Research Article

Effect of Cocoa Bean Shell Addition on Metabolite Profile and Antioxidant Activity of Herbal Infusions

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Cocoa bean shell (CBS) is a by-product with aromatic characteristics that can enhance the aroma and bioactivity of herbal infusions. This study was aimed to determine the effect of the addition of cocoa bean shell on the metabolite profile and antioxidant activity of infusions made with Ilex guayusa and Vernonanthura patens and their mixtures. Metabolite profile was analyzed by gas chromatography–mass spectrometry combined with multivariate analysis. Total polyphenol content and flavonoids were determined by the Folin-Ciocalteu method and by the flavonoid-AlCl3 complex, respectively. Antioxidant activities were measured by the decolorization assay of the 2,2-diphenyl-1-picrylhydrazyl radical and the ferric reducing antioxidant power. The results revealed that the addition of CBS increases the content of phenolic acids in the infusions (caffeic acid, 4-hydroxybenzoic acid, and pyrocatechol). Nonetheless, the antioxidant activity of the infusions decreased with the addition of CBS (16.21 to 2.74 TEAC). Carboxylic acids and derivatives, major compounds present in the infusions prepared with V. patens, were the metabolites that showed the highest correlation with the antioxidant activity. This study suggests that the infusions made with CBS present a profile of metabolites different from the infusions of I. guayusa, V. patens, and their mixtures.

1. Introduction

Herbal infusion is a widely consumed beverage and represents an important source of polyphenols [1]. Those bioactive compounds have been related to biological activities such as antioxidant, anti-inflammatory, and anticarcinogenic [2–4].

Ilex guayusa is a holly species that is consumed as a stimulant beverage attributable to the high caffeine content [5]. Although there is limited information about the biological properties of I. guayusa, recent studies have reported the species as antioxidant and anti-inflammatory because of the presence of polyphenols, flavonoids, xanthines, and carotenoids [6–11].

Vernonanthura patens is a wild plant distributed from Mexico to Argentina [12]. Leaves are used to prepare decoctions to calm headaches and for treating certain types of cancer [13]. Several compounds have been identified in the species such as pentacyclic triterpenoids, polyphenols, tannins, and flavonoids [14–16].

Cocoa bean shell (CBS) is a by-product of the cocoa industry [17]. The estimated generation of this material is about 700 thousand tons [18, 19]. Due to its nutritional characteristics such as fiber content, polyphenols, and lipid profile, many authors have focused on the use of this material to create value-added products [20–25]. For instance, Kraft Food has developed a patent to use CBS as a food additive that can improve the viscosity of dairy products and accentuate the chocolate flavour [26].

Therefore, the present study was aimed to establish the metabolite profile of herbal infusions prepare with I. guayusa,
2. Materials and Methods

2.1. Reagents and Chemicals. Folin–Ciocalteu’s phenol reagent 2 N, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), aluminium chloride hexahydrate (AlCl3), N,O-Bis(trimethylsilyl)tri-fluoroacetamide (BSTFA), quercetin, gallic acid, sodium nitrite, and methanol were acquired from Sigma–Aldrich (St. Louis, MO, USA). Saturated alkanes standard (C7-C40) was purchased from Supelco (Bellefonte, PA, USA). Sodium hydroxide, hydrochloric acid, and ethanol were obtained from J.T. Baker (Phillipsburg, NJ, USA). Sodium carbonate (Na2CO3) was purchased from Fisher Scientific (Lisbon, Portugal), acetic acid was from Panreac (Barcelona, Spain), and ferric chloride (FeCl3) was from Mallinckrodt (New York, NY, USA). Water was purified in a Milli-Q water purification system Millipore (Bedford, MA, USA).

2.2. Preparation of Herbal Infusions. An augmented simplex-centroid mixture design with three components and ten formulations was employed in order to evaluate the addition of CBS in the infusions. According to Table 1, different proportions of CBS, *I. guayusa*, and *V. patens* were used. CBS were provided by Maquita Cushunchic-MCCH (nonprofit foundation), Guayaquil, Guayas. *I. guayusa* (voucher No. CIBE020), and *V. patens* (voucher No. CIBE037) leaves were obtained from Taisha, Morona Santiago, and Marcabelí, El Oro, respectively. Samples of plant material were authenticated by the National Herbarium of Ecuador. Then, the remaining plant material was dried, ground, and sieved individually. Afterward, herbal formulations were prepared by pouring 200 mL of boiled distilled water over 2 g of raw material (CBS, *I. guayusa* and *V. patens*) for 5 min without mixing. Infusions preparation were filtered through a paper Whatman #1 and kept at -17°C until its use.

