Antihypertensive Assay-Guided Fractionation of Syzygium polyanthum Leaves and Phenolics Profile Analysis Using LC-QTOF/MS

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ABSTRACT
Introduction: Syzygium polyanthum leaves extract that contains gallic acid as the major phenolic compound has shown significant antihypertensive effect, however the amount of gallic acid was inversely-related with magnitude of this effect. This study aimed to conduct bioassay-guided fractionation of S. polyanthum leaves with gallic acid as a reference compound, and to screen for other possible compounds responsible for the antihypertensive effect. Methods: S. polyanthum leaves were extracted using n-hexane, ethyl acetate, methanol, and water. The most active crude extract was fractionated using column chromatography and analyzed for total phenolic content (TPC) (n=3). Crude extracts and the derived fractions were intravenously administered into pentobarbital-anaesthetized Spontaneously Hypertensive Rats (n=5) for recording of blood pressure parameters. Liquid Chromatography-Quadrupole Time-Off-Flight/Mass Spectrometry was used for determination of chemical composition. One-way and two-way ANOVA were used for statistical analysis using GraphPad® PRISM Version 6. Results: Fractionation of aqueous S. polyanthum leaves extract (ASP) afforded nine fractions, later combined into three fractions (F1ASP, F2ASP, and F3ASP) based on the thin-layer chromatography profiles. ASP has the highest TPC while F2ASP has the lowest TPC. All fractions exhibited significant antihypertensive property, but F2ASP was the most active fraction. Few phenolics with related antihypertensive effects such as 1-galloyl glucose (a gallic acid-derivative majorly found in F2ASP and F3ASP), and other compounds such as polysatins, sesamol, brazillins, eugenol, ellagic acid, kukoamine A, and cyclocurcumin were found across all active fractions. Conclusion: These phenolics may partly contribute to the antihypertensive effect of S. polyanthum leaves, thus further isolation study is recommended. Key words: Antihypertensive, Bioassay-guided, LCMS, Syzygium polyanthum, Total phenol content (TPC).

INTRODUCTION
Hypertension is a major public health problem. According to the World Health Organization,1 an uncontrolled rise in blood pressure may predispose a patient to a heart attack which will eventually lead to heart and kidney failures, stroke, and cognitive impairment. It was estimated that the worldwide prevalence of hypertension exceeded 1.3 billion, representing 31 % of all adults.2 Throughout the years, the condition of raised blood pressure among the hypertensive patients was uncontrolled.3 While there are available antihypertensive drugs in the market, the global condition remains stagnant since the treatment is expensive, thus an average or a poor society did not afford to receive the best treatment regime. In addition, the concomitant drugs’ side effects such as dizziness, abnormal heart rate, sore throat, sexual dysfunction, thrombocytopenia, and hyperglycemia1 are undesirable, and this untoward reaction actually occurs more easily when drugs are used in combination.3 The expensive cost and the side effects of the currently-available antihypertensive drugs have enforced the research for new alternative antihypertensive drugs which should be at least equally effective, but yet inexpensive.

Some natural compounds from medicinal plants were found to exhibit significant antihypertensive effect, however, there is also a huge number of potential medicinal plants with antihypertensive properties that remains to be explored. Syzygium polyanthum (Wight) Walp, also known as ‘salam’ or ‘serai kayu’ is one of the medicinal herbs that is traditionally consumed as an alternative treatment for reducing blood pressure among Malay folks. S. polyanthum has been known as an antihypertensive medicinal plant and this is strongly supported by previous findings. Sukrasno et al7 reported the hypotensive effect of orally-administered aqueous extract of S. polyanthum leaves in normotensive Wistar rats. S. polyanthum leaves extracts have shown a significant reduction in blood pressure of anaesthetized Spontaneously Hypertensive Rats (SHR) and normal Wistar Kyoto (WKY) when intravenously administrated.8 When fed orally, S. polyanthum leaves extract significantly reduced the systolic blood pressure in SHR.9-10 Histological studies showed significant improvement in Bowman’s capsule and glomerulus morphology of

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treated SHR’s kidney, comparable to normal kidney structure and slight improvement of pedicels and thoracic aorta.10,11

Ramli et al11 suggested that the reduction in systolic blood pressure (SBP) in hypertensive rats might be due to the major composition of phenolics in the extract. Ismail et al11 showed the presence of gallic acid, a major phenolic compound present in S. polyanthum leaves extract. Gallic acid was previously reported to normalize blood pressure of diabetic rats12 and attenuate hypertension in NG-nitro-L-arginine methyl ester-induced hypertensive rats.13 However, our previous study showed there was no correlation between the amount of gallic acid with the magnitude of antihypertensive effect for the tested S. polyanthum leaves extracts, suggestive of the presence of synergism between compounds that contributes to the net antihypertensive effect.9 Therefore, this study aimed to perform bioassay-guided fractionation of S. polyanthum leaves to screen for other potential bioactive compounds responsible for its antihypertensive effect.

MATERIALS AND METHODS

Plant authentication

The leaves, flowers, buds, and stem parts of S. polyanthum were sent for authentication at UKMB Herbarium, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The plant was verified as Syzygium polyanthum Wight Walp. on 2nd May 2017. The voucher herbarium specimen (PIUM 0282) was deposited in Herbarium, Kulliyyah of Pharmacy, International Islamic University Malaysia, Kuantan, Pahang, Malaysia.

Animal

Seventy-five of 3-month old male Spontaneously Hypertensive Rats (SHR), weighing around 250 to 280 grams were placed in standard rat cages and acclimatized for 7 days in standard environmental conditions (25 °C with 60-70 % humidity) on a 12-hour light-dark cycle. Tap water and rat pellet were given ad libitum and the animal bedding was changed once a week. All experimental protocols regarding the animal study were approved by the Animal Ethics Committee, Universiti Sains Malaysia (UMS/Animal Ethics Approval/2016/102) (757).

Sample extraction

Two kg of S. polyanthum leaves were collected from Taman Pertanian Jubli Perak Kuantan, Pahang, Malaysia. The leaves were left dried in a drying cabinet for a week at 50 °C. After a week, the dried leaves were ground into powder using a laboratory blender prior to extraction. The powdered sample was extracted using ultra-sound assisted extraction (UAE) method as described by Rahim et al.14 This method enhances solvent penetration through plant cells with the aid of sound waves,15, 16 and usually provides sufficient yield for phenolic compounds.17

Hot distilled water (80 °C) and also solvents with varying polarities including n-hexane, ethyl acetate, and methanol were used to prepare a mixture of methanol and water (50:50) to enhance the solubility of compounds.9 These spraying reagents were % sulphuric acid spraying reagent, vanillin-sulphuric acid reagent and a drop of formic acid. The spots were visualized using a UV lamp, 50 % sulphuric acid spraying reagent, vanillin-sulphuric acid reagent and ferric chloride spraying detection reagent. These spraying reagents were used to enhance the separation of the four spots. Galactic acid was used as a reference compound (standard) based on finding from our previous study that gallic acid was found as a major phenolic compound in the aqueous and methanolic extracts of S. polyanthum leaves.9 The spots and the standard were visualized under UV lamp (Leybold Didactic GmbH, Germany) of short and long wave and by ferric chloride spraying detection reagent.

For fractionation, a 30 cm-height of silica column using a 25 mm glass column was prepared by mixing 35 g of silica gel 60 (0.063-0.200 mesh) with 100 % ethyl acetate. The column was allowed to stand overnight for complete packing. ASP slurry was prepared in a combined solvent mixture of methanol and water (50:50) to enhance the solubility of methoxylated and hydroxylated compounds.19 Then, the ASP slurry was run in the column chromatography using a gradient elution technique with a binary solvent system of ethyl acetate and methanol, allowing polarity changes during the fractionation. Gradient elution usually offers better speed, separation, and retention reproducibility compared to isocratic elution for wide range polarities of organic compounds.20

The gradient solvent system of ethyl acetate (100 %), ethyl acetate: methanol (7:3), ethyl acetate: methanol (5:5), ethyl acetate: methanol (3:7), and methanol (100 %) were sequentially employed and finally, the column was washed with 100 % methanol. Nine fractions were collected in a 15 ml centrifuge tube and characterized by TLC profiling with a solvent system of ethyl acetate: methanol (9:5.0:5.5) with a drop of formic acid. The spots were visualized using a UV lamp, 50 % sulphuric acid spraying reagent, vanillin-sulphuric acid reagent and ferric chloride spraying detection reagent. These spraying reagents were

Bioassay-guided fractionation

Since ASP was the crude extract with the most prominent antihypertensive effect, it was then subjected to fractionation. Before fractionation, the thin layer chromatography (TLC) is performed to study the characteristics of the extract and to optimize the solvent system to achieve a good separation during fractionation.14 TLC plates (8 x 8”) were firstly cut into a measurement of 10 cm x 2 cm and were allowed to dry overnight at 37 °C in an incubator oven (Memmert, Germany). The crude extract was firstly developed with 100 % n-hexane, ethyl acetate, dichloromethane, methanol, and acetonitrile. The crude extract was then run with the solvent system of ethyl acetate: methanol: acetonitrile (8:1:1) with an additional one drop of formic acid. The additional one drop of formic acid was used to enhance the separation of the four spots. Galactic acid was used as a reference compound (standard) based on finding from our previous study that gallic acid was found as a major phenolic compound in the aqueous and methanolic extracts of S. polyanthum leaves.9

For fractionation, a 30 cm-height of silica column using a 25 mm glass column was prepared by mixing 35 g of silica gel 60 (0.063-0.200 mesh) with 100 % ethyl acetate. The column was allowed to stand overnight for complete packing. ASP slurry was prepared in a combined solvent mixture of methanol and water (50:50) to enhance the solubility of methoxylated and hydroxylated compounds.19 Then, the ASP slurry was run in the column chromatography using a gradient elution technique with a binary solvent system of ethyl acetate and methanol, allowing polarity changes during the fractionation. Gradient elution usually offers better speed, separation, and retention reproducibility compared to isocratic elution for wide range polarities of organic compounds.20

The gradient solvent system of ethyl acetate (100 %), ethyl acetate: methanol (7:3), ethyl acetate: methanol (5:5), ethyl acetate: methanol (3:7), and methanol (100 %) were sequentially employed and finally, the column was washed with 100 % methanol. Nine fractions were collected in a 15 ml centrifuge tube and characterized by TLC profiling with a solvent system of ethyl acetate: methanol (9:5.0:5.5) with a drop of formic acid. The spots were visualized using a UV lamp, 50 % sulphuric acid spraying reagent, vanillin-sulphuric acid reagent and ferric chloride spraying detection reagent. These spraying reagents were

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prepared according to the methods stated in Pirring and Mohrig et al. Similar fractions (similar TLC profile) were pooled and combined to give the final three fractions designated as F1ASP, F2ASP, and F3ASP. These fractions were then dried in an incubator oven (Memmert, Germany) and stored at -20 °C in a refrigerator (SuperFreezer 340W, 1D, Korea) for further analysis.

