Trad. Med. J., January – April 2019
Vol. 24(1), p 59-64
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 19-11-2018
Revised : 21-01-2019
Accepted : 15-04-2019

Metabolomic Study of Three Species in Zingiberaceae Family based on 1H-NMR

Dinar S.C. Wahyuni[1*], Magdalena Kristanti[1], Soerya D. Marliyana[2], Yudi Rinanto[3]
[1]Pharmacy Department, Faculty of Mathematics and Natural Science, Universitas Sebelas Maret, Surakarta, Indonesia
[2]Chemistry Department, Faculty of Mathematics and Natural Science, Universitas Sebelas Maret, Surakarta, Indonesia
[3]Biology Education, Faculty of Teacher Training and Education, Universitas Sebelas Maret, Surakarta, Indonesia

ABSTRACT

Zingiberaceae is an economical plant and widely used as traditional medicines. Its rhizomes have been reported to have biological effects due to their metabolites content. However, metabolites profiling in Zingiberaceae has not been reported comprehensively. Therefore, this study aimed to profile primary and secondary metabolite of three species Zingiberaceae rhizomes (*Zingiber amaricans* BI, *Zingiber officinale* Roscoe, and *Alpinia purpurata* (Vieill.) K.Schum using ^{1}H-NMR-based metabolomic approach. All samples were collected from local farmer located in Nguter, Sukoharjo, Central Java. Multivariate statistical analysis and ANOVA applied to measure the differences. It resulted that metabolite profiling discriminated between Zingiber and Alpinia samples. Fructose, α-glucose, β-glucose, sucrose, malic acid, alanine, valine, and shogaol contributed in discrimination between *Z. amaricans* BI, *Z. officinale* Roscoe, and *A. purpurata* (Vieill.) K.Schum. Sugar (α-glucose, β-glucose, fructose, and sucrose) and malic acid were significantly higher in *Alpinia* than in Zingiber samples. Relative concentration of amino acids (alanine and valine) and shogaol were significantly higher in *Z. officinale*. This result might be useful for databases and supplementary informations in *Z. amaricans*, *Z. officinale*, and *A. purpurata* taxonomy classifications.
Keywords: Zingiberaceae; *Z. amaricans* BI; *Z. officinale* Roscoe; *A. purpurata* (Vieill.) K.Schum; metabolomic; ^{1}H-NMR

INTRODUCTION

Zingiberaceae has been widely for years as traditional medicine in Asia. Two economic genera of Zingiberaceae are *Zingiber* and *Alpinia*. The former includes *Zingiber amaricans* BI and *Zingiber officinale* Roscoe which grow extensively in Indonesia. It has been reported to have anti-inflammatory (Thomson *et al.,* 2002), anticancer (Sang *et al.,* 2009), immunomodulator (Keong *et al.,* 2010) and antibacterial (Karuppiah and Rajaram 2012). Besides, *Alpinia purpurata* (Vieill.) K.Schum belong to *Alpinia* genus which has been reported to have antimicrobial (Kochuthressia *et al.,* 2010 and Santos *et al.,* 2012), antioxidant and anticancer (Raj *et al.,* 2012), mycobacterium tuberculosis (Villaflores et al. 2010) and larvicidal (Santos *et al.,* 2012). Those biological activities are due to metabolites profiling in biological samples (Fernie *et al.,* 2004 and Watkins and German, 2002).