2.3. Metabolite Profile by GC-MS. Metabolite profile was performed by gas chromatography–mass spectrometry (GC–MS) (Agilent Technologies 7890A GC system and 5975C inert XL MSD with a triple axis detector), using a DB-5MS capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness, Agilent Technologies, Inc.) and helium as a carrier gas at a flow rate of 0.6 mL/min [27]. Briefly, 5 mg of freeze-dried infusion was mixed with 200 μL of BSTFA, and incubated in a water bath at 70°C for 2 hours. After derivatization, 2 μL of samples were injected at 280°C with splitless mode, and three biological replicates of each sample were measured. The initial oven temperature was held at 70°C for 5 minutes, after it was increased to 130°C at 15°C/min, then it was increased to 160°C at 4°C/min (held for 15 minutes), and finally, it was increased to 300°C at 10°C/min (held for 15 minutes). The MSD transfer line was 285°C, and the ion source temperature was 230°C. Electron ionization of 70 eV was used, and the raw data compounds were collected with the full scan mode (40-700 amu) in the quadrupole mass analyzer.

2.4. GC–MS Data Processing and Compound Identification. Raw data files were converted to NetCDF/AIA (*.cdf) format using the ChemStation GC/MSD Data Analysis Software (Agilent Technologies, Palo Alto, CA, USA). MzMine 2 (version 2.2.9) was employed for mass spectra detection, chromatographic building, deconvolution, and alignment [28]. Next, the resulting data sets were imported into MetaboAnalyst 3.0 for multivariate statistical analysis [29]. Compounds were tentatively identified by matching mass spectra with the information available in the NIST 11 Wiley 9 database and by comparing the estimated retention index using a series of n-alkanes (C7-C40) [30, 31].

2.5. Antioxidant Capacity of Herbal Infusions. Total phenolic content (TPC) was determined spectrophotometrically using Folin–Ciocalteu method [32], and the results were expressed as mg Gallic Acid Equivalent (GAE)/L. Total flavonoid content (TFC) was estimated according to the aluminium chloride colorimetric method [33], and the results were expressed as mg Quercetin Equivalent (QE)/L. Scavenging activity of CBS infusion against DPPH free radical was calculated by referenced method [34], and results were expressed as an equivalent of mM Trolox (TEAC). Finally, the Ferric Reducing Antioxidant Power (FRAP) was evaluated by the reducing power of ferric-tripyridyl-triazine (Fe3+-TPTZ) complex to ferrous form (Fe2+) method [35]. Then, a standard curve of Trolox was calculated, and the results were expressed as equivalent of mM Trolox (TEAC).

2.6. Statistical Analysis. All experiments were conducted in triplicate and values were expressed as mean ± standard deviation (SD). Statistical significance was analyzed through ANOVA and Tukey test at *p* < 0.05 using the statistical software package Minitab 16 (Minitab Inc., State College, PA, USA).

