incubated at 95°C for 30 minutes before the supernatant separated. The supernatant was then inserted in a plastic tube and mixed with deuterium oxide (100 µL) comprising phosphate buffer (pH = 6). The solution was then mixed after that put in the NMR tube.

2.3.2. NMR Analysis

A Varian INOVA 500 MHz NMR spectrometer was applied to record the NMR spectra of the extract of *C. arabica* var. sigararutang. ^{1}H NMR spectrum was measured with a water suppression technique, 128 scans and 64K data points, yielding 8012 Hz spectra width. This measurement was conducted with acquisition time of 2.72 s and the delay time of 2 s. 2D NMR techniques, including ^{1}H-^{1}H Correlation Spectroscopy (COSY), Total Correlation Spectroscopy (TOCSY), and J-Resolved were applied to verify the detected signals in the ^{1}H NMR spectrum. COSY spectrum was measured with 4 scans and 512 increments. TOCSY spectrum was recorded with 4 scans and 256 increments, while J-resolved measurement was carried out with 4 scans and 128 increments. NMR raw data were treated with ACD/Labs 12.0 software (Toronto, Canada).

3. Results and discussion

The ^{1}H NMR spectrum of the coffee samples, as depicted in Figure 1**,** exhibited a heavy overlapping in the more shielding region from 1.88 ppm to 4.10 ppm, lead to the challenging in the metabolite identification. 2D NMR techniques, namely COSY, TOCSY, and J-resolved, decreased the problems of the overlapped signals and greatly eased the determination of protons of the coffee sample.

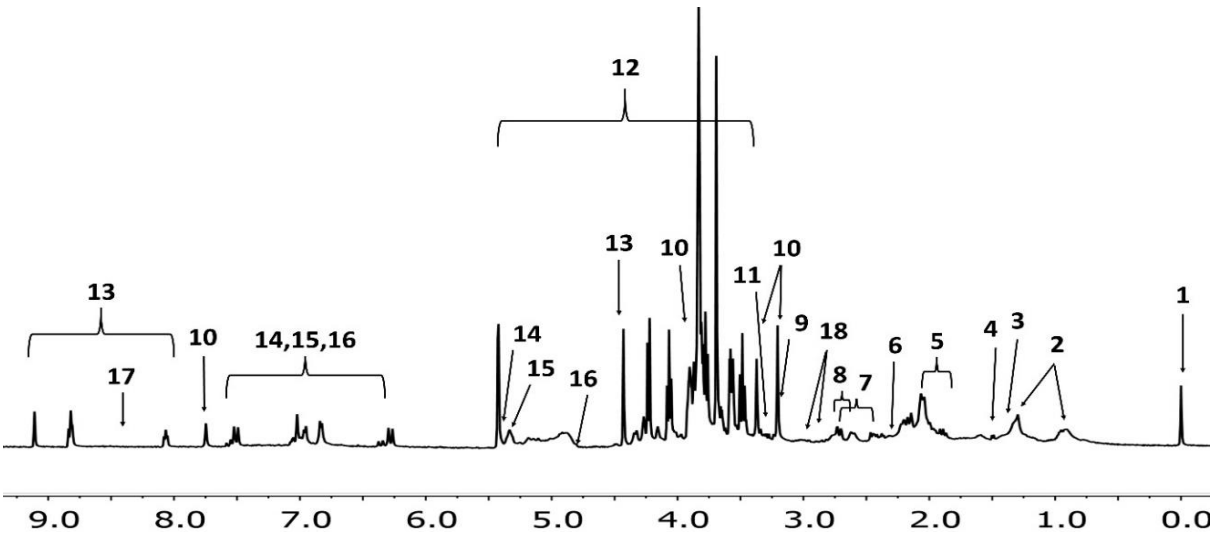

Figure 1: Metabolite signal interpretations in the ^{1}H NMR spectra of *C.arabica* var.sigarar utang. 1. TSP; 2. Lipids; 3. Lactic acid; 4. Alanine; 5. Quinic acid; 6. GABA; 7. Malic acid; 8. Citric acid; 9. Choline; 10. Caffeine; 11. Myo-inositol; 12. Sucrose; 13. Trigonelline; 14. 3-CQA; 15. 5-CQA; 16. 4-CQA, 17. Formic acid; 18. Asparagine.

In the mixture extract, COSY and TOCSY techniques able to detect the proton signals originated from the same molecule. Thus, it provided the correlation information of the adjacent hydrogens in a molecule. Meanwhile, 2D J-resolved explained the splitting and coupling constant of proton signals which were not clear in the ^{1}H NMR spectrum containing complex compounds. In all, 17 metabolites were identified in the water extract of *C. arabica* var. sigararutang coffee beans including lipids, alanine, lactic acid, caffeine, malic acid, GABA, citric acid, choline, quinic acid, myo-inositol, trigonelline, 3-CQA, 5-CQA, 4-CQA, sucrose, formic acid, and asparagine. This paper discussed the structural elucidation of 17 coffee metabolites using the 1H NMR, COSY, TOCSY, and J-resolved spectra in detail.