Determination of antihypertensive effect of crude extracts and fractions

This in vivo antihypertensive study was conducted based on several previous studies. A BIOPAC Data Acquisition System, attached to an arterial pressure transducer with an amplifier recorder (MP30, BIOPAC Data Acquisition System) was employed for measurement of blood pressure parameters and the data were displayed using BIOPAC Student Lab Pro™ v3.6.7.

Each rat was weighed using a laboratory weighing balance and anaesthetized with 60 mg/kg sodium pentobartal via intraperitoneal injection. The reflex of the rat was checked by pinching the tail and the toe. The rat was placed in a rat's container until no reflex reaction occurred. Later, the rat was brought to the surgery table before performing a tracheotomy. An additional amount of 10 mg/kg sodium pentobartal was given throughout the experiment to maintain the anaesthetic condition whenever necessary. The body temperature of rats was maintained at 37 ± 1 °C using an overhead lamp. The skin on the anterior side of the neck was carefully cut-off using a surgical scissor. A small incision was made (1.5-2 cm) on the skin layers of the anterior side of the neck. A slit incision was made on the rat platysma muscles. By using two forceps with teeth, the skin was separated via blunt dissection technique while taking extra precautions not to disturb the larynx, hyoid bone, and thyroid cartilage. The trachea was then identified and forceps were used to slightly pull up the trachea and then a thread was eventually passed underneath it. The front part of the trachea was then half-incised for the insertion of modified intravenous drip tubing. The tube was thick with a length around 3 to 4 cm. The thread under the trachea was then used to fix the inserted tube to the trachea. Tracheotomy was performed to aid the respiration process since the employed sodium pentobartal usually increases the bronchial secretion. Continuous monitoring of the rats' respiration was performed throughout the experiment.

After tracheotomy, cannulation of the carotid artery was performed. The dark red, elastic, rounded, and thick vessel of the carotid artery was identified along the vagus nerve which was white-in-color on either side of the trachea. Separation of the vagus nerve, connective tissue, and longus capitis (a longitudinal bundle of muscle located adjacent to the trachea) was carried out. The cephalic end of the carotid artery was identified along the vagus nerve which was white-in-color on either side of the neck. A slit incision was made on the rat platysma muscles. By using two forceps with teeth, the skin was separated via blunt dissection technique while taking extra precautions not to disturb the larynx, hyoid bone, and thyroid cartilage. The trachea was then identified and forceps were used to slightly pull up the trachea and then a thread was eventually passed underneath it. The front part of the trachea was then half-incised for the insertion of modified intravenous drip tubing. The tube was thick with a length around 3 to 4 cm. The thread under the trachea was then used to fix the inserted tube to the trachea. Tracheotomy was performed to aid the respiration process since the employed sodium pentobartal usually increases the bronchial secretion. Continuous monitoring of the rats' respiration was performed throughout the experiment.

The total phenolic content of the ASP crude extract and the three derived fractions (F1ASP, F2ASP, and F3ASP) were determined using Folin–Ciocalteau assay with AC5 reagent grade gallic acid as a standard. Two-hundred µl of sample for ASP, F1ASP, F2ASP, and F3ASP, and gallic acid (as a standard) were pipetted into individual test tubes. Eight-hundred µl of distilled water and 500 µl of Folin’s Reagent were added together into the test tubes containing-samples and standard. Each sample was prepared in triplicates. The standard was prepared from 30 to 200 µg/ml of gallic acid dissolved in AR methanol. All samples (ASP, F1ASP, F2ASP, and F3ASP) were prepared in 1 mg/ml of AR methanol. All of them were allowed to stand in the dark for 5 minutes. After that, 1.5 ml of 20 % w/v sodium carbonate (Na₂CO₃) was added and all the mixtures were incubated at room temperature in a dark condition for 2 hours. Two ml of prepared mixtures of samples (ASP, F1ASP, F2ASP, and F3ASP) and standard (gallic acid, 30 to 200 µg/ml) were then transferred into a plastic cuvette for measurement. The absorbance was measured at the wavelength of 760 nm against a blank (distilled water) using a UV–VIS spectrophotometer (Perkin Elmer, Malaysia). Blainski et al. reported that the maximum absorption can be produced at this specific wavelength. Moreover, the long-wavelength absorption of the chromophores minimizes the interference of the sample matrix that is often coloured. The measured absorbance for standard (gallic acid, 30 to 200 µg/ml) and each respective sample in triplicates were averaged and a standard curve graph was plotted.

LC-QTOF/MS analysis for identification of phenolic compounds in the most active crude extract and active fractions

Identification of the compounds in the ASP, F1ASP, F2ASP, and F3ASP were conducted using a modified method described by Terpinc et al. LC-MS instrument used was a Waters, VION Ion Mobility QTOF MS. HPLC system was a binary pump with solvent gradient of water (A) and
Statistical analysis

The recorded MAP, SBP and DBP changes were expressed as mean percent changes ± standard error of mean (S.E.M.). All statistical tests were analyzed using GraphPad Prism version 6 software. A two-way ANOVA test was performed to determine the significant differences between multiple doses of extracts and fractions. Unpaired T-test was done only to ensure there was no significant difference (P>0.05) if the plateau effect occurred on high dosages. A post-hoc Sidak test was performed for multiple pairwise comparisons between the doses. The ED₅₀ values for MAP, SBP, and DBP reductions by ASP and fractions were computed by the software based on the constructed dose-response plateau effect occurred on high dosages. A post-hoc Sidak test was done only to ensure there was no significant difference (P>0.05) if the plateau effect occurred on high dosages. A post-hoc Sidak test was performed for multiple pairwise comparisons between the doses. The ED₅₀ values for MAP, SBP, and DBP reductions by ASP and fractions were computed by the software based on the constructed dose-response curves. TPC was analyzed by one way ANOVA, followed by post-hoc Sidak multiple comparison test between the doses. All tests were two-tailed and a P value less than 0.05 was considered significant (P<0.05).

RESULTS AND DISCUSSION

Yield of extraction

In total, 1.45 kg of dried S. polyanthum leaves used in this study. The mean average yield for HSP, ESP, MSP and ASP were 1.72 ± 0.83 %, 6.39 ± 1.25 % and 5.00 ± 2.59 %, respectively. It was observed that methanol gave the highest yield among the four extracts whereas the hexane gave the lowest yield. In agreement with this finding, Jumaat et al. reported that their extraction with n-hexane, a solvent with a polarity index (P') of 0.1 gave low extraction yield as compared to methanol. Extraction with n-hexane is crucial to break down the cell wall which is coated with the non-polar phospholipids. Ethyl acetate, a solvent with a polarity index (P') of 4.4, dissolves any hydrophilic, lipophilic compounds and hydrophobic chain lipids such as waxes and fats while methanol is a solvent with a polarity index (P') of 5.1 that partially dissolves some other non-water soluble compounds and extracts polar compounds like sugars, amino acids, glycosides and phenolic compounds with low and medium polarity. Water, on the other hand, is a universal solvent that is widely being used in extracting phytochemicals from traditional medicine. It mostly dissolves proteins, carbohydrates, and glycosides. The extraction with water, a solvent with a polarity index (P') of 10.2 usually did not dissolve any hydrophobic hydrocarbon compound. This is perhaps the reason that the yield of water extract was lower as compared to methanol. Thus, optimal temperature (80 °C) and ultrasound wave from sonication in the ultrasound-assisted extraction technique plays an important role to enhance water as a solvent to permeate the plant cell wall. Do et al. and Dhawan and Gupta also showed a lower percentage yield of water extract compared to methanol. This was probably due to the non-solubility of neutral lipids (non-polar hydrophobic) in water, while methanol dissolves a higher amount of polyphenols compared to water due to its inherent efficiency to degrade cell wall comprising of non-polar components. Tiwari et al. suggested the presence of active polyphenol oxidase enzyme in water extract which may be responsible for degradation of some polyphenols in water extract, whereas the enzyme is non-active in methanol extract. This may justify the higher yield in methanol extract as compared to water extract.

Bioassay-guided fractionation

Fractionation was done on ASP, the most prominent crude extract found in the first phase of the antihypertensive study. When ASP crude extract and the reference compound (gallic acid) was run with TLC using a solvent system of ethyl acetate: methanol: acetonitrile (8:1:1) with one drop of formic acid, four different spots were visualized with good separation when viewed under the UV lamp and sprayed with FeCl₃ reagent (Figure 1). These spots were identified with Rₖ values of 0.21, 0.24, 0.66, and 0.70. Only a spot with an Rₖ value of 0.21 appeared to be slightly-tailing. The reference compound, gallic acid resulted

![Figure 1: TLC profiles of ASP crude extract and reference (standard) gallic acid with the mobile system of ethyl acetate: methanol: acetonitrile (8:1:1) with one drop of formic acid with detection using A) UV short wave (254 nm), B) UV long wave (365 nm) and C) FeCl₃ reagent.](image-url)
in a very huge spot at R$_f$ = 0.68. In comparison to that, from the four spots developed for the crude ASP extract, two spots with R$_f$ = 0.66 and R$_f$ = 0.7 were very close to gallic acid spots, and they were probably pyrogallic acid, a derivative of gallic acid which was later identified in LC-MS chromatogram of ASP crude extract and F1ASP. Pyrogallic acid is a compound that can be derived from gallic acid via decarboxylation reaction. Based on these TLC profiles, a combination of ethyl acetate and methanol were selected as the binary solvent system for fractionation of ASP using column chromatography.

