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PHYTOCHEMICAL ANALYSIS OF COMMERCIAL *Ziziphus mauritiana* LEAF TEAS BY LIQUID CHROMATOGRAPHY COUPLED WITH ION TRAP/TIME-OF-FLIGHT MASS SPECTROMETRY

(Analisis Fitokimia Teh Daun *Ziziphus mauritiana* Komersial oleh Kromatografi Cecair Bergabung dengan Perangkap Ion/Spektrometri Jisim Masa Penerbangan)

Siti Umairah Mokhtar*, Nurul Fadilah Suardi, Ulia Maisarah Musni, Fathul Aiman Fahmi, Muhammad Syahmi Faiz Shamsuddin

Faculty of Industrial Science and Technology, University Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, 26300 Gambang, Pahang, Malaysia

**Corresponding author: umairah@umpsa.edu.my*

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Abstract

Ziziphus mauritiana (Z. mauritiana) tea is a medicinal herbal tea that is widely sold and consumed especially in Malaysia due to the belief of this herbal tea's beneficial health effects. However, there are limited comprehensive studies on the phytochemicals profiling of this herbal tea that have been published to date. Therefore, this study investigates the effects of different infusion times and volumes of water on the phytochemical's profiles of *Z. mauritiana* tea infusions. Phytochemical screening was conducted to identify the presence of saponin and flavonoid while the total phenolic content (TPC) of the herbal tea was quantified using the Folin-Ciocalteu assay method. The gallic acid standard calibration curve showed good correlation coefficient, R^2, at 0.9987. From the TPC study, it was indicated that 10 minutes of infusion time of Z. *mauritiana* infused tea with 200 mL water resulted in higher TPC (32.01 mg GAE/g) compared to other infusion times (15 and 20 minutes) and volume of water (50 and 150 mL). For chemical profiling of phytochemical compounds in tea infusion, the advanced chromatographic method of Liquid Chromatography Mass Spectrometry-ion-trap-time of flight (LCMS-IT-TOF) was applied to provide highly accurate analysis for phytochemical analysis and revealed the identification of 14–23 compounds, including phenolic, saponin, terpenoid, glycosides and alkaloid compounds in the *Z. mauritiana* infused tea sample.

Keywords: *Ziziphus mauritiana*, herbal tea, phytochemical, LCMS-IT-TOF, phenolic

Abstrak

Teh *Ziziphus mauritiana* (*Z. mauritiana*) ialah teh herba perubatan yang banyak dijual dan digunakan terutamanya di Malaysia kerana wujudnya kepercayaan bahawa teh herba ini mempunyai kesan kesihatan yang baik. Namun, sedikit sahaja kajian komprehensif yang telah diterbitkan setakat ini mengenai pemprofilan fitokimia teh herbanya. Oleh itu, kajian ini menyiasat kesan masa infusi dan isi padu air yang berbeza terhadap profil fitokimia infusi teh *Z. mauritiana*. Saringan fitokimia telah dijalankan untuk mengenal pasti kehadiran saponin dan flavonoid serta jumlah kandungan fenolik (TPC) teh herba yang dikuantifikasikan dengan menggunakan metode asai Folin-Ciocalteu. Keluk penentukuran piawai asid galik menunjukkan pekali korelasi yang baik, R^2, dengan nilai R^2 pada 0.9987. Daripada kajian TPC tersebut, ditunjukkan bahawa infusi selama 10 minit di dalam teh *Z.*

mauritiana dengan 200 mL air menghasilkan TPC yang lebih tinggi (32.01 mg GAE/g) berbanding dengan tempoh infusi (15 dan 20 minit) dan isi padu air (50 dan 150 mL) yang lain. Untuk pemprofilan kimia sebatian fitokimia di dalam infusi teh, kaedah kromatografi lanjutan iaitu Kromatografi Cecair Spektrometri Jisim-ion-perangkap-masa penerbangan (LCMS-IT-TOF) telah digunakan untuk menyediakan analisis yang sangat tepat untuk analisis fitokimia dan mendedahkan pengesanan 14 –23 sebatian termasuk sebatian fenolik, saponin, terpenoid, glikosida, dan alkaloid di dalam sampel teh *Z. mauritiana*.