Metabolite identification was initiated by detecting the signals of the main metabolites in the green coffee beans. Then, it was continued with the identification of the other minor compounds. Sucrose was found as the major compound in the green coffee beans. The proton signals from sucrose were identified easily. The proton signals of sucrose were detected in the chemical shift at δ 5.43 (1H, d, J = 5.03 Hz, H-1), δ 3.58 (1H, dd, J = 10.57 Hz, J = 5.03 Hz, H-2), δ 3.79 (1H, t, J = 9.51 Hz, H-3), δ 3.49 (1H, t, J = 9.51 Hz, H-4), δ 3.88 (1H, dd, J = 6.46 Hz, J = 3,25 Hz, H-5), δ 3.84 (1H, m, H-6′ /H-6), δ 3.70 (2H , s, H-1'), δ 4.23 (1H, d, J = 9.27 Hz, H-3′), δ 4.08 (1H, t, J = 9.51 Hz, H-4′), and δ 3.90 (1H, dd, J = 6.50 Hz, J = 3.66 Hz, H-5′). The correlation between sucrose hydrogens amplified the assignment of the proton signals, as depicted in the cross-peak 13 to 19 in the COSY spectrum (**Figure 2**). The cross-peak 16 in Figure 2 was the characteristic linkage of the anomeric H atom (δ 5.43 ppm) and H2 of sucrose. The H2 signal of sucrose was split into a doublet of doublet multiplicity with a coupling constant of 10.57 Hz ($J_{2,3}$) and 5.03 Hz ($J_{2,1}$), confirming that H2 of sucrose was at an axial bond and H1 was at an equatorial bond.