### 3. Results and Discussion

3.1. Multivariate Analysis of Herbal Infusions Using Different Mixture of Raw Materials. Metabolite profiles of ten

| Formulation | CBS (%) | *I. Guayusa* (%) | *V. patens* (%) |
|-------------|---------|------------------|-----------------|
| F1          | 0,33    | 0,33             | 0,33            |
| F2          | 0       | 1                | 0               |
| F3          | 0       | 0                | 1               |
| F4          | 0,167   | 0,667            | 0,167           |
| F5          | 0       | 0,5              | 0,5             |
| F6          | 0,5     | 0,5              | 0               |
| F7          | 0,5     | 0                | 0,5             |
| F8          | 0,667   | 0,167            | 0,167           |
| F9          | 1       | 0                | 0               |
| F10         | 0,167   | 0,167            | 0,667           |
formulations obtained by a mixture design of experiment were assessed by CG–MS in order to determine the effect of CBS addition on herbal infusions chemical composition of *I. guayusa* and *V. patens* medicinal plants. The principal component analysis (PCA) explained 72.40% of the total of variability (Figure 1(a)). The PC1 (62.40%) separated F6 and F9 from F1, F2, F3, F4, F5, F7, F8, and F10. The separation can be attributed to the quantity of CBS present in the infusions F6 and F9, which is higher than in the other formulations. However, F7 and F8 samples present an equal or higher content of CBS but they were not grouped by the presence of *V. patens*. Additionally, PC2 (10%) separated F1, F2, F3, and F4 from F5, F7, and F8, which indicated that *I. guayusa* and *V. patens* present a similar chemical profile, in contrast to CBS. Hierarchical cluster analysis (HCA) of all detected metabolites showed a similar pattern to that suggested by the PCA and divided the samples into two clusters at a distance of two in the dendrogram (Figure 1(b)).

3.2. Comparison of Metabolites in Herbal Infusions. Twenty-two metabolites were selected based on the variable importance in the projection (VIP) > 2 and *p* value < 0.05 in partial least squares discriminant analysis (PLS-DA). Carboxylic acids and derivatives (9), phenols (5), and sugar and sugar alcohols (3) were identified to discriminate the chemical composition of herbal infusions (Table 1). According to the heat map (Figure 2), carboxylic acids and derivatives were predominant in the formulations that contains *V. patens* as the main raw material (F3, F10, and F5). Among the phenolic acids, they were more abundant in the formulation (F9) that contains CBS (cafeic acid, hydroxy-benzoic acid, and pyrocatechol), followed by formulation (F2) with *I. guayusa* (quercetin 7,3′, 4′-trimethyl ether) and *V. patens* (F3) (hydroquinone). Sugar and sugar alcohols were found in the formulations with higher content of CBS (F9, F8, and F6).

3.3. Correlation between Bioactivity and Metabolite Composition of Herbal Infusions. Correlation map between antioxidant activity (DPPH, FRAP), TPC, TFC, and metabolite profile was performed in order to determine the potential compounds that are related to the biological activity of the infusions. According to Figure 3, fourteen compounds including linoleic acid, myristic acid, palmitic acid, stearic acid, 1-monopalmitin, 1-monolinolein, 1-monocestarin, hydroquinone, quercetin 7,3′, 4′-trimethyl ether, and five nonidentified compounds showed a positive correlation with antioxidant activity, TPC, and TFC. On the other hand, eight compounds identified as nonadecanoic acid, hydroxybenzoic acid, pyrocatechol, caffeic acid, d-lactose, sucrose, l-threitol, and acetic acid were negatively correlated to antioxidant activity, TPC, and TFC. Additionally, a strong positive correlation was observed between antioxidant activity and TPC and TFC.

3.4. Antioxidant Activity, TPC, and TFC of Herbal Infusions. Antioxidant activity (DPPH, FRAP), TPC, and TFC of ten
formulations of herbal infusions are showed in Figure 4. The antioxidant activity ranged from 8.74 (F3) to 1.41 (F1) mM TEAC for the DPPH assay and from 16.21 (F3) to 3.12 (F1) mM TEAC for FRAP assay. Higher values of TPC and TFC were observed in F3 (3306.04 mg GAE/L and 388.19 mg QE/L), and lower values were registered in F9 (428.18 mg GAE/L and 42.64 mg QE/L).

In this research, we studied how the addition of CBS in herbal infusion affects the chemical composition and antioxidant activity of beverages made with *I. guayusa* and *V. patens*. Despite, CBS was rich in phenolic compounds; the addition of this raw material only increased the content of caffeic acid and hydroxy-benzoic acid, which are compounds that have exhibited relevant biological activities. Caffeic acid has been related to the prevention of acute neuroinflammation-induced [36]. Moreover, analgesic and anti-inflammatory activities have been reported for hydroxy-benzoic acid [37]. On the other hand, infusions made with *I. guayusa* were characterized by the presence of quercetin 7, 3′, 4′-trimethyl ether, a methyl flavone that is reported for the first time to the species. Palmitic acid and stearic acid were also predominant in *I. guayusa* and have been reported in *I. paraguariensis* [38, 39]. Palmitic acid has been associated with the antimicrobial and antioxidant activities of *Scenedesmus intermedius* [40]. *V. patens* infusions presented high amounts of linoleic acid, myristic acid, hydroquinone, 1-monopalmitin, 1-monolinolein, and 1-monostearin. Unsaturated fatty acid, linoleic acid, has

![Image of heatmap](https://example.com/heatmap.png)

**Figure 2:** Heatmap representation of metabolite correlations in herbal infusions. Correlations coefficients were calculated based on Pearson’s correlation.
exhibited an inhibitory effect on AgRP expression suggesting that the compound can help to reduce food intake and treat obesity [41].