Metabolites in Zingiber and Alpinia genera are mostly terpenoid and phenolic compounds. The former yield volatile oil which varies from 1%-3%. These are mainly monoterpenoids and sesquiterpenoids such as β-phellandrene, cineol, geraniol, β-bisabolene, zingiberol etc (Watkins and German 2002). Shogaol is a phenolic compound which is the main compound in *Zingiber officinale* (Kumar, Karthik, and Rao 2011). Besides gingerols, paradol and gingerdiol are detected in Zingiber genus. Major components of the rhizome oil of Alpinia were 1.8-cineole, chavicol, α-selinene (Chan and Wong 2015). Phenolic content in Alpinia were kaempferol 3-O-glucuronide and rutin (Villaflores *et al.,* 2010). Dominant volatile compounds in Alpinia genus were α-pinene, β-pinene and β-caryophyllene (Santos *et al.,* 2012). Metabolite profiling both primary and secondary compounds in Zingiber and Alpinia genera have been little reported. Therefore, a study of comprehensive analysis metabolites in those two genera need to be further investigated.

Comprehensive analysis of metabolites present in biological samples called metabolomic. Chemical analysis applied should be unbiased and high-throughput analytical method so that it could provide all information about metabolite (Gomez-Casati, Zanor, and Busi 2013). Nuclear Magnetic Resonance (NMR) spectroscopy is now increasingly popular in metabolome analysis. Despite its less sensitive, the benefit of NMR in metabolomic are non-destructive, simple sample preparation, and rapid sample preparation (Krishnan, Kruger and Ratcliffe, 2004 and Ali *et al.,* 2011). Besides, multivariate data analysis usually applied in combination with metabolomic analysis. Metabolomic based NMR has been used to analyze

***Corresponding author : Dinar S.C. Wahyuni**
Email : dinarsari_cw@staff.uns.ac.id

various plants. It has been used to analysis metabolites profiling of grape berry (Ali, Maltese, Fortes *et al.,* 2011), green tea (Lee *et al.,* 2015), *Polygonatum* species (Lee *et al.,* 2016).

This study aimed to investigate metabolite profiling of three species of Zingiberaceae family, *Z. amaricans* BI, *Z.* officinale Roscoe, and *A. purpurata* (Vieill.) K.Schum). Both primary and secondary metabolites identified and quantified to determine differences among samples. Additional 2D-NMR techniques (*J*-resolved and COSY) applied to support metabolites identification.

METHODOLOGY

Materials

All chemical reagents were deuterated degree for NMR analysis purposes. Methanol-d_4 (CD_3OD) 99.8% (Merck), Deuterium oxide (D_2O) >99.9% atom (Merck), Sodium deuteroxide (NaOD), and 3-(Trimethylsilyl) propionic acid-d_4 sodium salt.

Zingiberaceae family rhizomes (*Zingiber amaricans* BI, *Zingiber officinale* Roscoe, and *Alpinia purpurata* (Vieill.) K.Schum) were collected from local farmer located in Nguter, Sukoharjo, Central Java. Plant determination has been done in Biology Laboratory of Universitas Sebelas Maret, Surakarta.

Methods

Sample Preparation and Extraction

Three replicates of each sample were used for NMR metabolomics. The standard metabolomics protocol of sample preparation and ^{1}H-NMR profiling described by Kim *et al.,* (2010). Each sample was washed and air-dried. These samples were cut to smaller size and blended using blender for about 1-2 minutes then lyophilized for about 4x24 hours, afterwards. The dried rhizomes then ground to a fine powder using pestle and mortar. A sample of 30 mg of lyophilized sample was transferred to an Eppendorf tube to which 0.9 mL of methanol-d_4 (630 µL) and D_2O (270 µL) (KH_2PO_4 buffer, pH 6.0), containing 0.01% TSP-d_4 were added. The mixture was vortexed at room temperature for 1 minutes, ultrasonicated for 20 minutes, and centrifuged at 13,300 rpm at room temperature for 10 minutes. About 600 µL of the supernatant was transferred to NMR tube and use for the ^{1}H-NMR analysis.