When the sample was loaded with the starting solvent system (25 ml of 100% ethyl acetate), three different colours of bands of brown, green, and yellow started to appear. The first (25 ml of 100% ethyl acetate) and the second ratio solvent system (50 ml of 70% ethyl acetate and 30% methanol mixture) allowed the bands to separate. However, the brown and green bands were strongly attracted to the silica column even though the third ratio solvent system (50 ml of 50% ethyl acetate and 50% methanol mixture) has been added. The strong attraction was most probably due to the strong polarity of compounds. For the third ratio solvent system (50 ml of 50% ethyl acetate), three different colours of bands of brown, green, and yellow started to appear. The first (25 ml of 100% ethyl acetate) as F1, F2, F3, and F4 were eluents collected when the ratio of solvent system used was ethyl acetate: methanol (3:7); F6, F7, and F8 were eluents collected when the ratio of the solvent system used was ethyl acetate: methanol (5:5); F3, F4, and F5 were eluents collected when the ratio of solvent system used was ethyl acetate: methanol (7:3); F6, F7, and F8 were eluents collected when the ratio of the solvent system was 100% methanol; while F9 was an eluent collected by washing the column again with 100% methanol. The yield of each fraction was shown in Table 1.

Table 1: Yield of fractions derived from ASP crude extract.

| Solvents (Gradient elution) | Ethyl acetate (100%) | Ethyl acetate: methanol (7:3) | Ethyl acetate: methanol (5:5) | Ethyl acetate: methanol (3:7) | Methanol (100%) | Methanol (Wash) (100%) |
|-----------------------------|----------------------|------------------------------|-----------------------------|-------------------------|----------------|------------------------|
| Volume of binary solvent (ml) | 25 | 50 | 50 | 50 | 50 | 100 |
| Fractions (Weight in grams) | - | - | F1 (0.34) | F2 (0.84) | F3 (0.48) | F4 (0.45) | F5 (0.76) | F6 (0.64) | F7 (0.88) | F8 (0.52) | F9 (2.57) |
| New fractions after pooling | - | - | Fraction 1 (F1ASP) (2.11g) | Fraction 2 (F2ASP) (2.80g) | - | - | - | - | - | - |
| Percentage yield (%) | - | - | 7.03 % | 9.33 % | 8.57 % | - | - | - | - | - | - |

Table 2: Summary of the spots developed on TLC profiles of F1 to F9 and reference (standard) gallic acid under different visualization agents.

| Visualization methods/reagents | Description | S1 (R$_f$ = 0.20 ± 0.01 cm) | S2 (R$_f$ = 0.10 ± 0.02 cm) | S3 (R$_f$ = 0.30 ± 0.01 cm) | S4 (R$_f$ = 0.31 ± 0.02 cm) | S5 (R$_f$ = 0.50 ± 0.04 cm) | S6 (R$_f$ = 0.64 ± 0.03 cm) | S7 (R$_f$ = 0.76 ± 0.03 cm) | Gallic acid (R$_f$ = 0.68 ± 0.03 cm) |
|-------------------------------|-------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| UV 254 | Visualization of the quenched organic compounds including aromatic and conjugated double bonds. | - | - | - | - | - | - | - | - |
| UV 365 | Visualization of most polycyclic compounds. | - | - | - | - | - | - | - | - |
| FeCl$_3$ reagent | General detection on phenolic compounds. | - | - | - | - | - | - | - | - |
| 50 % sulphuric acid | General detection on sterols. | - | - | - | - | - | - | - | - |
| Vanillin-sulphuric acid | General detection on higher alcohol, aldehydes, and ketones. | - | - | - | - | - | - | - | - |
an Rf value of 0.76 ± 0.03 cm. After pooling, F2ASP gave the highest yield of 2.80 g while F1ASP gave the lowest yield of 2.11 g (Table 1).

Antihypertensive effects of S. polyanthum leaves crude extracts and fractions

Three blood pressure parameters were measured in this study which includes mean arterial pressure (MAP), systolic blood pressure (SBP), and diastolic blood pressure (DBP). MAP is the average arterial pressure throughout one cardiac cycle and it is usually influenced by cardiac output and systemic vascular resistance. SBP is the pressure measured during systole or heart contraction, while DBP is a pressure during diastole or relaxation period. While MAP is a better indicator of perfusion to vital organs than systolic blood pressure (SBP), SBP is a bigger risk factor than DBP for cardiovascular disease in elderly patients. Considering the individual importance of each parameter, this study includes all these three blood pressure parameters.

Figure 3 shows dose-response curves for MAP, SBP, and DBP of SHR when administered with HSP, ESP, MSP, and ASP crude extracts. The mean baselines for MAP, SBP, and DBP (n=20) before intravenous administration of these crude extracts were 185.77 ± 5.05 mmHg, 218.41 ± 5.86 mmHg, and 157.04 ± 5.27 mmHg, respectively. The negative control (vehicle that dissolved the extracts) did not give any significant changes to the baseline of all blood pressure parameters (n = 5).

ASP crude extract caused significant reductions in MAP at 40 mg/kg and 70 mg/kg by 16.66 ± 1.51 % (P<0.001) and 20.12 ± 1.19 % (P<0.001), respectively. ESP crude extract only caused a significant reduction in MAP only at the highest dose of 70 mg/kg by 15.39 ± 3.58 %. On the other hand, there was no significant reduction of MAP for HSP crude extract.

There were also significant reductions in SBP by ASP crude extract at 10 mg/kg, 40 mg/kg and 70 mg/kg by 21.97 ± 3.79 % (P<0.05), 35.76 ± 4.74 % (P<0.001) and 73.75 ± 6.93 % (P<0.001), respectively (Figure 3B). On the other hand, MSP crude extract caused a significant reduction in SBP at 40 mg/kg and 70 mg/kg by 20.58 ± 1.50 % (P<0.001) and 16.19 ± 1.80 % (P<0.001), respectively. Similar to reductions in MAP, ESP crude extract only caused a significant reduction in SBP of SHR only at the highest dose of 70 mg/kg by 16.51 ± 3.82 % (P<0.001) while HSP gave no significant reduction in SBP at all.

Meanwhile for DBP, both ASP and MSP crude extracts gave significant reductions in DBP at 40 mg/kg and 70 mg/kg. ASP significantly reduced DBP by 37.31 ± 4.21 % (P<0.001) at 40 mg/kg and by 72.94 ± 7.76 % (P<0.001) at 70 mg/kg (Figure 3C). While for MSP, it significantly reduced DBP by 17.79 ± 2.24 % (P<0.001) at 40 mg/kg and by 21.84 ± 1.23 % (P<0.001) at 70 mg/kg. ESP crude extract could only give a significant reduction (P<0.01) at 70 mg/kg by 15.39 ± 3.58 %. However, there was no significant difference observed in DBP when administered with HSP crude extract at all dosages.

From the pattern of dose-response curves in Figure 3, HSP crude extract did not exhibit any significant antihypertensive effect and since it only extracted non-polar compounds, this has suggested that non-polar compounds did not significantly contribute to the antihypertensive effect of S. polyanthum leaves.
effect for S. polyanthum leaves. On the other hand, it was observed that ASP crude extract has the most prominent antihypertensive effect as it caused more reduction in all blood pressure parameters, especially at dosages of 40 mg/kg and 70 mg/kg when compared to other crude extracts. In summary, though ESP and MSP have significantly reduced the blood pressure of SHR, the antihypertensive effects by these two extracts were not as prominent as ASP. Besides the fact that ASP showed the most prominent antihypertensive effect, aqueous extraction is usually advantageous from the pharmacological point of view. Aqueous extract usually is the safest solvent with less toxicity for animal study40, and at the same time it is cost-effective and is usually used to mimics the traditional preparation. Considering all these findings, ASP was further fractionated in the subsequent study.

The subsequent antihypertensive study was conducted to identify the most active fraction. By using the same blood pressure parameters, the effect of fractions (F1ASP, F2ASP, F3ASP) was evaluated and compared with the original crude ASP extract. Normal saline (0.90 %) was used as the negative control while captopril (5 mg/kg) was used as the positive control. Figure 4 shows the magnitude of changes in MAP, SBP, and DBP when administered with the three fractions, in comparison with those exhibited by negative and positive controls as well as with the original ASP crude extract.

The mean baselines for MAP, SBP, and DBP (n=20) of SHR in this experiment were 171.19 ± 4.54 mmHg, 198.71 ± 4.09 mmHg, and 144.51 ± 3.94 mmHg, respectively. As shown in Figure 4A, there was no significant reduction observed on MAP of SHR with normal saline. Crude ASP extract at doses of 30, 40, 50, and 60 mg/kg significantly reduced MAP of SHR by 23.30 ± 1.08 % (P<0.01), 32.85 ± 3.75 % (P<0.001), 25.78 ± 7.09 % (P<0.01), and 29.24 ± 9.10 % (P<0.01), respectively. Meanwhile for the fractions, F1ASP at doses of 30, 40, 50, and 60 mg/kg significantly reduced MAP by 25.41 ± 3.57 % (P<0.01), 32.05 ± 6.66 % (P<0.001), 25.46 ± 4.55 % (P<0.05), and 27.39 ± 2.42 % (P<0.001), respectively. F2ASP at dosages of 20, 30, 40, 50, and 60 mg/kg significantly reduced MAP by 30.87 ± 6.70 % (P<0.001), 37.94 ± 5.84 % (P<0.001), 36.66 ± 5.41 % (P<0.001), 35.45 ± 0.93 % (P<0.001), and 27.65 ± 9.98 % (P<0.001), respectively. Nevertheless, for F3ASP, the MAP was significantly reduced only at two doses of 30 and 40 mg/kg by 25.64 ± 5.67 % (P<0.001) and 33.70 ± 5.45 % (P<0.001), respectively. In addition, 5 mg/kg of captopril (positive control) has significantly reduced MAP by 29.29 ± 3.18 % (P<0.001, Figure 4A). In comparison to positive control, the significant reduction in MAP by ASP (30, 40, 50 and 60 mg/kg), F1ASP (30, 40, 50, and 60 mg/kg), F2ASP (20, 30, 40, 50, and 60 mg/kg), and F3ASP (30 and 40 mg/kg) was not significantly different with the reduction by captopril at 5 mg/kg. This finding has indicated a comparable reduction in MAP between the positive control with ASP and the fractions at these dosages.