Antioxidant activity (DPPH, FRAP) was decreased with the addition of CBS. Nevertheless, F8 that presents an important content of CBS exhibited an antioxidant activity for the DPPH assay as high as the formulation elaborated using only V. patens (F3). According to previous study [42], DPPH activities reported for chamomile infusion (0.90 ± 0.02 mM TEAC) is lower than the values found for all the formulations of this study. Spearmint infusion (3.33 ± 0.11 mM TEAC) reports a higher antioxidant activity than F9 and F10 but lower than the other formulations. Black tea infusion presents a lower activity (5.13 ± 0.08 mM TEAC) than F3, F5, F7, and F8, and all ten formulations present a lower antioxidant activity than green tea infusion (24.62 ± 0.49 mM TEAC). In the case of FRAP activity, chamomile infusion presents a lower antioxidant activity (1.03 ± 0.14 mM TEAC) than the samples of this investigation. Only the formulation that consisted of 100% of CBS (F9) showed a lower FRAP value than spearmint (5.33 ± 0.09 mM TEAC) and black tea infusion (3.38 ± 0.01 mM TEAC). Green tea infusion has a higher FRAP value (24.98 ± 0.41 mM TEAC) than the formulations of this research.

CBS infusion (F9) presents a TPC higher than Sideritis syriaca (Greek Mountain tea infusion) [43], Moringa oleifera [44], and Matricaria chamomilla [45] infusions. However, TCP was lowered by the addition of CBS in V. patens and I. guayusa infusions. TPC of all formulations, with the exception of F9, are higher than the values reported for Mentha piperita, Eucalyptus globules, and Salvia fruticose but are lower than black and green tea [43]. In the case of TFC, the values described for the species V. patens, I. guayusa, and CBS are reported for the first time in the literature.

The compounds that mainly contribute to the antioxidant activity of the infusions are carboxylic acids and derivatives. Fatty acids have been reported as bioactive compounds in herbal medicines [46]. Linoleic, myristic, and palmitic acids have shown antioxidant activity by analysis of model liposome oxidation [47]. However, linoleic acid has not
shown radical quenching activity against DPPH [48]. Additionally, in previous studies, stearic acid has not presented antioxidant activity [47]. In addition, phenolic compounds have also been reported as antioxidants [49]. Nevertheless, it was unexpected that caffeic acid did not show a positive correlation with antioxidant activity, because this compound has exhibited relevant biological activity such as antioxidant and anti-inflammatory [50].

4. Conclusions

This study showed that the addition of CBS to herbal infusions of *I. guayusa* and *V. patens* could increment the variety of polyphenols found in these raw materials. However, the addition of this by-product decreased the antioxidant activity of the infusions. *I. guayusa* and *V. patens* presented a metabolite profile clearly different from CBS, and antioxidant activity was correlated principally to the presence of carboxylic acids. These findings indicate that CBS is a good source of phenolic compounds.

Data Availability

All data have been placed in the manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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References

[1] M. J. Rodriguez Vaquero, L. R. Tomassini Serravalle, M. C. Manca de Nadra, and A. M. Strasser de Saad, "Antioxidant capacity and antibacterial activity of phenolic compounds from argentinean herbs infusions," *Food Control*, vol. 21, no. 5, pp. 779–785, 2010.
[2] K. D. Croft, “Dietary polyphenols: antioxidants or not?,” *Archives of Biochemistry and Biophysics*, vol. 595, pp. 120–124, 2016.

[3] M. S. Hossen, M. Y. Ali, M. H. A. Jahurul, M. M. Abdel-Daim, S. H. Gan, and M. I. Khalil, “Beneficial roles of high polyphenols against some human degenerative diseases: a review,” *Pharmacological Reports*, vol. 69, no. 6, pp. 1194–1205, 2017.