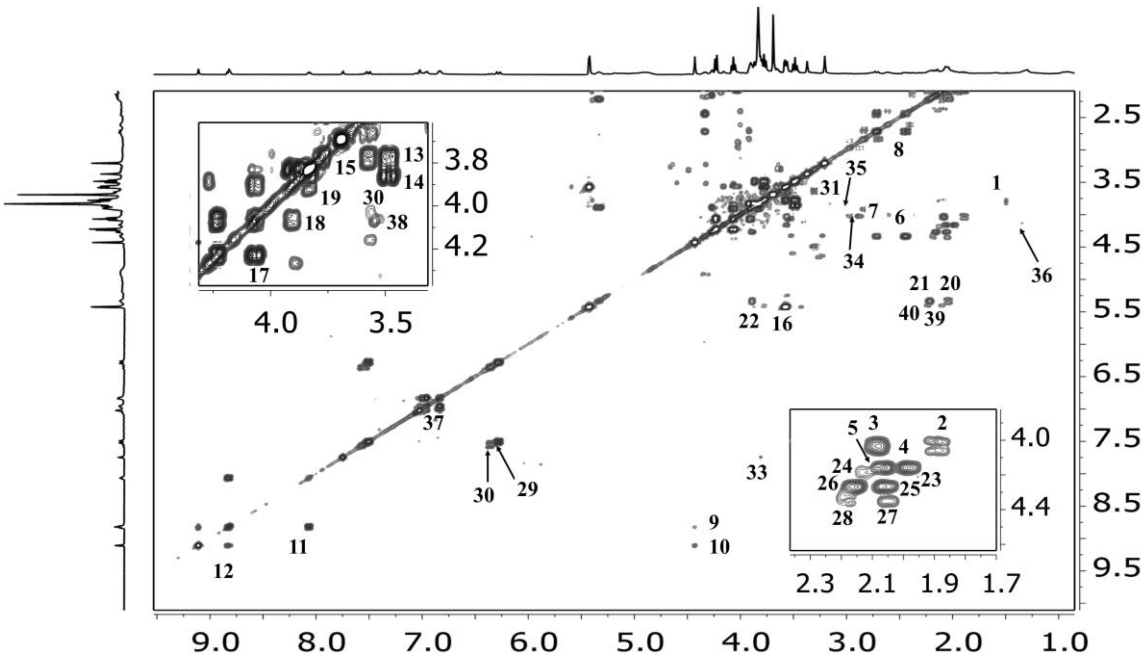

Figure 2: The signal associations of detected metabolites in the ^{1}H-^{1}H COSY spectrum. 1. H2/H3 of alanine; 2. H2a/H3 of quinic acid; 3. H2e/H3 of quinic acid; 4. H6a/H5 of quinic acid; 5. H6e/H5 of quinic acid; 6. H3/H2 of malic acid; 7. H3/H2 of malic acid; 8. H3/H3 of malic acid; 9. NCH_3/H6 of trigonelline; 10. NCH_3/H2 of trigonelline; 11. H5/H6 of trigonelline; 12. H4/H2 of trigonelline; 13. H4/H3 of sucrose; 14. H4/H5 of sucrose; 15. H2/H3 of sucrose; 16. H2/H1 of sucrose; 17. H4'/H3' of sucrose; 18. H4'/H5'of sucrose; 19. H5'/H6' of sucrose; 20. H6a/H5 of 5-CQA; 21. H6e/H5 of 5-CQA; 22. H5/H4 of 5-CQA; 23. H6a/H5 of 3-CQA; 24. H6e/H5 of 3-CQA;25. H2a/H3 of 5-CQA; 26. H2e/H3 of 5-CQA; 27. H2a/H3 of 4-CQA; 28. H2e/H3 of 4-CQA; ; 29. H2'/H3' of 5-CQA; 30. H2'/H3' of 3-CQA; 31 H2/H1 of myo-inositol; 32. H5/H4 of myo-inositol; 33. N7CH_3/H8 of caffeine; 34. H3/H2 of asparagine; 35. H3/H2 of asparagine; 36. H3/H2 of lactic acid; 37. H5'/H6' of 5-CQA; 38. H1/H2 of choline; 39. H2a/H3 of 3-CQA; and 40. H2e/H3 of 3-CQA

Trigonelline (1-methylpyridinium-3-carboxylate) was one of the alkaloids identified in this coffee sample. The proton signals in the pyridine ring of trigonelline were in the deshielding region, namely in the chemical shifts at 8.06 ppm (1H, t, J = 6 Hz, H-5), 8.80 ppm (1H, m, H-6), 8.82 ppm (1H, m, H-4) and 9.10 ppm (1H, s, H-2). The linkage of protons in the pyridine ring was confirmed by the cross-peaks of H5/H6 and H4/H2 in the COSY spectrum (Figure 2). N-methyl group of trigonelline in the ^{1}H NMR spectrum was detected at δ 4.43 ppm (3H, s, NCH_3). The correlations between NCH_3/H2 and NCH_3/H6 were confirmed at cross-peaks 12 and 13 in the TOCSY spectrum as seen in Figure 3. Another identified coffee alkaloid was caffeine. Caffeine is a trimethylxanthine in which the 3 methyl groups are located at carbons 1, 3, and 7. Signals of the 3 N-methyl groups of caffeine were identified at δ 3.17 ppm (3H, s, $N3CH_3$), 3.33 ppm (3H, s, $N1CH_3$), 3.88 ppm (3H, s, $N7CH_3$), meanwhile the aromatic proton signal of caffeine was identified at δ 7.72 ppm (1H, s, H-8).