NMR Measurement

^{1}H-NMR spectra were recorded at 25^{o}C on 400 MHz Agilent spectrometer. Deuterated water was used as the internal lock. Each ^{1}H-NMR spectrum consisted of 128 scans requiring 56 min and 48 s acquisition time with the following parameters: 0.29 Hz/point, pulse width (PW) of 90^o (6.8 µs), and relaxation delay (RD) of 2s. Two-dimensional J-resolved NMR spectra were acquired using eight scans per 64 increments for F1 (chemical shift axis) and eight scans for F2 (spin-spin coupling constant axis) using spectral widths of 64 Hz and 4807.7 Hz respectively. Both dimensions were multiplied by sine-bell functions (SSB = 0) before double complex Fourier transformation. J-resolved spectra were tilted by 45^o, symmetrized about F1, and then calibrated to TSP. ^{1}H-^{1}H correlated COSY spectra were acquired with a 1.0 s relaxation delay and 4807.7 Hz spectral width in both dimensions. The window function for the COSY spectra was Qsine (SSB = 0). MestRenova version 11.0.0 applied to identify metabolites in samples.

Data Analysis and Statistics

The ^{1}H NMR spectra were automatically reduced to ASCII files. Spectral intensities were scaled to internal standard and reduced to integrated regions of equal width (0.04) corresponding to the region of δ 0.0–9.98. Bucketing was performed by AMIX software with scaling on internal standard. Principal component analysis (PCA) with scaling based on Pareto was performed with the SIMCA-P software (version 15.0, Umetrics, Umeå, Sweden). The ANOVA and LSD Test for the ^{1}H-NMR signals was performed by SPSS.

RESULTS AND DISCUSSION

Metabolites Identification

^{1}H-NMR spectra of the methanol extract of *Z. amaricans* BI, *Z. officinale* Roscoe, and *A. purpurata* (Vieill.) K.Schum are shown in (Figure 1). In general, signals detected in three regions: amino acids at δ 2.0 – δ 0.5 ppm, organic acid at δ 3.0 – δ 2.0 ppm, sugars at δ 5.0 – δ 3.0 ppm, and phenolic at δ 7.5 – δ 6.0 ppm. The ^{1}H-NMR spectrum of *A. purpurata* (Vieill.) K.Schum were dominated by the signals of sugars and organic acid. Metabolites identified using ^{1}H-NMR spectra and confirmed by 2D NMR spectra (J-resolved and COSY). The dominant primary metabolites present in the three species of Zingiberaceae family rhizomes extract included four carbohydrates (α-glucose, β-glucose, fructose, and sucrose), amino acids (alanine, valin, and threonine), organic acid (malic acid), and phenolic (shogaol). Signal assignment is listed in (Table I).

Multivariate data analyses (MvDA)

The ^{1}H-NMR data were subjected to multivariate data analyses Principle Component Analysis (PCA). PCA scoring plot was used to