Meanwhile for SBP, ASP crude extract at dosages of 30, 40 and 50 mg/kg significantly reduced SBP by 27.16 ± 3.02 % (P<0.01), 32.80 ± 3.55 % (P<0.001), and 26.19 ± 4.09 % (P<0.01), respectively (Figure 4B).
For the fractions, F1ASP only significantly reduced SBP at two dosages of 40 and 60 mg/kg by 33.79 ± 7.35 % (P<0.001) and 24.25 ± 3.78 % (P<0.05), respectively. Meanwhile F2ASP at dosages of 20, 30, 40, 50, and 60 mg/kg significantly reduced SBP by 28.72 ± 6.78 % (P<0.01), 38.54 ± 7.26 % (P<0.001), 36.92 ± 5.53 % (P<0.001), 30.56 ± 3.08 % (P<0.05), and 30.93 ± 15.17 % (P<0.01), respectively. With the similar pattern observed for MAP changes, the SBP was significantly reduced by F3ASP only at two dosages of 30 and 40 mg/kg by 32.87 ± 10.32 % (P<0.05), and 30.93 ± 15.17 % (P<0.01), respectively. In spite of that, for F3ASP, DBP was not significantly different than the reduction by captopril (5 mg/kg) (Figure 4C). These significant reductions in DBP by ASP (30, 40, 50, and 60 mg/kg), F1ASP (30, 40, 50, and 60 mg/kg), F2ASP (20, 30, 40, 50, and 60 mg/kg), and F3ASP (30 and 40 mg/kg) for all dosages were not significantly different than the reduction by positive control (captopril) at 5 mg/kg (Figure 4B). These findings showed a comparable reduction in SBP of SHR by the positive control with ASP and the fractions at these dosages.

As illustrated in Figure 4C, ASP at doses of 30, 40, and 60 mg/kg significantly reduced DBP by 26.30 ± 3.45 % (P<0.001), 35.08 ± 4.37 % (P<0.001), and 27.04 ± 9.65 % (P<0.05), respectively. For the fractions, only two dosages of F1ASP at 40 and 60 mg/kg significantly reduced DBP by 26.36 ± 6.99 % (P<0.001) and 23.67 ± 3.06 % (P<0.01), respectively. F2ASP at dosages of 20, 30, 40, 50, and 60 mg/kg significantly reduced DBP by 30.37 ± 6.83 % (P<0.001), 35.48 ± 5.01 % (P<0.001), 35.81 ± 4.86 % (P<0.001), 29.78 ± 6.343 % (P<0.01), and 28.52 ± 10.48 % (P<0.001), respectively. In spite of that, for F3ASP, DBP was also significantly reduced only at two doses of 30 and 40 mg/kg by 22.73 ± 4.98 % (P<0.01), and 31.80 ± 5.33 % (P<0.001), respectively. Five mg/kg of captopril (positive control) significantly reduced DBP by 28.52 ± 10.48 % (P<0.001) (Figure 4C). These significant reductions in DBP by ASP (30, 40, 50, and 60 mg/kg), F1ASP (40, 50, and 60 mg/kg), F2ASP (20, 30, 40, 50, and 60 mg/kg), and F3ASP (30 and 40 mg/kg) were not significantly different than the reduction by captopril (5 mg/kg). This finding has also indicated a comparable reduction in DBP by the positive control with ASP and the fractions at these dosages.

Dose-response curves for the effect of each fraction on MAP, SBP, and DBP were then constructed and then compared with ASP crude extract (Figure 5). Both ASP and F1ASP started to cause significant reductions in MAP, SBP, and DBP at 30 mg/kg, then it caused a maximum reduction in MAP, SBP, and DBP at 40 mg/kg, and then the curve has started to become plateau afterward. In contrast to ASP and F1ASP, F2ASP started to produce significant MAP, SBP and DBP reduction at a low dose of 20 mg/kg, and then the effect has become plateau from 30 mg/kg until 60 mg/kg. In fact, the maximum reduction in MAP by F2ASP at 30 mg/kg was actually higher (P<0.05) than the other fractions and also ASP crude extract (Figure 5A). F3ASP showed the same trend as ASP and

Figure 4: Effects of ASP, F1ASP, F2ASP, F3ASP, captopril (positive control), and normal saline (negative control) on A) MAP, B) SBP, and C) DBP of anaesthetized SHR (n=5). Mg/kg: Milligram per kilogram, MAP: Mean arterial pressure, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, mmHg: Millimeters Mercury, a: P<0.05, b: P<0.01, c: P<0.001, all vs. normal saline and #P>0.05, all vs. Captopril, 5 mg/kg (this shows comparable effect with positive control drug). All data were analyzed using two-way ANOVA with post-hoc Sidak multiple comparison test.
F1ASP at low dosages, and then the effects became reduced at 50 mg/kg and maintained at 60 mg/kg. It is postulated that for F3ASP, there are different receptors involved at low and high dosages. When the reduction in blood pressure has reached maximum through activating the first receptor, then at the higher dosages, the fraction might activate another receptor system that causes attenuation of the antihypertensive effect. The involvement of receptors can be further investigated through in-depth pharmacodynamics studies.

To analyze the potency of the ASP crude extract and fractions, their ED$_{50}$ was then determined using GraphPad® Prism Version 6.00 software based on the constructed dose-response curves. ED$_{50}$ is an effective dose that produces 50% of the maximal effect. The ED$_{50}$ values for MAP, SBP and DBP reduction by F2ASP were 14.16 mg/kg, 19.17 mg/kg and 13.80 mg/kg, respectively. These ED$_{50}$ values were actually lower than the ED$_{50}$ values for ASP crude extract (29.48 mg/kg, 29.18 mg/kg and 30.15 mg/kg, respectively). On the other hand, F3ASP's pattern of reduction in blood pressure differed from the other fractions. After the antihypertensive effect by F3ASP reached maximum, then the effect was significantly reduced at subsequent dosages (50 mg/kg and 60 mg/kg). Thus, the ED$_{50}$ value of F3ASP could not be determined in this study. Altogether, F2ASP was more potent as compared to ASP and F1ASP and thus was considered as the most active fraction. The high potency of F2ASP might be due to the high concentration of the bioactive compound in this fraction compared to its crude extract itself. In agreement, Idris et al also found a higher antihypertensive effect of the fraction compared to crude extract and suggested the probability of an increased concentration of active compounds during the partitioning process.

**Total phenolic content**

This study examined the total phenolic content of the crude ASP extract and the three derived fractions (F1ASP, F2ASP, and F3ASP) by using Folin-Ciocalteu assay. Folin-Ciocalteu assay was generally a modified method from analysis of protein and is widely used for determination of the total phenolic content of various plant extracts. This assay was chosen to be used in this study as it is commercially available and has a standard procedure. This assay utilizes Folin-Ciocalteau reagent that determine phenols and easily-oxidized substances by forming a blue color complex form, reducing the yellow color of heteropoly phosphomolibdate-tungstate anions. The concentration of phenols can be determined by the blue color formed. However, this reaction's mechanism is not solely used for specific determination of only phenolics, instead, it can be used for determining any reducing compounds that can react with the phosphotungstic reagent.

Figure 6 shows the standard curve of gallic acid with an R$^2$ value of 0.992. The TPC for ASP, F1ASP, F2ASP, and F3ASP is shown in Table 3. ASP crude extract had the highest total phenolic content (232.81 ± 0.67 mg GAE/g), followed by F1ASP (76.15 ± 3.75 mg GAE/g), F3ASP (36.45 ± 1.35 mg GAE/g) and lastly F2ASP (30.52 ± 5.83 mg GAE/g). These TPCs were significantly different (P< 0.001) than ASP, in which all of them have lower TPC than ASP.
of both F2ASP and F3ASP were not significantly different. Thus, the order of TPC of S. polyanthum leaves from the highest to the lowest order was ASP>F1ASP>F3ASP>F2ASP. Since the reaction of Folin’s reagent also based on a redox reaction, the TPC assay would detect all substances that were oxidized, and this may include several potential reductants such as the reducing sugars glucose and fructose. This might cause significant effect on the accuracy of the TPC assay. Note that from LC-MS analyses, the ASP crude extract and all fractions contained a lot of glucosides (glucose bounded to another functional group) in which the glucose part may affect the reactions. Besides, this assay involves an oxidation reaction where the blue chromophore is formed by a phosphotungstic-phosphomolybdenum complex in which the maximum absorption depends on the alkaline solution and the concentration of phenolic compounds oxidized. Thus, the TPC assay would only detect phenolics that can function as reductants in a redox-linked colorimetric method. In addition, less availability of hydroxyl group or non-oxidized phenolics could also contribute to the low concentration of phenolics and eventually affected the total phenolic content analyzed.

In comparison with previous studies on the TPC of S. polyanthum leaves, it was found that the TPC of water extract of S. polyanthum leaves collected in Singapore was 11.21 mg GAE, a value which was lower than our present finding. Safriani et al. also reported a lower TPC of water extract (=40.0 mg GAE/g) compared to the current findings. However, Har and Ismail found that the methanolic extract of S. polyanthum leaves contained 1,125 mg GAE/g, which indicated for higher TPC than our ASP crude extract (232.81 ± 0.67 mg GAE/g). The higher phenolic content of methanol extract compared to the water extract used in our current study was probably due to the higher efficiency of methanol in extracting polyphenol. Methanol actually causes cell wall degradation causing more polyphenols to be released from the cells.

Phenolic compounds identified using LC-QTOF/MS

The different magnitude of antihypertensive effects by the extracts and the fractions may be affected by their varying phytochemical composition. Thus, LC-QTOF/MS analyses were then run for the most prominent crude extract, ASP, as well as for the three derived fractions (F1ASP, F2ASP, and F3ASP). Since this LC-QTOF/MS analysis was conducted in negative mode, only compounds with negative ions at high pH were detected. Figure 7 showed the LC-MS chromatograms of the blank (methanol) and ASP crude extract while Table 4 listed all the eluted compounds. In total, there were 216 peaks eluted out using the binary gradient elution with some redundant compounds detected as different peaks and at different retention times. Thus, in total, only 93 single compounds were actually detected in this analysis. In terms of composition, ASP crude extract was composed of gallotannins, phenolic acids, glucosides, flavonoids, and simple phenols. The highest intensity compound (highest percent response) was 2,4,7-trihydroxy-9,10-dihydrophenanthrene which was eluted at 11.90 min with an intensity of 14.36 %. Another two highest intensity compounds were oshmannside H (4.33 %) and sinapaldehyde (3.56 %). Meanwhile compound with the least intensity was 3, 4-dihydroxyphenethyl-3-O-β-D-glucopyranoside by 0.06 %.