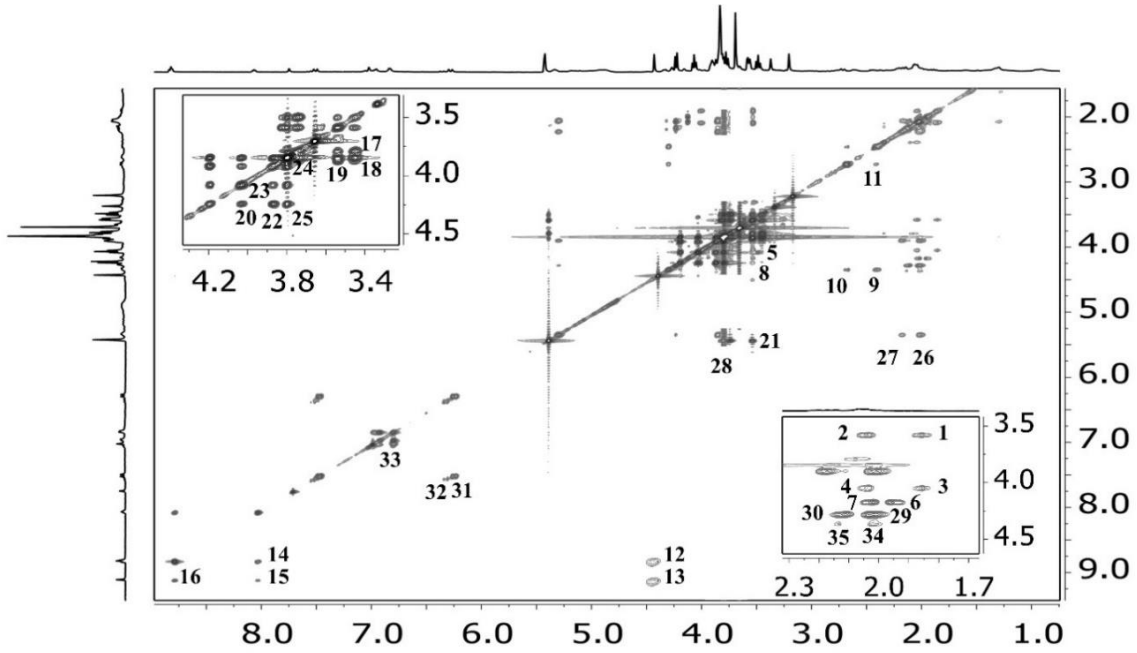

Figure 3: The signal associations of detected metabolites in the TOCSY spectrum. 1. H2a/H4 of quinic acid; 2. H2e/H4 of quinic acid; 3. H2a/H3 of quinic acid; 4. H2e/H3 of quinic acid; 5. H4/H3 of quinic acid; 6. H6a/H5 of quinic acid; 7. H6e/H5 of quinic acid; 8. H4/H5 of quinic acid; 9. H3/H2 of malic acid; 10. H3/H2 of malic acid; 11. H3/H3 of malic acid; 12. NCH_3/H6 of trigonelline; 13. NCH_3/H2 of trigonelline; 14. H5/H6 of trigonelline; 15. H5/H2 of trigonelline; 16. H4/H2 of trigonelline; 17. H4/H3 of sucrose; 18. H4/H5 of sucrose; 19. H2/H3 of sucrose; 20. H4'/H3' of sucrose; 21. H2/H1 of sucrose; 22. H5'/H3' of sucrose; 23. H5'/H4' of sucrose; 24. H5'/H6' of sucrose; 25. H6'/H3' of sucrose; 26. H6a/H5 of 5-CQA; 27. H6e/H5 of 5-CQA; 28. H5/H4 of 5-CQA; 29. H2/H3 of 5-CQA; 30. H2/H3 of 5-CQA; 31. H2'/H3' of 5-CQA; 32. H2'/H3' of 3-CQA; 33. H5'/H6' of 5-CQA; 34. H2/H3 of 4-CQA and 35. H6/H5 of 4-CQA.

Chlorogenic acids are the most common of phenolic metabolite in coffee beans and derived from the condensation of caffeic acid and quinic acid. Methylene groups of 3 chlorogenic acid isomers, namely 4-CQA, 5-CQA, and 3 CQA, were identified at δ 1.88-2.20 ppm of the 1D NMR spectra. The COSY (Figure 2) and TOCSY (Figure 3) were completely used as a guide to distinguish proton signals of chlorogenic acid isomers and the free quinic acid. The chemical shifts of the methylene and methine groups of 3 chlorogenic acid isomers were slightly more deshielding than the chemical shifts of these groups on quinic acid. This was due to the withdrawal electron effect of the ester bond connecting the quinic acid moiety and caffeoyl moiety of chlorogenic acids. Proton signals at quinic acid moiety of 5-CQA were identified at δ 2.07/2.20 ppm (2H, m, H-6), δ 2.04/2.16 ppm (2H, m, H-2), δ 3.90 ppm (1H, dd, J = 9.9 Hz, J = 4.5 Hz, H-4), δ 4.24 ppm (1H, d, J = 5 Hz, H-3), δ 5.33 ppm (1H, m, H-5). Meanwhile the proton signals of

the free quinic acid were confirmed at δ 1.89 ppm (1H, dd, J = 16.3 Hz, J = 3.1 Hz, H-2), δ 2.07 ppm (1H, m, H-2e/H6e), δ 3.55 ppm (1H, dd, J = 9.3 Hz, J = 3.7 Hz, H-4), δ 4.15 ppm (1H, ddd, J = 10.5 Hz, J = 9.5 Hz, J = 2.5 Hz, H- 5), δ 4.03 ppm (1H, ddd, J = 10.81 Hz, J = 9.33 Hz, J = 2.5 Hz, H-3), δ 1.98 ppm (1H, dd, J = 14.1 Hz, J = 11.9 Hz, H-6a). Proton linkages of a quinic acid moiety of 5-CQA were indicated by cross-peaks of H2/H3, H6/H5, and H4/H5 in Figure 2. At the same time, proton linkage of the free quinic acid were determined by cross-peaks of H2/H3, H6/H5 in Figure 2 and amplified by cross-peaks of H4/H5, H3/H4, H2/H4, H6/H4, H2/H3 and H6/H5 in Figure 3. Meanwhile, the olefinic proton signals of 5-CQA had been confirmed at δ 6.28 ppm and δ 7. 51 ppm. These two proton signals have a doublet multiplicity with a large constant coupling of approximately 16.04 Hz, as depicted in Figure 4. The large constant coupling suggested a trans configuration of the 5-CQA olefinic protons. The benzene ring of CQAs were detected at the δ 6.80 ppm to 6.99 ppm. Another CQAs, were elucidated with the same method. The olefinic protons of 4-CQA and 3-CQA were identified at δ 6.36 ppm and δ 7.56 ppm. Akin to 5-CQA, the H2/ H3 correlations of 4-CQA and 3-CQA (cross-peak 27 and 39 in Figure 2, respectively) confirmed the assignment of these protons in the quinic acid moiety of 4-CQA and 3-CQA.