Kata kunci: *Ziziphus mauritiana*, teh herba, fitokimia, LCMS-IT-TOF, fenolik

Introduction

The use of herbs or medicinal plants to develop products for medicines and supplements has increased rapidly over the past few decades due to the various medicinal benefits of those plants. Herb plants consist of unpurified plant extracts that consist of various constituents to cure certain types of diseases and enhance general health and well-being [1]. According to the World Health Organization (WHO), medicinal plants is a natural plant material that has been used in both developed and developing countries for many years because of their natural composition contained in the plant cause the least complications and more than 80% of the population in the world depend more on traditional drugs derived from traditional herb plants [2].

Ziziphus mauritiana plant is commonly referred to as Bidara by Malaysians and belongs to the Rhamnaceae family. This tree is native to Southeastern Asia's warm climate region [3]. It can withstand adverse conditions such as salinity, drought, and waterlogging, making it adaptable to a variety of soil types [3]. Bidara leaves, fruit, seed, and other plant parts have been used in traditional medicine and rituals to treat a variety of diseases. Malay people believed that cleansing a deceased body with Bidara leaves softens the body, strengthens the skin, and slows the decaying process [3].

In addition, fresh or dried flowers, fruits, leaves, seeds, and roots can all be used to make herbal teas by pouring boiling water over them and soaking it for a few minutes. After that, the herbal tea is strained, sweetened, and served. Many companies sell herbal teas due to their major source of dietary antioxidants, and scientists have been studying polyphenolic compounds, as well as vitamins and carotenoids, for decades [4]. However, the suitability and health effects of drinking this herbal tea still lacking to date and the bioactive compounds contained in this infused herbal tea remain unclear. The safety and quality of unregistered herbal tea for human health is a major concern. Contaminants from natural and anthropogenic sources frequently reduce the quality of medicinal herbs and herbal remedies, causing side effects and even fatal death. Indeed, many herbal products lack sufficient information on their acute and chronic toxicity, standardization, stability, and quality. Therefore, if the plant material is intended for human consumption, it is very crucial to ensure the chemical profile as well as metallic and nonmetallic elements in the herbal extract or herbal tea.

A study on the identification of phytochemical compounds in *Ziziphus* jujube leaf tea has been conducted previously by using HPLC-DAD-ESI-TOFMS. Based on the study, reported that the infusion tea does not contain caffeine and theophylline but is rich in flavonoids with possible sedative activity. It revealed that this infusion has good radical scavenging ability and can be a healthy beverage for the public [5]. Based on the study, the application of advanced mass spectrometry provides higher sensitivity and accuracy in the identification of both targeted and non-targeted compounds [6].

Therefore, this study aims to explore the effects of different infusion parameters towards the phytochemical content as well as to investigate the chemical profile of *Z. Mauritiana* tea infusion by identifying the primary phytochemical compounds qualitatively. A hybrid technology LC-IT-TOF/MS was implemented to explore the phytochemical compounds in infused tea which can offer high speed and high accuracy determination.

Materials and Methods

Reagents and materials

Methanol, gallic acid, Folin-Ciocalteu reagent, sodium carbonate anhydrous powder and 2N sodium hydroxide

were obtained from Sigma Aldrich (St. Louis, USA). All of the chemicals and solvents were of high analytical grade. Three brands of herbal tea samples were purchased from online local shops namely brands A, B and C, respectively.

Infusion method of herbal tea

1 g of tea samples was mixed with 50, 100, 150, and 200 mL of hot distilled water (95 °C) to make the tea infusion. After 5 minutes of infusion, the solution was cooled to around 50 °C. After that, the infused teas were filtered using a Whatman paper filter No. 1 before analysis. This study was adjusted by varying the volume of water (50, 100, 150 and 200 mL) and infusion time (10, 15 and 20 minutes). To find the ideal conditions for infusing *Z. mauritiana* tea at different volumes and times, the infusion process was optimized using a constant mass, which was 1 g of *Z. Mauritiana* tea. Each sample was prepared in triplicate.

Qualitative test for saponin and flavonoid

Qualitative phytochemical analysis of saponin and flavonoid compounds in tea samples was conducted using standard methods [7]. For the saponin test, 2 mL of *Z. Mauritiana* herbal tea was mixed with 6 mL of water in a graduated cylinder. The mixture was vigorously shaken, and the presence of saponins was determined by the formation of persistent 2 cm foam. As for the flavonoid test, 2 mL of *Z. Mauritiana* herbal tea was mixed with 1 mL 2N sodium hydroxide. The presence of flavonoids is indicated by the presence of yellow color.