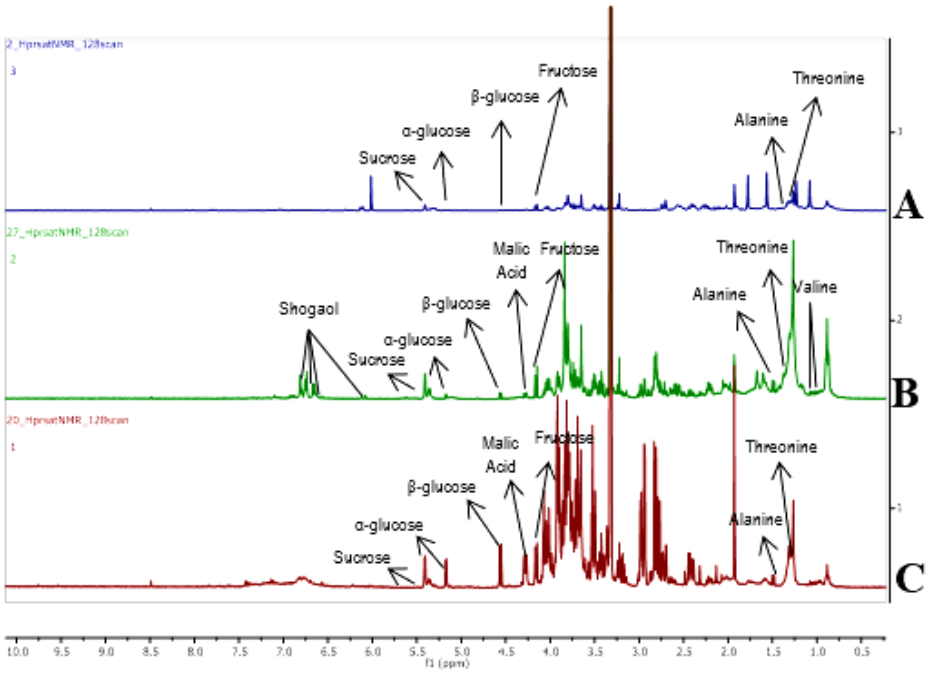

Figure 1. Representative ^{1}H-NMR spectra of the methanol extract of three species Zingiberaceae family rhizomes. (A) *Z. amaricans* BI; (B) *Z. officinale* Roscoe; (C) *A. purpurata* (Vieill.) K.Schum.

Table I. ^{1}H-NMR chemical shifts (δ) and coupling constants (Hz) of three species Zingiberaceae rhizomes metabolites identified by references and using 1D and 2D NMR spectra (CD_3OD–KH_2PO_4 in D_2O, pH 6.0). Abbreviation, s = singlet, d = doublet, t = triplet, dd = double-doublet, dt = double-triplet m = multiplet

Metabolite	**δ H (Multiplicity [a])**
α-glucose	5.17 ppm (d, *J* = 3.49 Hz), 3.44 ppm (dd, *J* = 9.57, 3.94 Hz)
β-glucose	4.55 ppm (d, *J* = 7.75 Hz), 3.18 ppm (dd, *J* = 10.29, 7.20 Hz)
Sucrose	5.41 ppm (d, *J* = 3.75 Hz), 3.49 ppm (dd, *J* = 9.98, 3.75 Hz), 3.74 ppm (t, *J* = 9.31 Hz), 3.42 ppm (t, *J* = 9.31 Hz), 4.16 ppm (d, *J* = 8.46 Hz)
Fructose	4.15 ppm (d, *J* = 8.86 Hz)
Valine	1.07 ppm (d, *J* = 7.05 Hz), 1.02 ppm (d, *J* = 7.25 Hz)
Alanine	1.49 ppm (d, *J* = 7.10 Hz)
Threonine	3.52 ppm (d, *J* = 4.44 Hz), 1.32 ppm (d, *J* = 6.85 Hz)
Malic acid	4.28 ppm (dd, *J* = 9.53, 3.32 Hz), 2.73 ppm (dd, *J* = 15.28, 3.32 Hz), 2.43 ppm (dd, *J* = 15.61, 9.53 Hz)
Shogaol	6.90 ppm (dt, *J* = 16.15, 6.59 Hz), 6.75 ppm (d, *J* = 8.06 Hz), 6.65 ppm (dd, *J* = 8.03, 2.73 Hz), 6.57 ppm (d, *J* = 2.23 Hz), 6.10 ppm (dt, *J* = 16.4, 1.53 Hz), 3.84 ppm (s), 2.79-2.57 ppm (m), 2.21 ppm (m), 0.88 ppm (t, *J* = 7.22 Hz)