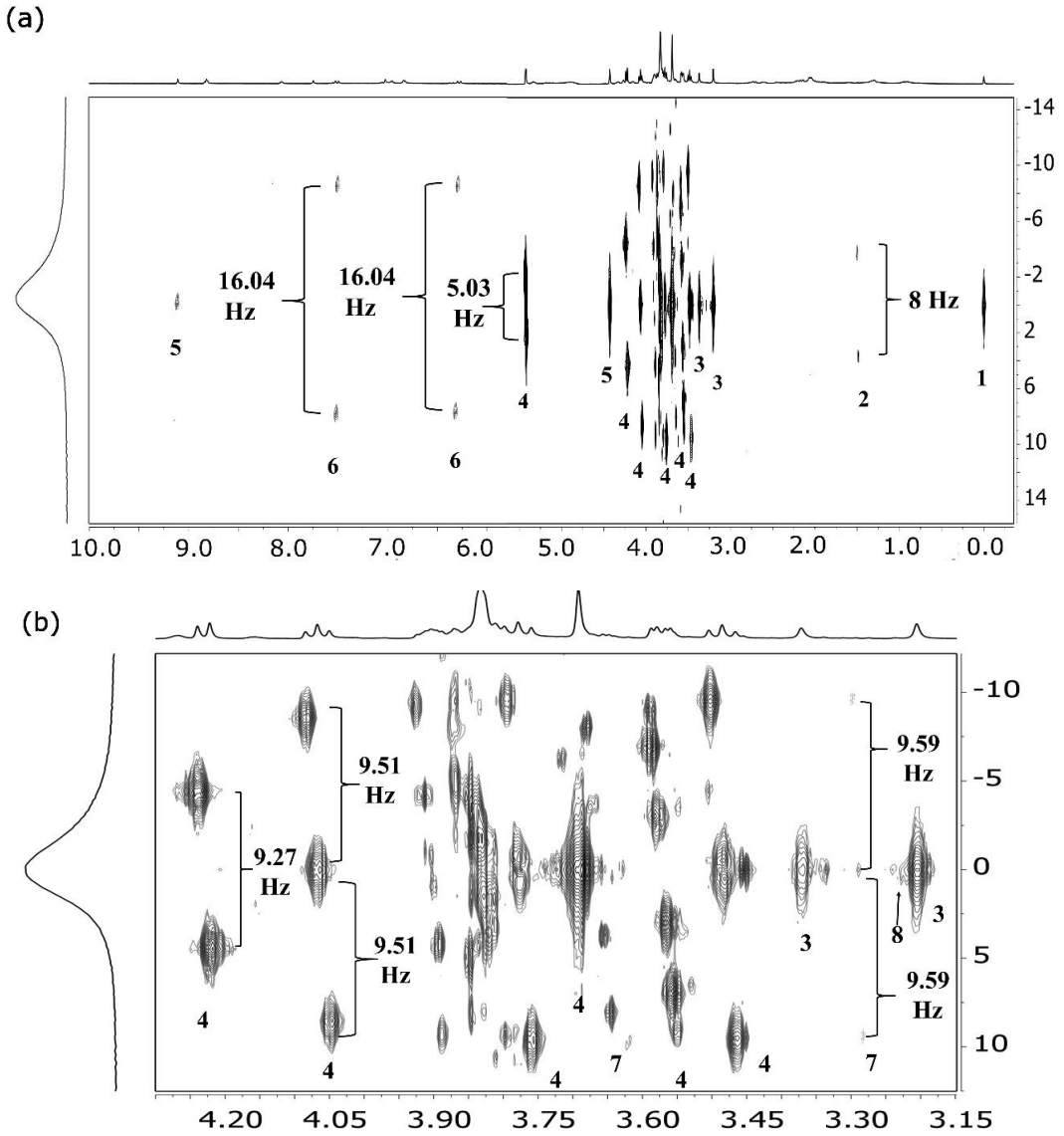

Figure 4: (a) 2D J-resolved spectrum of *C.arabica* var.sigararutang. **(b)** Expansion of J-resolved spectrum from 3.15 to 4.20 ppm. 1. TSP; 2. H-3 of Alanine; 3. $N3CH_3$ and $N1CH_3$ of Caffeine; 4. H-4, H-2, H-1', H-3, H-4', H-3', H-1 of Sucrose; 5. NCH_3 and H-2 of Trigonelline; 6. Oleofenic proton of CQAs; 7. H-2 and H-1 Myo-inositol; 8. $(NCH_3)_3$ of Choline.