Total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method with gallic acid as a control. A gallic acid standard solution with concentration ranges of 0.05 to 0.4 mg/mL was prepared for the standard calibration curve. In this method, 1 mL *Z. Mauritiana* herbal tea and 2.5 mL Folin-Ciocalteu (0.2 N) reagent were mixed. Then, 2 mL of sodium carbonate (37.7 g/L) was added and thoroughly mixed. Then, 7.5 mL of distilled water was added to dilute it. The mixture was covered with parafilm foil and incubated in a dark place for 2 hours at 30°C. The absorbance was then measured in a UV-Vis spectrophotometer (Shimadzu) at 760 nm. The obtained result was calculated and expressed as mg of gallic acid equivalents (mg GAE)/mL of tea infusion as stated in Equation 1.

$$Total\ Phenolic\ Content = \frac{\mathrm{CV}}{m} \quad (1)$$

Where C is the concentration of *Z. Mauritiana* leaves herbal tea (mg/mL), V is the volume of *Z. Mauritiana* herbal tea (mL) and m is the weight of *Z. Mauritiana* herbal tea (g).

Analysis by LCMS-IT-TOF

For the phytochemical screening of *Z. Mauritiana* herbal tea sample, LC-MS analysis was performed using the Shimadzu Prominence Series LC coupled to the LCMS-IT-TOF (Shimadzu, Kyoto, Japan). Prominence Series components included two LC-20AD pumps, SIL-20A Autosampler, and a CBM-20A System Controller. The column used for reversed-phase LC analysis was a Shimadzu Shimpack VP-ODS packed with 4.6 µm particles (4.6 x 150 mm). The instrument was controlled through an LCMS solution, and data analysis was performed using the same software. The mobile phases consisted of eluent A (0.1% formic acid in water, v/v) and eluent B (0.1% formic acid in acetonitrile, v/v) with a flow rate of 0.5 mL/min. The injection volume was 10 µL with a gradient elution at the flow rate of 0.5 mL/min: 1% B (0 ~ 1 min), and was linearly increased to 35% (1 ~ 16 min), 35-100% (16 ~ 18 min) and 100% (18 ~ 20 min). Re-equilibration duration was five minutes between individual runs.

Data analysis

All data acquired were processed by Shimadzu LC-solution software (Kyoto, Japan), Version 3.0. The experimental results were performed in triplicate. The processed data was compiled in the Microsoft Excel software and further Mean, SD, relative standard deviation (%RSD), and Percentage Relative Error (%RE) values were calculated.

Results and Discussion

Effect of different parameters to the infused herbal tea

Observation of color was conducted to view the effect

of different water volumes on the color intensity of tea infusion. All brands of tea revealed that the intensity of the color decreased as water volume increased as can be seen in Figure 1. It can be seen that 50 mL has darker color compared to 100 mL, 150 mL and 200 mL. This is due to the increased volume of water used for infusion resulting in the dilution of the chemical compounds.

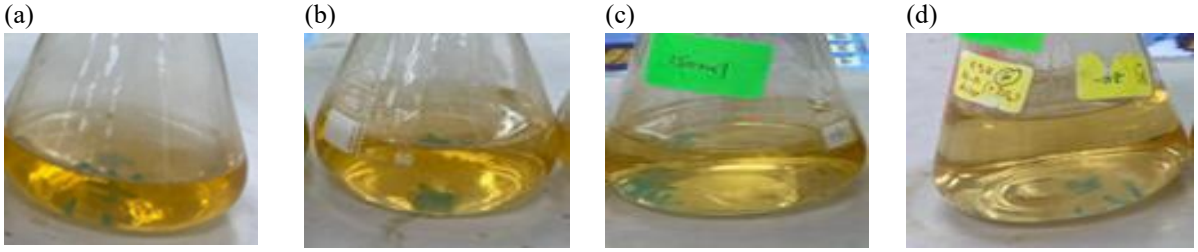

Figure 1. Color of infused *Z. mauritiana* tea at (a) 50 mL, (b) 100 mL, (c) 150 mL and (d) 200 mL

Furthermore, infusion tea with different infusion times (5, 10, 15 and 20 minutes) indicated that the intensity of the yellowish color of the infused tea decreased as infusion time increased. It revealed that 10 minutes of infusion time give darker color followed by 15 and 20 minutes of infusion time, respectively. According to previous studies, the flavor, color, and taste of tea beverages are said to be affected by a long infusion time. The infusion's turbidity increases as the infusion time increases due to the increase in protein and pectic structures, which are expressed at the same time [3]. Observing the turbidity through a UV spectrophotometer, the water-soluble compounds in the tea were found to pass quickly to the water, and the number of water-soluble compounds in the infusion increased slowly even as the infusion time increased [4]. Moreover, the brightness of the infusions decreased as infusion time increased. It can be stated that as the infusion time increases, undesirable compounds pass into the infusion, the emergence of some decomposed and degraded components, and increased turbidity cause a decrease in the brightness of the infusion. The number of compounds in the infusion increases with infusion time and these compounds affect the color of the tea, the change in color of the infusion is to be expected [5].