[4] G. L. Russo, I. Tedesco, C. Spagnuolo, and M. Russo, “Antioxidant polyphenols in cancer treatment: friend, foe or foil?,” *Seminars in Cancer Biology*, vol. 46, pp. 1–13, 2017.

[5] W. H. Lewis, E. J. Kennelly, G. N. Bass, H. J. Wedner, and M. P. Elvin-Lewis, “Ritualistic use of the holly Ilex guayusa by Amazonian Jivaro Indians,” *Journal of Ethnopharmacology*, vol. 33, no. 1–2, pp. 25–30, 1991.

[6] A. García-Ruiz, N. Baenas, A. M. Benítez-González et al., “Guayusa (Ilex guayusa L.) new tea: phenolic and carotenoid composition and antioxidant capacity,” *Journal of the Science of Food and Agriculture*, vol. 97, no. 12, pp. 3929–3936, 2017.

[7] M. D. Pardau, A. S. P. Pereira, Z. Apostolides, J. C. Serem, and J. Villacís-Chiriboga, “Effect of solvent-solvent partition on antioxidant activity and GC-MS profile of Ilex guayusa Loes. leaves extract and fractions,” *Natural Product Research*, pp. 1–5, 2021.

[8] Y. Arteaga-Crespo, M. Radice, L. R. Bravo-Sanchez, Y. García-Quintana, and L. Scalvenzi, “Optimisation of ultrasound-assisted extraction of phenolic antioxidants from Ilex guayusa Loes. leaves using response surface methodology,” *Heliyon*, vol. 6, no. 1, article e03043, 2020.

[9] P. M. Santana, M. Quijano-Avilés, I. Chóez-Guaranda et al., “Effect of drying methods on physical and chemical properties of Ilex guayusa leaves,” *Revista Facultad Nacional de Agronomía Medellín*, vol. 71, no. 3, pp. 8617–8622, 2018.

[10] P. I. Manzano, M. Miranda, Y. Gutiérrez, E. Santos, and R. Scull, “Estudio morfo-anatómico e identificación genética de Vochysia patens (Kunth) H. Rob. [Morpho-anatomical and fingerprinting study of Vochysia patens (Kunth) H. Rob.],” *Journal of Pharmacy & Pharmacognosy Research*, vol. 2, no. 5, pp. 119–128, 2014.

[11] M. S. Gachet, J. S. Lecaro, M. Kaiser et al., “Assessment of anti-protozoal activity of plants traditionally used in Ecuador in the treatment of leishmaniasis,” *Journal of Ethnopharmacology*, vol. 128, no. 1, pp. 184–197, 2010.

[12] P. Manzano Santana, T. Orellana León, M. Miranda Martínez, J. Abreu Payrol, O. Ruiz, and E. Peralta García, “Algunos parámetros farmacognósticos de Vochysia patens (Kunth) H. Rob. (Asteraceae) endémica de ecuador,” *Revista Cubana de Plantas Medicinales*, vol. 18, no. 1, pp. 131–139, 2013.

[13] P. I. Manzano, M. Miranda, J. Abreu-Payrol, M. Silva, O. Sterner, and E. L. Peralta, “Pentacyclic triterpenoids with antimicrobial activity from the leaves of Vochysia patens (Asteraceae),” *Emirates Journal of Food and Agriculture*, vol. 25, no. 7, pp. 539–543, 2013.

[14] I. Chóez-Guaranda, O. Ruiz-Barzola, J. Ruales, and P. Manzano, “Antioxidant activity optimization and GC-MS profile of aqueous extracts of Vochysia patens (Kunth) H. Rob. leaves,” *Natural Product Research*, vol. 34, no. 17, pp. 2505–2509, 2020.

[15] M. S. Fowler, “Cocoa beans: from tree to factory,” in *Industrial Chocolate Manufacture and Use*, pp. 10–47, Wiley-Blackwell, Oxford, UK, Fourth edition, 2009.

[16] ICCO, *May 2018 Quarterly Bulletin of Cocoa Statistics*, vol. 2017, https://www.icco.org/about-us/icco-news/384-fourth-quarterly-bulletin-of-cocoa-statistics.html.