Identifying minor metabolites of *C. arabica* var. sigararutang coffee was carried out using the same approach as identifying the major metabolites of coffee. Some of the minor coffee compounds identified were choline, malic acid, alanine, lactic acid, citric acid, formic acid,GABA, and asparagine. Malic acid is one of the natural acidic compounds present in the coffee beans. The attendance of malic acid in the coffee sample was identified through detecting its methylene signals at δ 2.46 ppm (1H, dd, J = 15 Hz, J = 5 Hz, H-3) and δ 2.71 ppm (1H, dd, J = 15 Hz, J = 5 Hz, H-3). Meanwhile, the methine group signal of malic acid was identified at δ 4.33 (1H, dd, J = 16.50 Hz, J = 6.50 Hz, H-2). The correlation of methylene and methine groups of malic acid was identified at cross-peaks 7 and 8 in Figure 2, respectively. The methylene groups of citric acid were confirmed at δ 2,58 (1H, d, J = 20,30 Hz, H-3) and δ 2,72 (1H, d, J = 20,30 Hz, H-3). Meanwhile, formic acid was identified clearly at δ 8.47 (1H, s, H-1). The signals of major and minor compounds identified in the 1D and 2D NMR spectrum of the sigagarutang coffee beans were summarized in Table 1.

Lactic acid was also identified by detecting its methyl group signal at δ 1.31 ppm (3H, d, J = 3.92 Hz, H-3) and the proton signal of C-chiral at δ 4,15 ppm (1H, d, J = 3.93 Hz, H-2). The cross-peak 36 in Figure 2 was the characteristic linkage of H3/H2 of lactic acid. The lactic acid signal at δ 1.31 ppm overlapped with the unknown lipid signal, forming a broad signal. The lipid signals in the ^{1}H NMR spectrum were confirmed at δ 0.94 ppm (–CH_2-CH_3, brs), δ 1.34 ppm ((–CH_2-)n, brs) as reported in the previous study[21], [22]. Three methyl groups of choline were identified at δ 3.21 ppm (9H, s, $N(CH_3)_3$), while its 2 methylene groups were detected at δ 3.49 ppm (2H, t, J = 9.51 Hz, H-1) and δ 4.08 (2H, t, J = 9.51 Hz, H-2). The correlation of choline protons was detected in the cross-peak 38 (COSY) in Figure 2.

Inositol belongs to the sugar family usually found in fruits and beans [23]. This compound has several isomers, and one of them is myo-inositol. The correlations detecting the presence of myo-inositol in the coffee samples were the signals in 3.28/3.62 ppm and 3.54/4.02 ppm of the COSY spectrum (seen on the cross-peaks 31 and 32 in Figure 2, respectively). These signals were weak and found in the absorption region of sugar protons in general. The four proton signals at δ 3.28 ppm (1H, t, J= 9.59 Hz, H-5), δ 3.54 ppm (H-5, t, J = 9.59 Hz, H-2), δ 3.62 ppm (1H, t, J = 9.59 Hz, H-4/H-6), δ 4.02 ppm (1H, dd, J = 11.45 Hz, J = 4.36 Hz, H-1/H-3) were confirmed as proton signals of myo-inositol. This identification result was confirmed by the reported works [4], [9].

Amino acids for instance asparagine, alanine, and GABA were also identified in the ^{1}H NMR spectrum of the coffee sample. Alanine had a typical doublet signal at δ 1.49 ppm (3H, d, J = 8 Hz, H-3). This signal was assigned to methyl protons of alanine, while the proton signal of its C-chiral was detected δ 3.81 ppm (1H, q, J = 7.5 Hz, H-2). The constant coupling of the methyl group of alanine was depicted in cross-peak 2 (Figure 4). Asparagine comprises an alpha carboxyl group and alpha-amino group in the main chain, as well as a carboxamide as a side chain [24]. The cross-peak 34 in figure 2 was assigned as the linkage between carboxamide and chiral proton of asparagine. Carboxamide signals of asparagine was detected at δ 2.89 ppm (1H, m, H-3α) and δ 2.97 ppm (1H, m, H-3β), while the proton signal of the C-chiral was identified at δ 4.04 ppm (1H, dd, J = 7.69 Hz, J = 4.02 Hz, H-2). Gamma-aminobutyric acid (GABA) is butanoic acid with the amino substituent located at C-4. This compound has three methylene groups identified at δ 1.90 ppm (2H, t, J = 12 Hz, H-3), 2.32 ppm (2H, m, H-2), and 3.02 ppm (2H, t, J = 12 Hz, H-4). Amino and carboxyl groups of GABA caused the proton chemical shifts of C2 and C4 were more slightly deshielding than the C3 proton signal.