Qualitative test for saponin

A qualitative test of saponin was conducted to identify the presence of saponin in *Z. mauritiana* tea with infused parameter of 50 mL water and 10 mins. Saponins are amphipathic glycosides distinguished phenomenologically by the soap-like foam produced when shaken in aqueous solutions and structurally by the presence of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. From this study, Brand A and C proved the presence of saponin after the mixture was shaken vigorously while Brand B does not record the presence as shown in Figure 2.

Based on previous studies on different tea plants, saponin content may vary but the processing of tea did not significantly affect them. Saponin can be found in many medicinal plants such as *alstonia scholaris, curcuma longa, jasmin sambac, phyllantus acidus* as well as *ziziphus mauritiana* [8]. This compound is beneficial as it has several pharmacological properties namely antimicrobial, antifungal, anti-inflammatory and antioxidant.

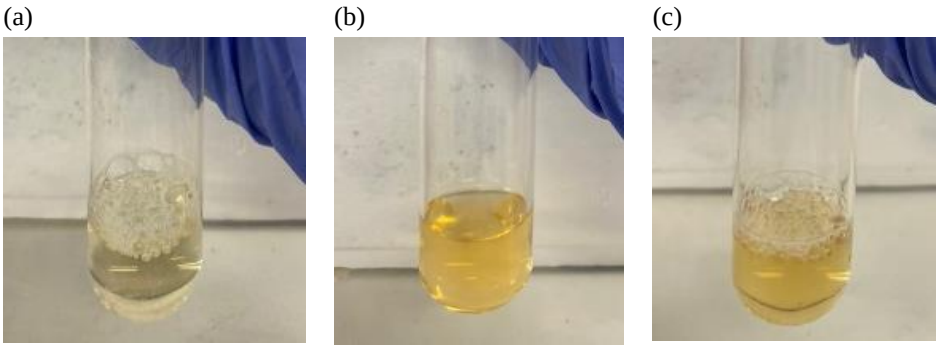

Figure 2. Saponin test in infused herbal tea at volume 50 mL and 10 min infusion time (a) Brand A (b) Brand B and (c) Brand C

Qualitative Test for Flavonoid

This test was conducted at infusion parameters; 50 mL water and 10 mins infusion time. The presence of flavonoids is indicated by the presence of an intense yellow color in the mixture as compared to blank infused tea [9]. Figure 3 shows that all brands contain flavonoids in herbal teas based on qualitative tests of flavonoids. Brand B shows the highest content of flavonoids due to the brownish-yellow color of the herbal tea of which is darker than Brand A, Brand C as well as the control (blank infused *Z. mauritiana* tea). Flavonoids and flavonols are typically yellow or ivory-colored pigments. Notably, the anthoxanthins content will impact the yellow colors [10]. Tea is particularly rich in three flavonoid classes: flavan-3-ols (or catechins), ooligomeric flavonoids (including thearubigins and theaflavins generated during fermentation), and flavonols (e.g. quercetin). These flavonoid oligomers in tea have very complex chemical structures that have yet to be fully characterized. The most common flavonol found in tea is quercetin [11, 12].

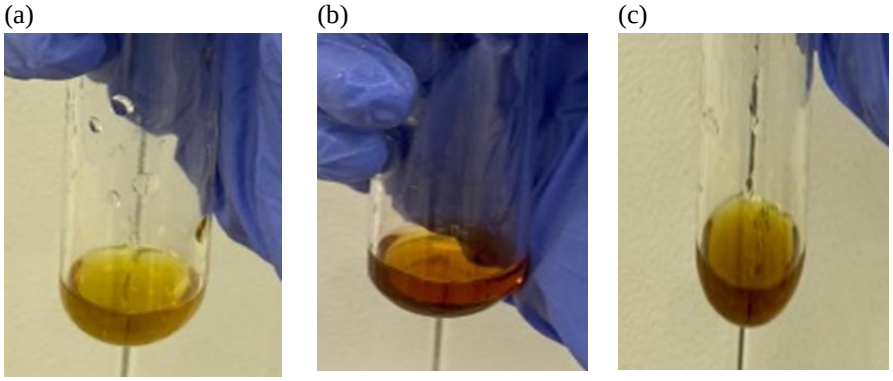

Figure 3. Flavonoid test in infused herbal tea at 10 mins infusion time and 50 mL of water volume (a) Brand A (b) Brand B and (c) Brand C

Effect of different water volumes on the TPC value of infused tea

Gallic acid was used as the standard calibration curve in TPC analysis and was measured using a UV-Vis Spectrophotometer at a wavelength of 765 nm. The correlation coefficient, R-value, obtained from the experiment showed a value of 0.9987 indicating excellent linearity of absorbance versus concentration.