Figure 8 shows LC-MS chromatograms for the blank methanol and F1ASP while Table 5 listed all the eluted compounds. In total, there were 76 peaks eluted out using the binary gradient elution with some redundant compounds. To be exact, only 46 single compounds that were detected in this analysis. The highest intensity compound (highest percent response) was ferroxin A at 5.76 min with an intensity of 8.44 %. Another two highest intensity compounds were 2,4,7-trihydroxyhydro-9,10-dihydrophenanthrene (7.79 %) and 1-galloyl-glucose (6.90 %). Meanwhile, the compound with the least intensity was cyclocurcumin with an intensity of 0.38 %.

Figure 9 shows the LC-MS chromatogram for the blank methanol and F2ASP while Table 6 listed all the eluted compounds. There were 13 peaks present in the chromatogram with few compounds that occurred in redundancy. Thus, there were only six compounds to be exact in F2ASP. These phytochemical compounds were either gallotannins, simple phenols, or isoflavonoids. The highest intensity of compound (highest percent response) in F2ASP was 1-galloyl-glucose; it was eluted at 1.33 min with an intensity of 20.24 %. Another two highest

Table 3: Total phenolic content of ASP crude extract and fractions.

| No. | Sample | Total phenolic content (GAE mg/gram) | Mean± SD |
|-----|--------|-------------------------------------|----------|
|     |        | Trial 1     | Trial 2     | Trial 3     |          |
| 1   | F1ASP  | 78.60       | 71.83       | 78.012      | 76.15 ± 3.75*** |
| 2   | F2ASP  | 25.01       | 36.63       | 29.907      | 30.52 ± 5.83 ***|
| 3   | F3ASP  | 38.01       | 35.67       | 35.674      | 36.45 ± 1.35 **  |
| 4   | ASP    | 232.04      | 232.083     | 233.29      | 232.81 ± 0.67 |

Note: Symbol*** showing P<0.001, all vs ASP using one way ANOVA with post-hoc Sidak multiple comparison test.
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**Figure 7:** Chromatogram of blank methanol and crude ASP extract.

**Figure 8:** Chromatogram of blank methanol and F1ASP.
Figure 9: Chromatogram of blank methanol and F2ASP.

Table 4: Phytochemical compounds in ASP crude extract using LC-MS.