Table 1. Assignments of the signals in the 1D and 2D NMR Spectrum of the *C.arabica* var. sigararutang coffee in D_2O solvent with buffer solution pH 6.0.

No	Metabolites	Parameters of NMR (chemical shift in ppm, multiplicity, and coupling constants in Hz)
1	Sucrose	δ 5.43 (1H, d, J = 5.03 Hz, H-1), δ 3.58 (1H, dd, J = 10.57 Hz, J = 5.03 Hz, H-2), δ 3.79 (1H, t, J = 9.51 Hz, H-3), δ 3.49 (1H, t, J = 9.51 Hz, H-4), δ 3.88 (1H, dd, J = 6.46 Hz, J = 3,25 Hz, H-5), δ 3.84 (1H, m, H-6′ /H-6), δ 3.70 (2H , s, H-1'), δ 4.23 (1H, d, J = 9.27 Hz, H-3′), δ 4.08 (1H, t, J = 9.51 Hz, H-4′), δ 3.90 (1H, dd, J = 6.50 Hz, J = 3.66 Hz, H-5′)
2	Trigonelline	δ 4.43 (3H, s, NCH_3), 8.06 (1H, t, J = 6 Hz, H-5), 8.80 (1H, m, H-6), 8.82 (1H, m, H-4), 9.10 (1H, s, H-2),
3	Caffeine	δ 3.17 (3H, s, $N3CH_3$), 3.33 (3H, s, $N1CH_3$), 3.88 (3H, s, $N7CH_3$), δ 7.72 (1H, s, H-8)
4	5-CQA	δ 2.07/2.20 (2H, m, H-6), δ 2, 04 /2.16 (2H, m, H-2), δ 3.90 (1H, dd, J = 9.9 Hz, J = 4.5 Hz, H-4), δ 4.24 (1H, d, J = 5 Hz, H-3), δ 5.33 (1H, m, H-5), δ 6.28 (1H, d, J = 16.04 Hz, H-2'), δ 7.51 (1H, d, J = 16.04 Hz, H-3'), δ 6.91 (1H, dd, J = 8.86 Hz, J = 3.51 Hz, H-5'), δ 6.81 (1H, d, J = 8.68 Hz, H-6'), δ 6.95 (1H, d, J = 8.68 Hz, H-9')
5	3-CQA	δ 2.00/2.07 (2H, m, H-6), δ 2.11/2.23 (2H, m, H-2), δ 4.19 (1H, dd, J = 6.9 Hz, J = 2 Hz, H-5), δ 5.40 (1H, m, H-3), δ 3.72 (1H, m,H-4), δ 6.40 (1H, d, J = 16,04 Hz, H-1'), δ 7.55 (1H, d, J = 16,04 Hz, H-2'), δ 6.81 (1H, d, J = 8.68 Hz, H-6'), δ 6.91 (1H, dd, J = 8.86 Hz, J = 3.51 Hz, H-5'), δ 6.95 (1H, d, J = 8.86 Hz, H-9')
6	4-CQA	δ 2.06/2.18 (2H, m, H-6), δ 2,04/2.18 (2H, m, H-2), δ 4.32 (1H, m, H-5), δ 4.36 (1H, m, H-3), δ 4,92 (1H, dd, J = 9,1 Hz, J = 2 Hz, H-4), δ 6.36 (1H, d, J = 16.04 Hz, H-1'), δ 7.55 (1H, d, J = 16.04 Hz, H-2'), δ 6.81 (1H, d, J = 8.68 Hz, H-6'), δ 6.91 (1H, dd, J = 8.86 Hz, J = 3.51 Hz, H-5'), δ 6.95 (1H, d, J = 8.68 Hz, H-9')
7	Quinic acid	δ 1.89 (1H, dd, J = 16.3 Hz, J = 3.1 Hz, H-2), δ 2.07 (1H, m, H-2e/H6e), δ 3.55 (1H, dd, J = 9.3 Hz, J = 3.7 Hz, H-4), δ 4.03 (1H, ddd, J = 10.81 Hz, J = 9.33 Hz, J = 2.5 Hz, H-3), δ 4.15 (1H, ddd, J = 10.5 Hz, J = 9.5 Hz, J = 2.5 Hz, H- 5), δ 1.98 (1H, dd, J = 14.1 Hz, J = 11.9 Hz, H-6a)
8	Malic acid	δ 2.46 (1H, dd, J = 15 Hz, J = 5 Hz, H-3), δ 2.71 (1H, dd, J = 15 Hz, 5 Hz, H-3), δ 4.33 (1H, dd, J = 16.50 Hz, J = 6.50 Hz, H-2)
9	Citric acid	δ 2.58 (1H, d, J = 20,30 Hz, H-3), δ 2.72 (1H, d, J = 20,30 Hz, H-3)
10	Formic acid	δ 8.47 (1H, s, H-1)
11	Lactic acid	δ 1.31 (3H, d, J = 3.92 Hz, H-3), δ 4.15 ppm (1H, d, J = 3.93 Hz, H-2)
12	Lipid	δ 0.94 (–CH_2-CH_3, brs), δ 1.34 ((–CH_2-)n, brs)
13	Choline	δ 3.21 (9H, s, $N(CH_3)_3$), δ 3.49 (2H, t, J = 9.51 Hz, H-1) and δ 4.08 (2H, t, J = 9.51 Hz, H-2)
14	Myo-Inositol	δ 3.28 (1H, t, J = 9.59 Hz, H-5), δ 3.54 (1H, t, J = 9.59 Hz, H-2), δ 3.62 (1H, t, J = 9.59 Hz, H-4/ H-6), δ 4.02 (1H, dd, J = 11,45 Hz, J = 4.36 Hz, H-1/H-3)
15	Alanine	δ 1.49 (3H, d, J = 8 Hz, H-3), δ 3.81 (1H, q, J = 7.5 Hz, H-2)
16	Asparagine	δ 2.89 (1H, m, H-3α), δ 2.97 (1H, m, H-3β), δ 4.04 (1H, dd, J = 7.69 Hz, J = 4.20 Hz, H-2)
17	GABA	δ 1.90 (2H, t, J = 12 Hz, H-3), 2.32 (2H, m, H-2), 3.02 (2H, t, J = 12 Hz, H-4)