Based on Figure 4, brand A had the most phenolic compounds, followed by brand B and brand C. Unlike the previous study, the amount of TPC increased by the increment of water volume for Brand A tea. More TPC values resulting from the increment of water volume in

Brand A might be due to better solvation of TPC in the sample as a result of interactions (hydrogen bonds) between the polar sites of the TPC molecule and the water [13]. Meanwhile, TPC for Brand B and C decreased as the volume increased to 200 mL. The phenolic compounds were expected to be reduced by the increment of water volume [6]. These results might be due to the other factors that potentially affect the phenolic content such as the drying process, topographical location of the planting and cultivation, season, climate and the storage conditions of the tea sample. This phenolic compound also contributes to antioxidant activity as well as contributes to the quality in terms of modifying color, taste, aroma and flavor [14].

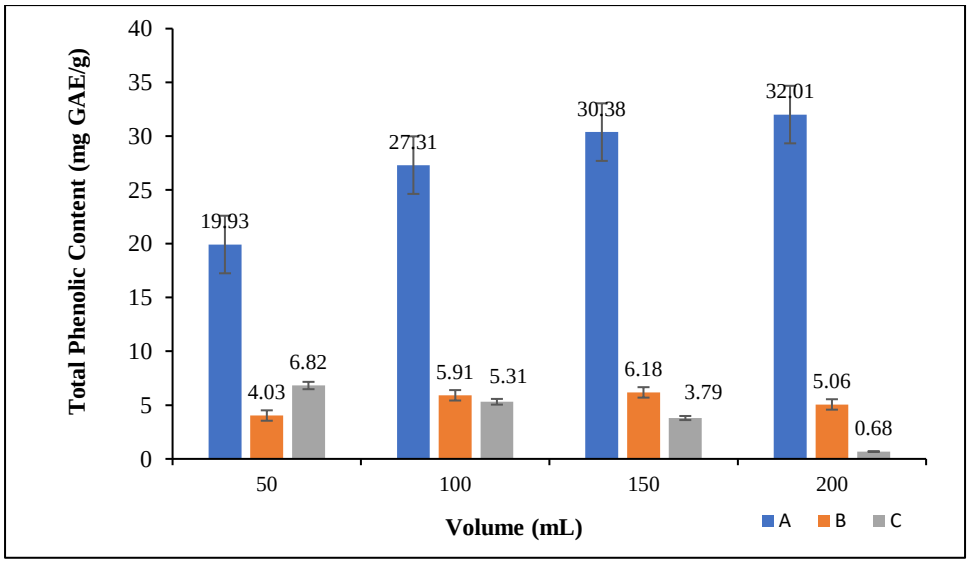

Figure 4. Total phenolic content for all types of brands with different volumes in mg GAE/g

Effect of different infusion time to TPC value of infused tea

Based on Figure 5, the amount of TPC in Brand A and B decreased by the increment of infusion time from 10 until 20 minutes. According to a previous study, increment of infusion time increases the chances of oxidation of phenolics due to their long exposure to unfavorable environmental factors such as temperature and oxygen [14]. However, TPC in Brand C increased at 15 minutes before being reduced at 20 minutes infusion time. As stated by a previous study, 10 minutes of infusion time for loosely packed teas is enough to infuse all the phenolic content into the water [15]. From this result, it concluded that *Z. mauritiana* Brand A significantly demonstrated the highest TPC values at different parameters as compared to Brands B and C. Brands B and C do not give much significant difference in the TPC value at different infusion times and water volumes.

LCMS-IT-TOF Analysis for *Z. Mauritiana* herbal teas

From the qualitative test and TPC test, it was shown that Brand A gave the highest amount of compounds compared to Brand B and C, thus, Brand A then continued for the analysis using advanced LCMS-IT-TOF. The identification and analysis of compounds detected were based on precursor ions and accurate mass measurement of either $[M+H]^+$, $[M+K]^+$, $[M+NH4]^+$ and $[M+Na]^+$. Table 1 indicated the identification of the active components in *Z. Mauritiana* herbal teas.