| No. | Compound                                      | Molecular Formula | RT (min) | Chemical Classes            | Response % | [M’]    |
|-----|-----------------------------------------------|-------------------|----------|-----------------------------|------------|---------|
| 1   | 1-Galloyl-glucose                             | C_{13}H_{16}O_{10} | 0.43     | Gallotannin                 | 0.15       | 331.07  |
| 2   | Cassialactone                                 | C_{16}H_{16}O_{6}  | 0.44     | Simple phenol               | 0.12       | 349.09  |
| 3   | Pyrogallic acid                               | C_{6}H_{6}O_{3}    | 0.53     | Phenolic acid derivative    | 0.11       | 125.02  |
| 4   | Pyrogallic acid                               | C_{6}H_{6}O_{3}    | 0.79     | Phenolic acid derivative    | 0.07       | 125.02  |
| 5   | 1-Galloyl-glucose                             | C_{13}H_{16}O_{10} | 0.91     | Gallotannin                 | 0.35       | 331.07  |
| 6   | 2,3-(S)-Hexahydroxydiphenol-D-glucose         | C_{20}H_{18}O_{14} | 1.05     | Glucoside                   | 0.35       | 481.06  |
| 7   | Pyrogallic acid                               | C_{6}H_{6}O_{3}    | 1.19     | Phenolic acid derivative    | 0.26       | 125.02  |
| 8   | Cassialactone                                 | C_{16}H_{16}O_{6}  | 1.26     | Simple phenol               | 0.10       | 349.09  |
| 9   | Pyrogallic acid                               | C_{6}H_{6}O_{3}    | 1.42     | Phenolic acid derivative    | 0.07       | 125.02  |
| 10  | Methyl-β-orsellinate                          | C_{9}H_{10}O_{4}   | 1.53     | Ester phenol                | 0.11       | 181.05  |
| 11  | 3,4-Dihydroxy phenethyl-3-O-β-D-glucopyranoside | C_{12}H_{16}O_{8}  | 2.01     | Glucoside                   | 0.06       | 333.08  |
| 12  | 2,4,5Trihydroxy benzaldehyde                  | C_{12}H_{16}O_{8}  | 2.07     | Simple phenol               | 0.11       | 153.02  |
| 13  | Cassialactone                                 | C_{16}H_{16}O_{6}  | 2.19     | Simple phenol               | 0.08       | 349.09  |
| 14  | 1-Galloyl-glucose                             | C_{13}H_{16}O_{10} | 2.24     | Gallotannin                 | 0.18       | 331.07  |
| 15  | 1-Galloyl-glucose                             | C_{13}H_{16}O_{10} | 2.31     | Gallotannin                 | 0.67       | 331.07  |
| 16  | Caesalpins J                                  | C_{17}H_{16}O_{6}  | 2.96     | Simple phenol               | 1.21       | 361.09  |
| 17  | Osmanthuside H                                | C_{19}H_{28}O_{11} | 3.07     | Glycoside phenol            | 0.92       | 431.16  |
| 18  | Feroxin A                                     | C_{17}H_{24}O_{8}  | 3.26     | 3-O Glucose                 | 0.19       | 401.14  |
| 19  | 3’-O-Methylbrazilin                           | C_{17}H_{16}O_{8}  | 3.36     | Simple phenol               | 0.20       | 345.10  |
| 20  | Feralolide                                    | C_{19}H_{28}O_{11} | 3.42     | Dihydro-isocoumarin         | 0.10       | 343.08  |
| 21  | Darendoside A                                 | C_{17}H_{28}O_{11} | 3.88     | Phenethyl alcohol glycosides| 2.99       | 431.16  |
| 22  | Caesalpins J                                  | C_{17}H_{16}O_{8}  | 3.93     | Simple phenol               | 1.18       | 361.09  |
| 23  | Tachioside                                    | C_{17}H_{16}O_{8}  | 3.98     | Phenolic glycoside          | 0.09       | 301.09  |
| 24  | Tachioside                                    | C_{17}H_{16}O_{8}  | 3.98     | Phenolic glycoside          | 0.11       | 301.09  |
| 25  | Dendroandrins B                               | C_{13}H_{16}O_{10} | 4.05     | Bibenzyl phenols            | 0.09       | 481.19  |
| 26  | Osmanthuside H                                | C_{19}H_{28}O_{11} | 4.33     | Glycoside Phenols           | 4.33       | 431.16  |
| 27  | Odorilavene                                   | C_{17}H_{24}O_{8}  | 4.36     | Isoflavene                  | 0.22       | 345.10  |
| 28  | Sesamol                                       | C_{7}H_{6}O_{3}    | 4.40     | Hydroquinone derivative     | 0.24       | 183.03  |
| 29  | 2,4,6-Tetrahydroxy-benzophenone               | C_{19}H_{16}O_{8}  | 4.40     | Benzophenone                | 0.22       | 245.05  |
| No. | Compound                          | Molecular Formula | Retention Time | Concentration | Mass (Da) |
|-----|-----------------------------------|-------------------|----------------|---------------|-----------|
| 30  | Haematoxylin                      | C_{16}H_{14}O_{6} | 4.41           | Phenocyanin   | 347.08    |
| 31  | Feroxin A                         | C_{16}H_{14}O_{6} | 4.42           | 3-O Glucoside | 401.14    |
| 32  | Caesalpins J                      | C_{17}H_{24}O_{8} | 4.49           | Simple phenol | 361.09    |
| 33  | Cistanoside H                     | C_{16}H_{22}O_{13}| 4.55           | Glucoside     | 503.18    |
| 34  | Polydactin                        | C_{16}H_{16}O_{6} | 4.58           | Glucoside     | 435.13    |
| 35  | Caesalpins J                      | C_{17}H_{16}O_{6} | 4.79           | Simple phenol | 361.09    |
| 36  | 10-O-Methyl protosappanin B       | C_{16}H_{14}O_{6} | 4.85           | Dibenzoxxocin derivative | 363.11 |
| 37  | Moracin F                         | C_{16}H_{14}O_{6} | 4.86           | 2-arylbenzofuran flavanoid | 285.08 |
| 38  | 2,3,5,6-Tetrahydroxystilbene-2,3-O-β-Dglucopyranoside | C_{26}H_{32}O_{14} | 4.93           | Glucoside     | 567.17    |
| 39  | Methyl-β-orsellinate              | C_{16}H_{12}O_{6} | 4.94           | Ester phenol  | 227.06    |
| 40  | Protosappanin C                   | C_{16}H_{14}O_{6} | 5.02           | Dibenzoxxocin derivative | 347.08 |
| 41  | 2,4,7-Trihydroxy-9,10-dihydrophenanthrene | C_{17}H_{24}O_{8} | 5.27           | Phenanthrene phenol | 273.08 |
| 42  | Kakool                            | C_{16}H_{16}O_{6} | 5.33           | Prophiophenone derivative | 239.06 |
| 43  | Kakool                            | C_{16}H_{16}O_{6} | 5.71           | Prophiophenone derivative | 239.06 |
| 44  | Aspidinol                         | C_{17}H_{20}O_{8} | 5.79           | Simple phenols | 269.10    |
| 45  | Feroxin A                         | C_{16}H_{14}O_{6} | 5.80           | 3-O Glucoside | 401.14    |
| 46  | Feroxin A                         | C_{16}H_{14}O_{6} | 5.80           | 3-O Glucoside | 401.14    |
| 47  | Feroxin A                         | C_{16}H_{14}O_{6} | 5.80           | 3-O Glucoside | 401.14    |
| 48  | Macurin                           | C_{17}H_{16}O_{6} | 5.92           | Benzoanoid phenolic | 261.04 |
| 49  | 4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one | C_{16}H_{14}O_{6} | 6.01           | Simple phenol | 267.09    |
| 50  | Moracin M-3'-O-β-Dglucopyranoside  | C_{16}H_{14}O_{6} | 6.07           | Glycoside     | 449.11    |
| 51  | Protosappanin A                   | C_{16}H_{14}O_{6} | 6.09           | Dibenzoxxocin derivative | 317.07 |
| 52  | Methyl-5-Ocaffeoylquinic acid     | C_{16}H_{14}O_{6} | 6.15           | Phenolic acid derivative | 413.11 |
| 53  | 3,4-Dihydroxy phenethanol         | C_{16}H_{16}O_{6} | 6.26           | Simple phenol | 153.06    |
| 54  | Cimidahurine                      | C_{16}H_{14}O_{6} | 6.27           | Phenylpropanoid glycoside | 315.11 |
| 55  | Cimidahurine                      | C_{16}H_{14}O_{6} | 6.27           | Phenylpropanoid glycoside | 315.11 |
| 56  | Aspidinol                         | C_{16}H_{14}O_{6} | 6.29           | Simple phenols | 223.10    |
| 57  | Protosappanin C                   | C_{16}H_{14}O_{6} | 6.30           | Dibenzoxxocin derivative | 347.08 |
| 58  | 10-O-Methyl-protosappanin B       | C_{16}H_{14}O_{6} | 6.41           | Dibenzoxxocin derivative | 363.11 |
| 59  | Cassialactone                     | C_{16}H_{14}O_{6} | 6.43           | Simple phenol | 349.09    |
| 60  | 2,7-Dihydroxy-4-methoxyphenanthrene-2-O-glucoside | C_{16}H_{14}O_{6} | 6.43           | Phenanthrene phenol | 447.13 |
| 61  | 1-O-Methyl-3,5 Odcificoeyquinic acid methyl ester | C_{16}H_{14}O_{6} | 6.45           | Phenolic acid | 589.16    |
| 62  | Kakool                            | C_{16}H_{14}O_{6} | 6.47           | Prophiophenone derivative | 239.06 |
| 63  | Aspidinol                         | C_{16}H_{14}O_{6} | 6.49           | Simple phenols | 223.10    |
| 64  | Dihydroeugenol                    | C_{16}H_{14}O_{6} | 6.53           | Simple phenols | 211.10    |
| 65  | Ciwujiatone                       | C_{16}H_{14}O_{6} | 6.59           | Lignan        | 433.15    |
| 66  | Phenol                            | C_{16}H_{14}O_{6} | 6.91           | Simple phenols | 139.04    |
| 67  | 2,7-Dihydroxy-4-methoxyphenanthrene-2-O-glucoside | C_{16}H_{14}O_{6} | 6.96           | Phenanthrene phenol | 447.13 |
| 68  | Nobilin D                         | C_{16}H_{14}O_{6} | 6.97           | Prenol lipid  | 305.10    |
| 69  | 3,7-Dihydroxy-2,4-dimethoxyphenanthrene-3-O-glucoside | C_{16}H_{14}O_{6} | 6.99           | Phenanthrene phenol | 477.14 |
| 70  | Cimidahurine                      | C_{16}H_{14}O_{6} | 7.05           | Phenylpropanoid glycoside | 315.11 |
| 71  | Kakool                            | C_{16}H_{14}O_{6} | 7.13           | Prophiophenone derivative | 239.06 |
| 72  | Kakool                            | C_{16}H_{14}O_{6} | 7.13           | Prophiophenone derivative | 239.06 |
| 73  | Phenol                            | C_{16}H_{14}O_{6} | 7.14           | Simple phenols | 139.04    |
| 74  | Phenol                            | C_{16}H_{14}O_{6} | 7.14           | Simple phenols | 139.04    |
| 75  | Kakool                            | C_{16}H_{14}O_{6} | 7.14           | Prophiophenone derivative | 239.06 |
| 76  | Tachioside                        | C_{16}H_{14}O_{6} | 7.15           | Phenolic glycoside | 301.09    |
| 77  | Phenol                            | C_{16}H_{14}O_{6} | 7.15           | Simple phenols | 139.04    |
| No. | Compound Name                                      | Structure                        | pKa | Molecular Formula | pIC50 | Molecular Weight |
|-----|---------------------------------------------------|----------------------------------|-----|------------------|-------|-----------------|
| 78  | Moracin F                                         | C_{16}H_{14}O_{5}                | 7.16 | 2-arylbenzofuran flavanoid | 0.69  | 285.08          |
| 79  | Gingerone                                         | C_{11}H_{14}O_{3}                | 7.26 | Simple phenols    | 0.41  | 239.09          |
| 80  | Dihydroeugenol                                    | C_{10}H_{14}O_{2}                | 7.28 | Simple phenols    | 0.53  | 211.10          |
| 81  | 2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucopyranoside | C_{20}H_{22}O_{9}               | 7.39 | Glucoside        | 0.07  | 405.12          |
| 82  | Cassialactone                                      | C_{16}H_{14}O_{5}                | 7.41 | Simple phenol     | 0.16  | 249.09          |
| 83  | Nobilone                                           | C_{14}H_{10}O_{4}                | 7.42 | Simple phenols    | 0.22  | 287.06          |
| 84  | Gingerone                                          | C_{11}H_{14}O_{3}                | 7.42 | Simple phenols    | 0.18  | 287.06          |
| 85  | Nobilone                                           | C_{14}H_{10}O_{4}                | 7.45 | Simple phenols    | 0.67  | 239.09          |
| 86  | Homeorbutin                                        | C_{10}H_{14}O_{2}                | 7.47 | Simple phenols    | 0.96  | 287.06          |
| 87  | Apocynin B                                         | C_{10}H_{14}O_{6}                | 7.50 | Simple phenols    | 0.09  | 331.10          |
| 88  | Dihydroeugenol                                     | C_{10}H_{14}O_{2}                | 7.55 | Simple phenols    | 0.42  | 211.10          |
| 89  | 3,4-Dimethoxyphenol                                | C_{10}H_{14}O_{2}                | 7.56 | Simple phenol     | 0.33  | 153.06          |
| 90  | Kakuo                                             | C_{16}H_{14}O_{5}                | 7.56 | Simple phenol     | 0.35  | 239.09          |
| 91  | Cimicidurine                                       | C_{10}H_{12}O_{2}                | 7.59 | Simple phenol     | 1.39  | 315.11          |
| 92  | Echinacoside                                       | C_{10}H_{12}O_{2}                | 7.60 | Simple phenol     | 1.60  | 287.06          |
| 93  | Sinapaldehyde                                      | C_{10}H_{12}O_{2}                | 7.60 | Simple phenol     | 2.42  | 211.10          |
| 94  | 3,4-Dimethoxyphenol                                | C_{10}H_{14}O_{2}                | 7.60 | Simple phenol     | 1.19  | 285.08          |
| 95  | Tachioside                                         | C_{10}H_{14}O_{2}                | 7.64 | Simple phenol     | 0.70  | 301.09          |
| 96  | 2-Hydroxy-4-methoxybenzaldehyde                    | C_{16}H_{16}O_{5}                | 7.70 | Simple phenol     | 0.34  | 151.04          |
| 97  | Ellagic acid                                       | C_{16}H_{14}O_{5}                | 7.72 | Phenolic acid     | 1.39  | 300.99          |
| 98  | Aspidinol                                          | C_{12}H_{16}O_{4}                | 7.81 | Simple phenol     | 0.09  | 269.10          |
| 99  | Moracin F                                          | C_{16}H_{14}O_{5}                | 7.83 | Flavanoid         | 0.22  | 285.08          |
| 100 | Tachioside                                         | C_{13}H_{18}O_{8}                | 7.84 | Simple phenol     | 0.19  | 301.09          |
| 101 | Phenol                                             | C_{13}H_{18}O_{8}                | 7.84 | Simple phenol     | 0.16  | 153.06          |
| 102 | Renifolin                                          | C_{14}H_{20}O_{8}                | 8.02 | Glucoside         | 0.25  | 397.15          |
| 103 | Nobilone                                           | C_{16}H_{14}O_{5}                | 8.12 | Simple phenol     | 0.22  | 287.06          |
| 104 | Protosappanin C                                   | C_{16}H_{14}O_{5}                | 8.16 | Dibenzoxyacid     | 0.09  | 301.07          |
| 105 | 3,4-Dihydroxybenzamide                             | C_{16}H_{14}O_{5}                | 8.18 | Amide phenol      | 0.09  | 152.04          |
| 106 | 3,4-Dihydroxybenzamide                             | C_{16}H_{14}O_{5}                | 8.18 | Amide phenol      | 0.09  | 152.04          |
| 107 | (±)-Isoduartin                                     | C_{16}H_{14}O_{5}                | 8.24 | Isolavanan        | 0.26  | 372.12          |
| 108 | Renifolin                                          | C_{16}H_{14}O_{5}                | 8.31 | Glucoside         | 0.10  | 397.15          |
| 109 | Aspidinol                                          | C_{16}H_{14}O_{5}                | 8.42 | Simple phenol     | 0.21  | 269.10          |
| 110 | Eugenol                                            | C_{16}H_{14}O_{5}                | 8.47 | Simple phenol     | 0.12  | 209.08          |
| 111 | Gingerone                                          | C_{16}H_{14}O_{5}                | 8.53 | Simple phenol     | 3.48  | 239.09          |
| 112 | Obovatol                                           | C_{16}H_{14}O_{5}                | 8.55 | Biphenolic        | 0.07  | 327.12          |
| 113 | 3,4-Dihydroxy-2,4-dimethoxyphenanthrene e-3-O-glucoside | C_{16}H_{14}O_{5}       | 8.64 | Glucoside         | 0.52  | 477.14          |
| 114 | 3,7-Dihydroxy-2,4-dimethoxyphenanthrene e-3-O-glucoside | C_{16}H_{14}O_{5} | 8.64 | Phenanthrene glucoside | 0.16  | 477.14          |