[17] O. Rojo-Poveda, L. Barbosa-Pereira, G. Zeppa, and C. Stévigny, “Cocoa bean shell—a by-product with nutritional properties and biofunctional potential,” *Nutrients*, vol. 12, no. 4, p. 1123, 2020.

[18] H. M. El-Saied, M. K. Morsi, and M. M. A. Amer, “Composition of cocoa shell fat as related to cocoa butter,” *Zeitschrift für Ernährungswissenschaft*, vol. 20, no. 2, pp. 145–151, 1981.

[19] R. Redgwell, V. Trovato, S. Merinat, D. Curti, S. Hediger, and A. Manez, “Dietary fibre in cocoa shell: characterisation of component polysaccharides,” *Food Chemistry*, vol. 81, no. 1, pp. 103–112, 2003.

[20] R. Martínez, P. Torres, M. A. Meneses, J. F. Figueroa, J. A. Pérez-Alvarez, and M. Viuda-Martos, “Chemical, technological and in vitro antioxidant properties of cocoa (Theobroma cacao L.) co-products,” *Food Research International*, vol. 49, no. 1, pp. 39–45, 2012.

[21] D. C. G. Okiyama, S. L. B. Navarro, and C. E. C. Rodrigues, “Cocoa shell and its compounds: applications in the food industry,” *Trends in Food Science and Technology*, vol. 63, pp. 103–112, 2017.

[22] P. Manzano, J. Hernández, M. Quijano-Avilés et al., “Polyphenols extracted from Theobroma cacao waste and its utility as antioxidant,” *Emirates Journal of Food and Agriculture*, vol. 29, no. 1, 2017.

[23] M. F. O. Avilés, A. Barragán, J. Hernández et al., “Optimisation of polyphenols extraction from cocoa bean shell using factorial design,” *Journal of Bio-Science*, vol. 26, pp. 49–54, 2019.

[24] D. Chronopoulos, R. Zuurbier, B. Brandsstetter, and C. Jung, *Food Comprising Alkalized Cocoa Shells And Method Therefor*, US20110151098A1, 2011.

[25] M. Saitta, S. Lo Curto, F. Salvo, G. Di Bella, and D. Dugo, “Gas chromatographic-tandem mass spectrometric identification of phenolic compounds in Sicilian olive oils,” *Analytica Chimica Acta*, vol. 466, no. 2, pp. 353–344, 2002.

[26] T. Plaskal, S. Castillo, A. Villar-Briones, and M. Orešić, “MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data,” *BMC Bioinformatics*, vol. 11, no. 1, p. 395, 2010.

[27] J. Xie and D. S. Wishart, “Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaAnalyst,” *Nature Protocols*, vol. 6, no. 6, pp. 743–760, 2011.

[28] B. Sevindik, “Stability of volatile compounds of Turkish saffron (Crocus sativus) after one-year storage,” *Journal of Raw Materials to Processed Foods*, vol. 1, no. 2, pp. 72–79, 2020.

[29] R. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, Allured Publishing Corporation, Carol Stream, Illinois, USA, 4th edition, 2007.

[30] A. L. Waterhouse, “Determination of Total Phenolics,” *Current protocols in food analytical chemistry*, vol. 6, no. 1, pp. I.1.1–I.1.8, 2002.
[33] B. Min, L. Gu, A. M. McClung, C. J. Bergman, and M. H. Chen, “Free and bound total phenolic concentrations, antioxidant capacities, and profiles of proanthocyanidins and anthocyanins in whole grain rice (Oryza sativa L.) of different bran colours,” *Food Chemistry*, vol. 133, no. 3, pp. 715–722, 2012.

[34] S. S. Ebada, R. A. Edrada, W. Lin, and P. Proksch, “Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates,” *Nature Protocols*, vol. 3, no. 12, pp. 1820–1831, 2008.

[35] H. Li, Z. Deng, H. Zhu et al., “Highly pigmented vegetables: anthocyanin compositions and their role in antioxidant activities,” *Food Research International*, vol. 46, no. 1, pp. 250–259, 2012.

[36] S. Basu Mallik, J. Mudgal, M. Nampoothiri et al., “Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice,” *Neuroscience Letters*, vol. 632, pp. 218–223, 2016.