Based on Table 1, LCMS-IT-TOF analysis successfully detected 14 compounds based on their accurate mass categorized as amine, fatty acids, phenolic glycosides, phenolic and alkaloid. As can be seen in Figure 6, it revealed that *m/z* 304.2968 gave the highest intensity of the mass compared to others. Based on the accurate mass, it revealed that nitrosoxacin A was identified. This compound belongs to the class of organic compounds

known as trialkylamines. The other four main measured masses in Figure 6(a) were *m/z* 288.2696, 332.3311, 367.1376 and 381.1430 identified as amine, phenolic and indole compounds, respectively. Abietylamine is tricyclic abietane diterpenoids which mainly found in plants and exhibits pharmacological activity such as antimalaria properties [16].

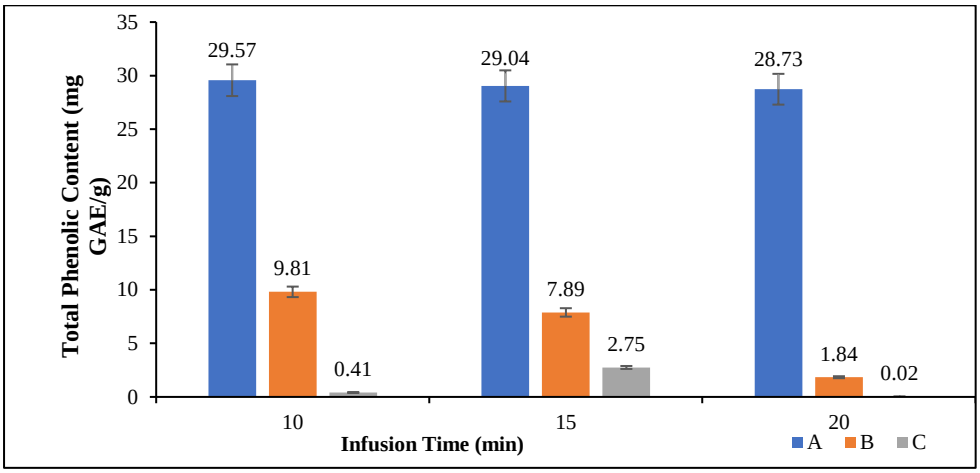

Figure 5. Total phenolic content for all types of brands with different infusion times in mg GAE/g

Meanwhile, dihydroconiferin belongs to the class of organic compounds known as phenolic glycosides, a plant metabolite that was quantitatively identified in a plant called *Lactuca orientalis* [17]. Varespladib (*m/z* 381.1430) is an anti-inflammatory agent which disrupts the first step of the arachidonic acid pathway of inflammation. All detected compounds in this sample were different compared to the previous study using the same herbal tea. There are three unknown compounds were identified to be further investigated for structure elucidation. The fragment ions can be used to study the elucidation of the proposed structures of the unknown compounds.

Based on Table 2, amount 23 compounds were identified using advanced LCMS-IT-TOF and categorized into four groups: saponin, terpenoids, glycosides, and phenols. As compared to Table 1, *Z. mauritiana* infused tea with different volumes of water affected the number of compounds detected. In 150 mL volume of water, it revealed that more compounds can infuse from the tea. Only nitrosoxacin A and camphor azine were detected in both infused samples at masses of *m/z* 304.2975 and *m/z* 318.2879. Camphor and its derivatives are considered among the most significant natural product, which has considerable pharmacological effects including antibacterial, antifungal, antiviral, anticancer and antidiabetic activity [18]. Based on previous studies, nitrosoxacin A and camphor azine were not detected in other infused herbal teas and no study has discovered these compounds in tea leaves. A further investigation on the potential and properties of nitrosoxacin A and camphor azine in *Z. mauritiana* tea can be conducted. Additionally, based on Figure 6, there are two possible molecular formulas detected at *m/z* 332 which showed a higher response, either $C_{23}H_{41}N$ and $C_{19}N_{41}NO_3$ at the volume of 200 mL (Figure 6a) and 150 mL (Figure 6b), respectively. Both molecular formulas indicated isomer compounds were identified and the possible compounds are amine and fatty acid.

In this study, it is noticed that changes in infusion parameters (volume and time) affected the number of compounds detected by LCMS-IT-TOF. Optimum infusion parameters play an important role in the infusion method to diffuse more compounds in the sample and thus be able to be detected by the chromatography method. From this, it can be concluded that the extractability of bioactive compounds from tea leaves depends on the infusion parameters.