analyze the metabolite pattern of three species. The PCA scoring plot clustered between *Z. amaricans* BI - *Z. officinale* Roscoe and *A. purpurata* (Vieill.) K.Schum at PC1 (63%) (Figure 2). Zingiber and Alpinia samples was grouped at positive and negative quadrant, respectively. It resulted metabolite profiling between Zingiber and Alpina samples differed. Between Zingiber samples, *Z. amaricans* BI and *Z. officinale* Roscoe differed by PC2 (27.8%). In consequence, metabolite profiling between Zingiber samples showed little different. Loading plot showed that Zingiber samples were much higher concentration of amino acids (alanine, valine, and threonine) and phenolic (shogaol) than Alpinia sample in positive value (Figure 3). On the other hands, in negative value, loading plot depicted much higher content of sugars (α-glucose, β-glucose, fructose, and sucrose) and organic acid (malic acid). It is in accordance to metabolic profiling and phylogenetic analysis of Zingiber species based on molecular and chemical markers (Jiang *et al.,* 2006). The metabolic profiles of Zingiber species were very different both qualitative and quantitatively. Moreover, it

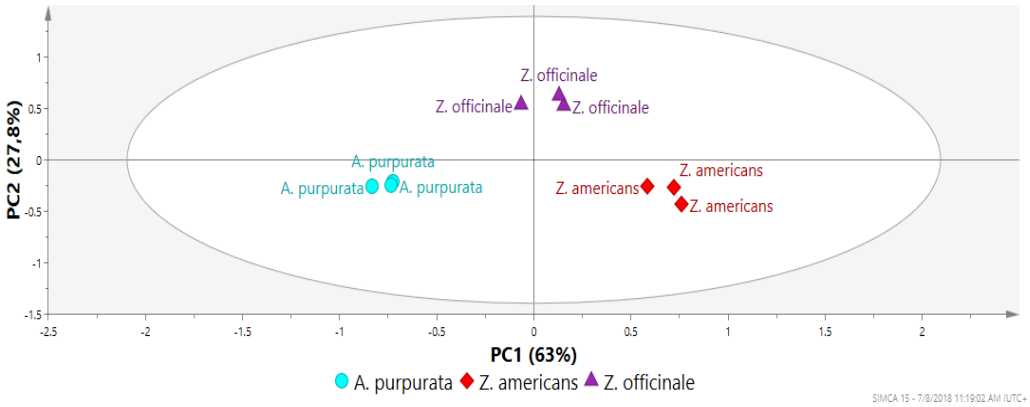

Figure 2. Scoring plot of PCA of *Z. amaricans* BI, *Z. officinale* Roscoe, and *A. purpurata* (Vieill.) K.Schum

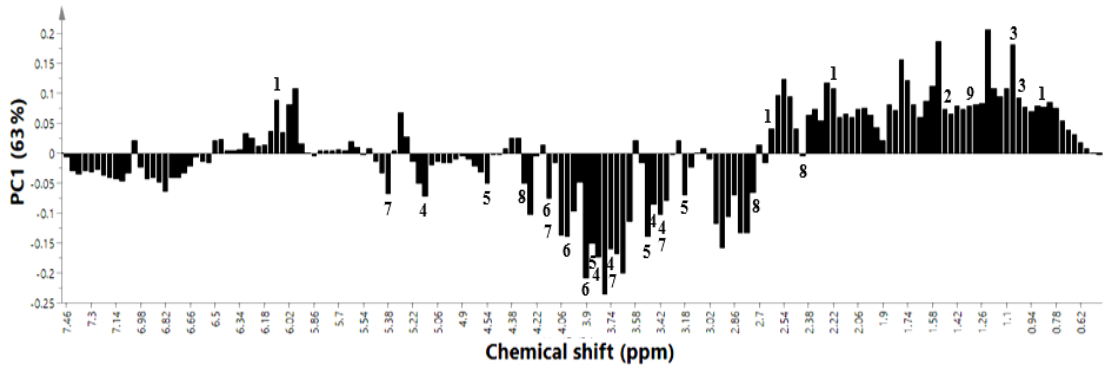

Figure 3. Loading plot of PCA of *Z. amaricans* BI, *Z. officinale* Roscoe, and *A. purpurata* (Vieill.) K.Schum indicated chemical shift that responsible to PCA scoring plot separation. 1: shogaol, 2: alanine, 3: valine, 4: α-glucose, 5: β-glucose, 6: fructose, 7: sucrose, 8: malic acid, 9: threonine.

explained metabolic profiling between Zingiber and Alpinia samples were clearly differed. Secondary metabolite, shogaol was detected only in Zingiber samples (Jiang *et al.,* 2006).