| 115 | 2,4,7-Trihydroxy-9,10-dihydrophenanthrene 4-(4'-Hydroxy-3,5'-dimethoxyphenyl)-3-buten-2-one | C_{16}H_{14}O_{5} | 9.02 | Simple phenol     | 0.24  | 267.09          |
| 116 | Torachrysone-8-O-β-D-glucopyranoside               | C_{16}H_{14}O_{5}                | 9.04 | Glucoside         | 0.12  | 407.14          |
| 117 | Brazilein                                          | C_{16}H_{14}O_{5}                | 9.08 | Simple phenol     | 0.10  | 285.08          |
| 118 | Moracin F                                          | C_{16}H_{14}O_{5}                | 9.09 | 2-arylbenezofuran flavanoid | 0.16  | 269.10          |
| 119 | Apocynin B                                         | C_{16}H_{14}O_{5}                | 9.15 | Simple phenol     | 0.68  | 269.10          |
| No. | Compound Name                          | Molecular Formula | Retention Time | Structure Type             | Area % | M/z    |
|-----|----------------------------------------|-------------------|----------------|----------------------------|--------|--------|
| 127 | 4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one | C₁₂H₁₄O₄       | 9.24           | Simple phenols             | 0.10   | 267.09 |
| 128 | 4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one | C₁₂H₁₄O₄       | 9.26           | Simple phenols             | 0.07   | 267.09 |
| 129 | Polydatin                              | C₁₀H₁₃O₄        | 9.42           | Glycoside                  | 0.12   | 435.13 |
| 130 | Echinacoside                           | C₁₂H₁₄O₄       | 9.42           | Phenyl propanoid glucoside | 1.57   | 631.26 |
| 131 | Sinapaldehyde                          | C₁₂H₁₄O₄       | 9.42           | Lignin intermediate        | 1.80   | 253.07 |
| 132 | 3,4-Dimethoxyphenol                    | C₁₀H₁₄O₄       | 9.43           | Simple phenol              | 0.06   | 153.06 |
| 133 | Cimifugoside                           | C₁₂H₁₄O₄       | 9.43           | Phenylpropanoid glucoside  | 0.36   | 315.11 |
| 134 | (+)-Isoduartin                         | C₁₀H₁₃O₄       | 9.43           | Isoflavan                 | 1.38   | 377.12 |
| 135 | Nobilone                               | C₁₂H₁₄O₄       | 9.51           | Simple phenols             | 0.11   | 267.06 |
| 136 | 4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one | C₁₂H₁₄O₄       | 9.53           | Simple phenol              | 0.12   | 267.09 |
| 137 | 4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one | C₁₂H₁₄O₄       | 9.54           | Simple phenol              | 0.60   | 267.09 |
| 138 | Kuzubutenolide A                       | C₁₀H₁₃O₄       | 9.64           | Glucoside                  | 0.10   | 459.13 |
| 139 | Moracin C                              | C₁₀H₁₃O₄       | 9.65           | Glucoside                  | 0.26   | 355.12 |
| 140 | Albaspidin AA                          | C₁₂H₁₄O₄       | 9.76           | Phloroglucinol derivative  | 0.24   | 449.15 |
| 141 | Renifolin                              | C₁₂H₁₄O₄       | 9.81           | Glucoside                  | 0.16   | 397.15 |
| 142 | Aspidinol                              | C₁₂H₁₄O₄       | 9.86           | Simple phenol              | 1.23   | 269.10 |
| 143 | Gingerone                              | C₁₂H₁₄O₄       | 9.87           | Simple phenol              | 0.70   | 239.09 |
| 144 | Nobilin B                              | C₁₂H₁₄O₄       | 10.07          | Prenol lipid               | 0.11   | 319.12 |
| 145 | Torachrysone-8-O-β-D glucopyranoside   | C₁₂H₁₄O₄       | 10.10          | Glucoside                  | 0.12   | 407.14 |
| 146 | Nobilin C                              | C₁₂H₁₄O₄       | 10.10          | Prenol lipid               | 0.16   | 379.14 |
| 147 | 1-Galloyl-glucose                      | C₁₂H₁₄O₄       | 10.14          | Gallotannin                | 0.09   | 331.07 |
| 148 | Asebotin                               | C₁₂H₁₄O₄       | 10.15          | Dihydrochalcone glucoside  | 0.24   | 449.15 |
| 149 | Renifolin                              | C₁₂H₁₄O₄       | 10.20          | Glucoside                  | 0.21   | 397.15 |
| 150 | 3,4-O-Dicaffeoylquinic acid            | C₁₂H₁₄O₄       | 10.33          | Phenolic acid              | 0.09   | 561.13 |
| 151 | 2-((3R,4R)-7-Hydroxy-4-(4-hydroxy-5-((R)-7-hydroxychroman-3-yl)-2-methoxyphenyl)-5-methylocyclohexa-1,4-diene-1,4-dione | C₁₂H₁₄O₄       | 10.36          | Simple phenol              | 0.09   | 555.17 |
| 152 | Asebotin                               | C₁₀H₁₃O₄       | 10.49          | Dihydrochalcone glucoside  | 0.09   | 449.15 |
| 153 | Sinapaldehyde                          | C₁₂H₁₄O₄       | 10.59          | Lignin intermediate        | 0.43   | 253.07 |
| 154 | 4,4'-Dihydroxy-9,10-dihydrophenanthrene| C₁₀H₁₃O₄       | 10.59          | Phenanthrene phenol        | 2.99   | 273.08 |
| 155 | p-Tolualdehyde                         | C₁₀H₁₃O₄       | 10.59          | Benzenoid                  | 0.07   | 167.04 |
| 156 | Ciwujianone                            | C₁₀H₁₃O₄       | 10.66          | Lignan                     | 0.13   | 433.15 |
| 157 | Ccaroin                                | C₁₀H₁₃O₄       | 10.66          | Simple phenol              | 0.29   | 289.07 |
| 158 | Albaspidin AA                          | C₁₂H₁₄O₄       | 10.67          | Phloroglucinol derivative  | 0.11   | 403.14 |
| 159 | Torachrysone-8-O-β-D glucopyranoside   | C₁₂H₁₄O₄       | 10.75          | Glucoside                  | 0.54   | 407.14 |
| 160 | Ciwujianone                            | C₁₂H₁₄O₄       | 10.81          | Lignan                     | 0.10   | 433.15 |
| 161 | Cassialactone                          | C₁₀H₁₃O₄       | 10.84          | Simple phenol              | 0.08   | 349.09 |
| 162 | Renifolin                              | C₁₂H₁₄O₄       | 10.90          | Glucoside                  | 0.34   | 397.15 |
| 163 | 4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one | C₁₂H₁₄O₄       | 10.91          | Simple phenol              | 0.12   | 267.09 |
| 164 | Sinapaldehyde                          | C₁₀H₁₃O₄       | 10.97          | Lignin intermediate        | 0.64   | 253.07 |
| 165 | 4,4'-Dihydroxy-3,5-dimethoxybenzyl     | C₁₀H₁₃O₄       | 11.03          | Simple phenol              | 0.28   | 319.12 |
| 166 | Protosappanin A                        | C₁₀H₁₃O₄       | 11.07          | Dibenzoxocin derivative    | 0.34   | 271.06 |
| 167 | Sinapaldehyde                          | C₁₂H₁₄O₄       | 11.13          | Simple phenols             | 0.33   | 223.10 |
| 168 | Thannilignan                           | C₁₀H₁₃O₄       | 11.15          | Lignan                     | 0.34   | 329.14 |
| 169 | Sinapaldehyde                          | C₁₀H₁₃O₄       | 11.17          | Lignin intermediate        | 0.64   | 253.07 |
| 170 | Cistanoside H                          | C₁₂H₁₃O₄       | 11.26          | Glucoside                  | 0.20   | 503.18 |
| 171 | Renifolin                              | C₁₂H₁₄O₄       | 11.30          | Glucoside                  | 0.15   | 397.15 |
| No. | Compound Description                                                                 | Molecular Formula | Retention Time (min) | Mass (m/z) | Relative Intensity | Molecular Mass |
|-----|----------------------------------------------------------------------------------------|-------------------|----------------------|------------|-------------------|---------------|
| 172 | Protosappanin A                                                                         | C_{15}H_{10}O_{5} | 11.43                | 271.06     | 1.18              |               |
| 173 | 2,3,5,4′-Tetrahydroxystilbene-2-O-(6′-O-α-d-glucopyranosyl)-β-Dglucopyranoside          | C_{19}H_{12}O_{14} | 11.53                | Glucoside  | 0.17              | 567.17        |
| 174 | Dendrocandin C                                                                          | C_{16}H_{10}O_{5} | 11.55                | Bibenzyl phenols | 0.38      | 289.11        |
| 175 | 3,4′-Dihydroxybenzamide                                                                | C_{17}H_{12}NO_{4} | 11.57                | 8380       | 0.27              | 152.04        |
| 176 | Thannilignan                                                                           | C_{19}H_{12}O_{5} | 11.78                | Lignan     | 0.18              | 375.15        |
| 177 | Cistanoside H                                                                          | C_{16}H_{12}O_{5} | 11.80                | Glucoside  | 0.08              | 503.18        |
| 178 | p-Tolualdehyde                                                                         | C_{17}H_{12}O_{5} | 11.88                | Benzenoid  | 0.57              | 167.04        |
| 179 | 2,4,7-Trihydroxy-9,10-dihydrophenanthrene                                               | C_{19}H_{12}O_{7} | 11.90                | Phenanthrene phenol | 14.36    | 273.08        |
| 180 | Tachioside                                                                             | C_{17}H_{12}O_{5} | 11.90                | Phenolic glycoside | 0.21     | 301.09        |
| 181 | Eugenol                                                                                | C_{19}H_{12}O_{7} | 11.96                | Simple phenol | 0.17     | 209.08        |
| 182 | 3,4-Dimethoxyphenol                                                                    | C_{17}H_{12}O_{7} | 11.97                | Simple phenol | 0.45     | 153.06        |
| 183 | Sinapaldehyde                                                                          | C_{17}H_{12}O_{7} | 11.97                | Lignin intermediate | 0.72    | 253.07        |
| 184 | 2,3,5,4′-Tetrahydroxystilbene-2-O-(6′-O-α-d-glucopyranosyl)-β-Dglucopyranoside          | C_{19}H_{12}O_{14} | 12.04                | Glucoside  | 0.12              | 567.17        |
| 185 | Phenol                                                                                 | C_{17}H_{12}O_{7} | 12.09                | Simple phenols | 1.15     | 139.04        |
| 186 | 10-O-Methyl protosappanin B                                                            | C_{17}H_{12}O_{7} | 12.16                | Dibenzoxocin derivative | 0.08   | 363.11        |
| 187 | Phenol                                                                                 | C_{17}H_{12}O_{7} | 12.36                | Simple phenols | 0.09     | 139.04        |
| 188 | 1,4-Dihydroxy-2-methoxybenzene                                                         | C_{20}H_{14}O_{5} | 12.37                | Benzenoid  | 1.10              | 139.04        |
| 189 | tran-Ferulaldehyde                                                                     | C_{22}H_{22}O_{3} | 12.37                | Aldehyde   | 0.10              | 223.06        |
| 190 | (3R)-3′,8-Dihydroxyvestitol                                                            | C_{23}H_{22}O_{9} | 12.51                | Isoflavane | 0.15              | 349.09        |
| 191 | Caesalpins J                                                                           | C_{17}H_{12}O_{7} | 12.59                | Simple phenol | 0.08     | 361.09        |
| 192 | Polydatin                                                                              | C_{17}H_{12}O_{7} | 12.67                | Glucoside  | 0.08              | 435.13        |
| 193 | 2,3,5,4′-Tetrahydroxystilbene-2,3-O-β-Dglucopyranoside                                 | C_{21}H_{14}O_{14} | 12.80                | Glucoside  | 0.16              | 567.17        |
| 194 | Aspidinol                                                                              | C_{17}H_{12}O_{7} | 12.88                | Simple phenol | 0.12     | 233.10        |
| 195 | Aspidinol                                                                              | C_{17}H_{12}O_{7} | 12.88                | Simple phenol | 0.35     | 233.10        |
| 196 | Protosappanin A                                                                        | C_{17}H_{12}O_{7} | 13.10                | Dibenzoxocin derivative | 0.35   | 271.06        |
| 197 | Darendoside A                                                                          | C_{19}H_{20}O_{11} | 13.15                | Phenethyl alcohol glycosides | 0.41 | 431.16        |
| 198 | Moracin M-3′-O-β-Dglucopyranoside                                                       | C_{16}H_{12}O_{7} | 13.38                | Glucoside  | 0.12              | 403.10        |
| 199 | Dendrocandin C                                                                        | C_{17}H_{12}O_{7} | 13.43                | Bibenzyl phenols | 0.09     | 289.11        |
| 200 | 7,2′,3′-Trihydroxy-4′-methoxy-isoflavan                                                 | C_{16}H_{12}O_{7} | 14.14                | Isoflavane | 0.28              | 287.09        |
| 201 | 2,3,5,4′-Tetrahydroxystilbene-2-O-(6′-O-α-d-glucopyranosyl)-β-Dglucopyranoside          | C_{19}H_{12}O_{14} | 14.27                | Glucoside  | 0.10              | 567.17        |
| 202 | Dendrocandin C                                                                        | C_{17}H_{12}O_{7} | 14.59                | Bibenzyl phenols | 0.10     | 335.11        |
| 203 | Aspidinol                                                                              | C_{17}H_{12}O_{7} | 15.01                | Simple phenol | 1.05     | 223.10        |
| 204 | Moracin M-3′-O-β-Dglucopyranoside                                                       | C_{16}H_{12}O_{7} | 16.84                | Glucoside  | 0.08              | 403.10        |
| 205 | Dendrocandin E                                                                        | C_{17}H_{12}O_{7} | 16.87                | Bibenzyl phenols | 0.13     | 321.10        |
| 206 | Xanthohumol                                                                            | C_{19}H_{20}O_{6} | 16.91                | Chalcone   | 0.08              | 399.15        |
| 207 | 2,7-Dihydroxy-4′-methoxyphenanthrene-2-O-glucoside                                     | C_{19}H_{20}O_{7} | 16.91                | Phenanthrene phenol | 0.15   | 447.13        |
| 208 | Kuzubutenolide                                                                        | C_{19}H_{20}O_{6} | 17.26                | Glucoside  | 0.07              | 505.13        |
| 209 | Cyclocurcumin                                                                          | C_{19}H_{20}O_{6} | 17.26                | Diarylheptanoid | 0.10    | 413.12        |
| 210 | Dendrocandin E                                                                        | C_{17}H_{12}O_{7} | 17.26                | Bibenzyl phenols | 0.15     | 321.10        |
| 211 | Nobilin A                                                                              | C_{17}H_{12}O_{7} | 17.26                | Prenol lipid | 0.09              | 349.13        |
| 212 | Syringylethanol                                                                        | C_{17}H_{12}O_{7} | 17.26                | Lignin     | 0.10              | 241.07        |
| 213 | Dendrocandin C                                                                        | C_{17}H_{12}O_{7} | 17.36                | Bibenzyl phenols | 0.07     | 335.11        |
| 214 | Polydatin                                                                              | C_{17}H_{12}O_{7} | 17.56                | Glucoside  | 0.08              | 435.13        |
| 215 | Moracin O                                                                              | C_{17}H_{12}O_{7} | 17.89                | Glucoside  | 0.17              | 371.11        |
| 216 | Gigantol                                                                               | C_{17}H_{12}O_{7} | 17.95                | Bibenzyl phenols | 0.07     | 305.10        |