Table 1. Number of compounds and its possible name detected in *Z. Mauritiana* herbal tea infused at the volume of 200 mL (10 minutes infusion time) by LCMS-IT-TOF.

No	Formula	Possible Detected Compounds	Ion	Measured *m/z*	Group of Compounds
1	$C_{20}H_{33}N$	Abietylamine	$[M+H]^+$	288.2696	Amine
2	$C_{16}H_{34}N_2O_2$	Nitrosoxacin A	$[M+NH_4]^+$	304.2968	Amine
3	$C_{20}H_{32}N_2$	Camphor Azine	$[M+NH_4]^+$	318.2893	Ketone
4	$C_{23}H_{41}N$	Benzylamine, N,N-dioctyl-	$[M+H]^+$	332.3311	Amine
5	$C_{17}H_{26}N_2O_4$	Bestatin Methyl Ester	$[M+Na]^+$	345.1777	Organic carbonic acids
6	$C_{19}H_{34}O_6$	Dodecanoic acid	$[M+H]^+$	359.2428	Fatty acids
7	$C_{16}H_{27}NO_8$	1-Aminoadamantane citrate monohydrate	$[M+H]^+$	362.1802	Amine
8	$C_{16}H_{24}O_8$	Dihydroconiferin	$[M+Na]^+$	367.1376	Phenolic glycosides
9	$C_{21}H_{20}N_2O_5$	Varespladib	$[M+H]^+$	381.1430	Indoles
10	$C_{30}H_{54}O_3$	2,3-Didodecoxyphenol	$[M+K]^+$	501.3701	Phenolic
11	$C_{26}H_{54}N_2O_7$	Glucoalkaloid	$[M+K]^+$	545.3584	Alkaloid
12	$C_{27}H_{57}NO_{10}$	Unknown	$[M+Na]^+$	578.3871	Unknown
13	$C_{29}H_{62}N_2O_7$	Unknown	$[M+K]^+$	589.4198	Unknown
14	$C_{30}H_{62}N_2O_{10}$	Unknown	$[M+Na]^+$	633.4245	Unknown

It also noted that even though TPC values showed the highest value in Brand A, the number of phenolic compounds detected by LC analysis is not much as expected. It can be due to the phenolic compounds being evaporated prior to LC analysis. Most phenolic compounds are volatile compounds which give the aroma to the herbal tea [19]. Sample preparation prior to LC analysis and the limited mass library could also be the reasons for only several phenolics being identified in this study. Hence, further studies are needed to comprehensively identify individual active compounds present in the infused *Z. mauritiana* tea prepared using different water volumes, infusion times as well as infusion temperatures, which might provide more details.

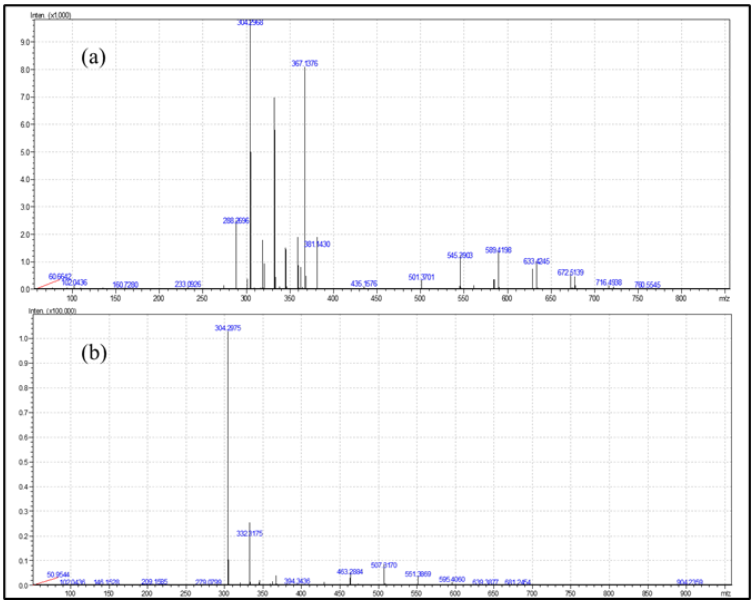

Figure 6. Mass spectra for *Z. mauritiana* infused tea (Sample A) (a) infused with 200 mL volume of water and (b) infused with 150 mL volume water

Table 2. Number of compounds and their possible name detected in *Z. Mauritiana* herbal tea infused at the volume of 150 mL (10 minutes infusion time) by LCMS-IT-TOF