The concentrations of some identified metabolites were determined relatively by comparing the corresponding metabolite signal integration to the integral of a reference signal (TSP 1 mM) [20]. Ten metabolites were successfully quantified, while the other metabolites had heavy overlapping signals making it difficult to quantify. Sucrose was reported as the biggest portion of the green coffee beans *C.arabica* var. sigararutang (6.62%, w/w) followed by 5-

CQA (2.76%, w/w) and caffeine (1.01% w/w). Meanwhile, alanine, GABA, and choline are minor coffee compounds whose abundance is below 0.1% (w/w). The concentration values of these metabolites were slight differences from the concentration of the green coffee beans reported in previous work [2,25]. The difference in the metabolite concentration values may be due to intrinsic differences in the coffee beans themselves, such as differences in the origin of the coffee beans, species, coffee processing methods, and analytical methods. The results of coffee metabolite quantification are depicted in table 2.

Table 2. Metabolite **c**oncentrations of *C. arabica* var. sigararutang calculated with the ^{1}H NMR quantitative method. Metabolite percentage was presented in mg/200 mg of green coffee bean sample

No.	Metabolites	Concentration (%)
1	Sucrose δ 5.43 (5.41-5.45)	6.62 ± 0.28
2	Trigonelline δ 9.10 (9.09-9.12)	0.47 ± 0.05
3	Caffeine δ 7.72 (7.70-7.74)	1.01 ± 0.04
4	5-CQA δ 6.31 (6.29-6.36)	2.76 ± 0.14
5	Quinic acid δ 4.15 (4.14-4.17)	0.83 ± 0.03
6	Malic acid δ 4.33 (4.31-4.34)	0.58 ± 0.06
7	Cholin δ 3.21 (3.18-3.22)	0.04 ± 0.01
8	Alanine δ 1.49 (1.48-1.51)	0.07 ± 0.01
9	GABA δ 3.02 (3.00-3.03)	0.08 ± 0.01
10	Asparagine δ 2.89 (2.87-2.90)	0.19 ± 0.03

4. Conclusion

Finally, the metabolite identification and quantification in the green bean extract of *C. arabica* var. sigararutang was carried out comprehensively using 1D and 2D NMR analysis. 1D ^{1}H NMR analysis was a useful method for identifying and quantifying metabolites in the extract coffee samples. The complexity in the ^{1}H NMR spectrum was completely resolved with TOCSY, COSY, and J-Resolved techniques. These results confirmed that the NMR spectroscopy analysis is a very fast and reliable method for analyzing samples containing complex metabolites. To the best of our knowledge, this is the first detailed study reporting the metabolite identification and quantification in this superior arabica coffee variety (sigararutang) with the NMR method.

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