Metabolites detected in spectra were mostly polar compounds due to its solvent combination of CD_3OD and D_2O. It was used in the experiment since first those solvents were commonly used in metabolomic study (Kim, Choi, and Verpoorte 2010b). Second, the solvent combination reflects the common solvent used in herbal medicine industry. Therefore, characteristic volatile metabolites such as β-phellandrene, cineol, geraniol, β-bisabolene, zingiberol, and terpineol of samples were not detected.

Metabolomic study based on NMR is able to profile metabolites of Zingiber and Alpinia samples. Metabolite profiling of Zingiber and Alpinia samples clearly discriminated. Both primary metabolites such as fructose, α-glucose, β-glucose, sucrose, malic acid, alanine, valine and secondary metabolites shogaol contributed in discrimination. This result might be useful for databases and supplementary information in *Z. amaricans* BI, *Z. officinale* Roscoe, and *A. purpurata* (Vieill.) K.Schum in taxonomy classifications.

CONCLUSION

Metabolomic study based on NMR is able to profile metabolites of Zingiber and Alpinia samples. Metabolite profiling of Zingiber and Alpinia samples clearly discriminated. Both primary metabolites such as fructose, α-glucose, β-glucose, sucrose, malic acid, alanine, valine and secondary metabolites shogaol contributed in discrimination. This result might be useful for databases and supplementary information in *Z. amaricans* BI, *Z. officinale* Roscoe, and *A. purpurata* (Vieill.) K.Schum in taxonomy classifications.

ACKNOWLEDGMENTS

The authors acknowledge financial support from Competitive Grant, PNBP, 2018, Universitas Sebelas Maret.