No	Formula	Possible Detected Compound	Ion	Measured m/z	Group of Compounds
1	$C_{16}H_{34}N_2O_2$	Nitrosoxacin A	$[M+NH_4]^+$	304.2975	Amine
2	$C_{21}H_{36}O$	3-Pentadecylphenol	$[M+H]^+$	305.2851	Phenol
3	$C_{20}H_{32}N_2$	Camphor azine	$[M+NH4]^+$	318.2879	Ketone
4	$C_{19}H_{30}N_2O$	5-(Nonyloxy)tryptamine	$[M+NH4]^+$	320.2675	Amine
5	$C_{19}H_{41}NO_3$	2-hydroxypropylazanium Hexadecanoate	$[M+H]^+$	332.3175	Fatty acid
6	$C_{20}H_{44}O_3$	Unknown	$[M+H]^+$	333.3362	Unknown
7	$C_{23}H_{18}N_2$	Unknown	$[M+Na]^+$	345.1360	Unknown
8	$C_{16}H_{36}N_2O_5$	Unknown	$[M+Na]^+$	359.2500	Unknown
9	$C_{24}H_{14}N_2O$	5,6-Diphenyl-2-diazoacenaphthene-1-one	$[M+NH4]^+$	364.1434	Acenaphthene
10	$C_{11}H_{24}N_2O_{10}$	2,2'-Bipyridinate Octahydrate	$[M+Na]^+$	367.1340	Pyridine
11	$C_{19}H_{24}O_8$	Aigialomycin B,	$[M+H]^+$	381.1532	Glucoside
12	$C_{20}H_{46}N_2O_3$	Unknown	$[M+Na]^+$	385.3378	Unknown
13	$C_{28}H_{43}N$	Bis(4-Octylphenyl)Amine,	$[M+H]^+$	394.3436	Amine
14	$C_{28}H_{38}N_{20}$	N,N-dihexyl-2-(2-phenyl-1H-indol-3-yl)acetamide	$[M+H]^+$	419.3052	Phenolic
15	$C_{22}H_{40}N_2O_6$	Unknown	$[M+H]^+$	429.2957	Unknown
16	$C_{28}H_{51}NO_2$	N-(2-Decyltetradecyl)-Maleimide	$[M+H]^+$	434.4018	Unknown
17	$C_{27}H_{48}O_3$	Trihydroxycoprostane	$[M+Na]^+$	443.3488	Saponin
18	$C_{21}H_{48}N_2O_7$	Unknown	$[M+Na]^{bc}$	Q1	Unknown
19	$C_{29}H_{44}O_5$	Triterpenoids	$[M+H]^+$	473.3277	Terpenoids
20	$C_{30}H_{50}O_6$	Protoaescigenin	$[M+H]^+$	507.3675	Saponin
21	$C_{27}H_{42}N_2O_6$	Hydrocortisone-21-lysinate	$[M+NH4]^+$	508.3360	Ester
22	$C_{27}H_{48}N_2O_8$	Serratamolide D	$[M+Na]^+$	551.3293	Unknown
23	$C_{29}H_{46}N_2O_7$	Kalimantacin C	$[M+NH4]^+$	552.3616	Fatty acid

Conclusion

In this study, infusion parameters such as volume of water and infusion time were successfully demonstrated to give different visual appearances and affect the total phenolic content (TPC) of infused *Z. Mauritiana* tea. This infused herbal tea from Brand A, B and C were compared qualitatively and quantitatively. Based on phytochemical screening, it proved the presence of flavonoid and saponin in all samples however saponin was absent in brand B. In TPC analysis, infused tea from brand A at different volumes (50, 150, 200 mL) and different infusion times (10, 15, 20 min) showed the highest TPC value compared to others. In this study, an efficient LCMS-IT-TOF method was successfully performed to identify and compare the phytochemical compounds in infused *Z. mauritiana* tea by using 150 mL and 200 mL water, respectively. It proved that less amount of water used to infuse resulted in a higher number of phytochemical compounds. Results showed that most of the compounds detected were categorized as amine, fatty acids, phenolic glycosides, phenolic, alkaloid, saponin, terpenoids and glycosides. Moreover, only nitrosoxacin A and camphor azine were detected in both infused samples. It is concluded that the different infusion parameters gave a significant effect on TPC value as well as the phytochemical compounds in the infused tea which can be led to different biological activities. Hence, further studies could be planned to focus on the novel compounds extracted from *Z. mauritiana* tea that might benefit human health as compared to other herbal tea.

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