[37] S. A. Khan, S. S. Chatterjee, and V. Kumar, “Low dose aspirin like analgesic and anti-inflammatory activities of mono-hydroxybenzoic acids in stressed rodents,” *Life Sciences*, vol. 148, pp. 53–62, 2016.

[38] E. B. Ferreira, F. de Assis Rocha Neves, M. D. Da Costa, W. A. Do Prado, L. de Araujo Funari Ferri, and R. B. Bazotte, “Comparative effects of Stevia rebaudiana leaves and stevioside on glycemia and hepatic gluconeogenesis,” *Planta Medica*, vol. 72, no. 8, pp. 691–696, 2006.

[39] A. H. P. Souza, R. C. G. Corrêa, L. Barros et al., “Phytochemicals and bioactive properties of Ilex paraguariensis: an in-vitro comparative study between the whole plant, leaves and stems,” *Food Research International*, vol. 78, pp. 286–294, 2015.

[40] M. Davoodbasha, B. Edachery, T. Nooruddin, S. Y. Lee, and J.-W. Kim, “An evidence of C16 fatty acid methyl esters extracted from microalga for effective antimicrobial and antioxidant property,” *Microbial Pathogenesis*, vol. 115, pp. 233–238, 2018.

[41] S. Wang, N. Xiang, L. Yang et al., “Linoleic acid and stearic acid elicit opposite effects on AgRP expression and secretion via TLR4-dependent signaling pathways in immortalized hypothalamic N38 cells,” *Biochemical and Biophysical Research Communications*, vol. 471, no. 4, pp. 566–571, 2016.

[42] A. Jiménez-Zamora, C. Delgado-Andrade, and J. A. Rufián-Henares, “Antioxidant capacity, total phenols and color profile during the storage of selected plants used for infusion,” *Food Chemistry*, vol. 199, pp. 339–346, 2016.

[43] A. K. Atoui, A. Mansouri, G. Boskou, and P. Kefalas, “Tea and herbal infusions: their antioxidant activity and phenolic profile,” *Food Chemistry*, vol. 89, no. 1, pp. 27–36, 2005.

[44] X. Coz-Bolaños, R. Campos-Vega, R. Reynoso-Camacho, M. Ramos-Gómez, G. F. Loarca-Piña, and S. H. Guzmán-Maldonado, “Moringa infusion (Moringa oleifera) rich in phenolic compounds and high antioxidant capacity attenuate nitric oxide pro-inflammatory mediator in vitro,” *Industrial Crops and Products*, vol. 118, pp. 95–101, 2018.

[45] D. A. A. Kogiannou, N. Kalogeropoulos, P. Kefalas, M. G. Polissiou, and A. C. Kaliora, “Herbal infusions; their phenolic profile, antioxidant and anti-inflammatory effects in HT29 and PC3 cells,” *Food and Chemical Toxicology*, vol. 61, pp. 152–159, 2013.

[46] E. Karimi, H. Z. E. Jaafar, A. Ghazemzadeh, and M. Ebrahimi, “Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of Labisia pumila Benth,” *Biological research*, vol. 48, no. 1, p. 9, 2015.

[47] G. E. Henry, R. A. Momin, M. G. Nair, and D. L. Dewitt, “Antioxidant and cyclooxygenase activities of fatty acids found in food,” *Journal of Agricultural and Food Chemistry*, vol. 50, no. 8, pp. 2231–2234, 2002.

[48] N. Fagali and A. Catalá, “Antioxidant activity of conjugated linoleic acid isomers, linoleic acid and its methyl ester determined by photoemission and DPPH techniques,” *Biophysical Chemistry*, vol. 137, no. 1, pp. 56–62, 2008.

[49] M. Lesjak, I. Beara, N. Simin et al., “Antioxidant and anti-inflammatory activities of quercetin and its derivatives,” *Journal of Functional Foods*, vol. 40, pp. 68–75, 2018.

[50] R. Shiozawa, Y. Inoue, I. Murata, and I. Kanamoto, “Effect of antioxidant activity of caffeeic acid with cyclodextrins using ground mixture method,” *Asian Journal of Pharmaceutical Sciences*, vol. 13, no. 1, pp. 24–33, 2018.