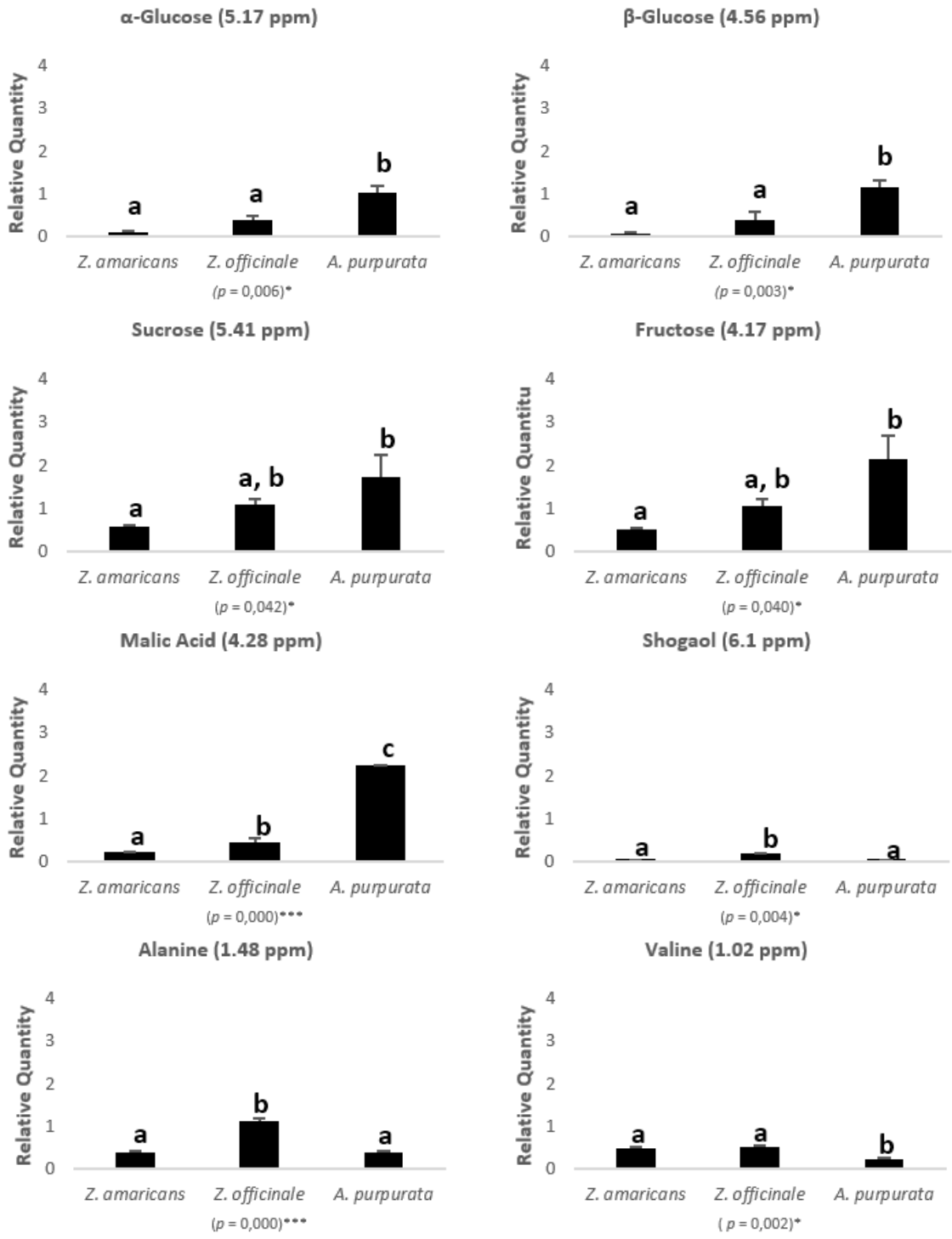

Figure 4. Relative quantification of metabolites in *Z. amaricans* BI, *Z. officinale* Roscoe, and *A. purpurata* (Vieill.) K.Schum. Difference in letters shows a significant value at 95% confidence level.

REFERENCES

Ali, Kashif, Federica Maltese, Ana Margarida Fortes, *et al.,* 2011. "Monitoring Biochemical Changes during Grape Berry Development in Portuguese Cultivars by NMR Spectroscopy." *Food Chemistry* 124(4):1760–69.

Ali, Kashif, Federica Maltese, Reinhard Toepfer, Young Hae Chotablei, and Robert Verpoorte. 2011. "Metabolic Characterization of

Palatinate German White Wines According to Sensory Attributes, Varieties, and Vintages Using NMR Spectroscopy and Multivariate Data Analyses." *Journal of Biomolecular NMR* 49(3–4):255–66.

Chan, Eric Wei Chiang and Siu Kuin Wong. 2015. "Phytochemistry and Pharmacology of Ornamental Gingers, Hedychium Coronarium and Alpinia Purpurata: A Review." *Journal of Integrative Medicine* 13(6):368–79.

Fernie, Alisdair R., Richard N. Trethewey, Arno J. Krotzky, and Lothar Willmitzer. 2004. "Metabolite Profiling: From Diagnostics to Systems Biology." *Nature Reviews Molecular Cell Biology* 5:763.

Gomez-Casati, Diego F., Maria I. Zanor, and María V Busi. 2013. "Metabolomics in Plants and Humans: Applications in the Prevention and Diagnosis of Diseases." *BioMed Research International* 2013.

Jiang, Hongliang *et al.,* 2006. "Metabolic Profiling and Phylogenetic Analysis of Medicinal Zingiber Species: Tools for Authentication of Ginger (Zingiber Officinale Rosc.)." *Phytochemistry* 67(15):1673–85.

Karuppiah, Ponmurugan and Shyamkumar Rajaram. 2012. "Antibacterial Effect of Allium Sativum Cloves and Zingiber Officinale Rhizomes against Multiple-Drug Resistant Clinical Pathogens." *Asian Pacific Journal of Tropical Biomedicine* 2(8):597–601.

Keong, YEAP SWEE *et al.,* 2010. "Immunomodulatory Effects of Zerumbone Isolated from Roots of Zingiber Zerumbet." *Pak J Pharm Sci* 23(1):75–82.

Kim, Hye Kyong, Young Hae Choi, and Robert Verpoorte. 2010a. "NMR-Based Metabolomic Analysis of Plants." *Nature Protocols* 5(3):536–49.

Kim, Hye Kyong, Young Hae Choi, and Robert Verpoorte. 2010b. "NMR-Based Metabolomic Analysis of Plants." *Nature Protocols* 5(3):536.

Kochuthressia, K. P., S. John Britto, M. O. Jaseentha, L. Joelri Michael Raj, and S. R. Senthilkumar. 2010. "Antimicrobial Efficacy of Extracts from Alpinia Purpurata (Vieill.) K. Schum. against Human Pathogenic Bacteria and Fungi." *Agriculture and Biology Journal of North America* 1(6):1249–52.

Krishnan, P., N. J. Kruger, and R. G. Ratcliffe. 2004. "Metabolite Fingerprinting and Profiling in Plants Using NMR." *Journal of Experimental Botany* 56(410):255–65.

Kumar, Gaurav, L. Karthik, and K. V. Bhaskara Rao. 2011. "A Review on Pharmacological and Phytochemical Properties of Zingiber Officinale Roscoe (Zingiberaceae)." *Journal of Pharmacy Research* 4(9):2963–66.

Lee, Jang-Eun *et al.,* 2015. "Metabolomic Unveiling of a Diverse Range of Green Tea (Camellia Sinensis) Metabolites Dependent on Geography." *Food Chemistry* 174:452–59.

Lee, Min Young *et al.,* 2016. "Discrimination of Polygonatum Species and Identification of Novel Markers Using 1H NMR-and UPLC/Q-TOF MS-based Metabolite Profiling." *Journal of the Science of Food and Agriculture* 96(11):3846–52.

Raj, Chinthamony Arul, Paramasivam Ragavendran, Dominic Sophia, Muthaiyan Ahalliya Rathi, and Velliyur Kanniappan Gopalakrishnan. 2012. "Evaluation of in Vitro Antioxidant and Anticancer Activity of Alpinia Purpurata." *Chinese Journal of Natural Medicines* 10(4):263–68.

Sang, Shengmin *et al.,* 2009. "Increased Growth Inhibitory Effects on Human Cancer Cells and Anti-Inflammatory Potency of Shogaols from Zingiber Officinale Relative to Gingerols." *Journal of Agricultural and Food Chemistry* 57(22):10645–50.

Santos, Geanne K. N. *et al.,* 2012. "Essential Oils from Alpinia Purpurata (Zingiberaceae): Chemical Composition, Oviposition Deterrence, Larvicidal and Antibacterial Activity." *Industrial Crops and Products* 40:254–60.

Thomson, M. *et al.,* 2002. "The Use of Ginger (Zingiber Officinale Rosc.) as a Potential Anti-Inflammatory and Antithrombotic Agent." *Prostaglandins, Leukotrienes and Essential Fatty Acids* 67(6):475–78.

Villaflores, Oliver B *et al.,* 2010. "Phytoconstituents from Alpinia Purpurata and Their in Vitro Inhibitory Activity against Mycobacterium Tuberculosis." *Pharmacognosy Magazine* 6(24):339.

Watkins, Steven M. and J. Bruce German. 2002. "Metabolomics and Biochemical Profiling in Drug Discovery and Development." *Current Opinion in Molecular Therapeutics* 4(3):224–28.