Note: RT: Retention Time, \([M+]:\) Molecular ion mass (m/z)
### Table 5: Phytochemical compounds in F1ASP using LC-MS.

| No. | Compound                               | Molecular Formula | RT (min) | Chemical Classes   | Response % | [M+]     |
|-----|----------------------------------------|-------------------|----------|--------------------|------------|----------|
| 1   | 1-Galloyl-glucose                       | C_{13}H_{16}O_{10} | 0.44     | Gallotannin        | 2.25       | 331.07   |
| 2   | 1-Galloyl-glucose                       | C_{13}H_{16}O_{10} | 0.74     | Gallotannin        | 0.38       | 331.07   |
| 3   | Pyrogallic acid                         | C_{6}H_{10}O_{5}  | 0.80     | Phenolic acid      | 0.49       |          |
| 4   | 1-Galloyl-glucose                       | C_{13}H_{16}O_{10} | 1.01     | Gallotannin        | 0.99       | 331.07   |
| 5   | 1-Galloyl-glucose                       | C_{13}H_{16}O_{10} | 1.27     | Gallotannin        | 2.46       | 331.07   |
| 6   | 1-Galloyl-glucose                       | C_{13}H_{16}O_{10} | 1.58     | Gallotannin        | 6.90       | 331.07   |
| 7   | 1-Galloyl-glucose                       | C_{13}H_{16}O_{10} | 2.22     | Gallotannin        | 0.49       | 331.07   |
| 8   | 2,4,5-Trihydroxybenzaldehyde           | C_{5}H_{6}O_{3}   | 2.34     | Benzaldehyde       | 0.43       | 153.02   |
| 9   | 2,6-Di-O-galloyl-β-D-glucose           | C_{20}H_{20}O_{14}| 3.03     | Gallotannin        | 0.70       | 483.08   |
| 10  | Polydatin                               | C_{16}H_{16}O_{8} | 3.33     | Glucoside          | 0.73       | 435.13   |
| 11  | 2,6-Di-O-galloyl-β-D-glucose           | C_{20}H_{20}O_{14}| 3.77     | Gallotannin        | 1.64       | 483.08   |
| 12  | Polydatin                               | C_{16}H_{16}O_{8} | 3.84     | Glucoside          | 2.57       | 435.13   |
| 13  | 2,6-Di-O-galloyl-β-D-glucose           | C_{20}H_{20}O_{14}| 4.22     | Gallotannin        | 0.63       | 483.08   |
| 14  | Tetrahydroxystilbene-2, 3-O-β-D-glucoypyranside | C_{16}H_{20}O_{14} | 4.32     | Glucoside          | 0.53       | 568.17   |
| 15  | Sesamol                                 | C_{7}H_{6}O_{3}   | 4.34     | Hydroquinone derivative | 2.75       | 183.03   |
| 16  | 2,3,5,4'-Tetrahydroxystilbene-2, 3-O-β-D-glucoypyranside | C_{16}H_{20}O_{14} | 4.44     | Glucoside          | 0.38       | 567.17   |
| 17  | 2,6-Di-O-galloyl-β-D-glucose           | C_{20}H_{20}O_{14}| 4.65     | Gallotannin        | 0.86       | 483.08   |
| 18  | 2,6-Di-O-galloyl-β-D-glucose           | C_{20}H_{20}O_{14}| 4.88     | Gallotannin        | 2.63       | 483.08   |
| 19  | 2,6-Di-O-galloyl-β-D-glucose           | C_{20}H_{20}O_{14}| 4.95     | Gallotannin        | 3.15       | 483.08   |
| 20  | 2,6-Di-O-galloyl-β-D-glucose           | C_{20}H_{20}O_{14}| 5.16     | Gallotannin        | 0.50       | 483.08   |
| 21  | 6'-O-Galloylhomoarbutin                 | C_{16}H_{16}O_{8} | 5.19     | Galloglucoside     | 0.52       | 483.08   |
| 22  | Darendoside A                           | C_{21}H_{22}O_{11}| 5.48     | Phenylen alcohol glucosides | 0.39       | 431.16   |
| 23  | Aspidinol                               | C_{12}H_{16}O_{4} | 5.76     | Simple phenols     | 0.78       | 269.10   |
| 24  | Feroxin A                               | C_{12}H_{16}O_{4} | 5.76     | 3-O Glucoside      | 8.44       | 401.14   |
| 25  | Feroxin A                               | C_{12}H_{16}O_{4} | 5.76     | 3-O Glucoside      | 0.44       | 401.14   |
| 26  | Moracin M-3'-O-β-D-glucopyranoside     | C_{12}H_{16}O_{4} | 5.82     | Glucoside          | 0.77       | 449.11   |
| 27  | Meliadanoside B                        | C_{16}H_{16}O_{4} | 5.87     | Glucoside          | 1.86       | 373.11   |
| 28  | 1,3,6-Trigalloyl-β-D-glucose           | C_{20}H_{20}O_{14}| 6.38     | Gallotannin        | 2.99       | 635.09   |
| 29  | Feroxinid                              | C_{16}H_{16}O_{4} | 6.99     | Simple phenol      | 1.98       | 329.14   |
| 30  | Thannilignan                           | C_{16}H_{16}O_{4} | 8.54     | Lignan             | 0.47       |          |
| 31  | 3,7-Dihydroxy-2,4-dimethoxyphenanthren| C_{16}H_{20}O_{14} | 8.54     | Phenanthrene glucoside | 0.62       | 477.14   |
| 32  | Thannilignan                           | C_{16}H_{16}O_{4} | 8.68     | Lignan             | 0.38       | 329.14   |
| 33  | Kurzubutenolide A                      | C_{20}H_{16}O_{7} | 8.82     | Glucoside          | 1.88       | 459.13   |
| 34  | Smilaxin                               | C_{16}H_{16}O_{4} | 9.09     | Steroid glucoside  | 0.50       | 315.09   |
| 35  | Polydatin                              | C_{16}H_{16}O_{4} | 9.09     | Glucoside          | 0.52       | 435.13   |
| 36  | Renifolin                              | C_{16}H_{16}O_{4} | 9.27     | Glucoside          | 0.44       | 397.15   |
| 37  | Protosappanin A                        | C_{16}H_{16}O_{4} | 9.45     | Dibenzoazocin derivative | 0.37       | 271.06   |
| 38  | 1-O-Methyl-3,5-O-dicaffeoylquinic acid | C_{12}H_{18}O_{12}| 9.67     | Phenolic acid      | 3.35       | 543.15   |
| 39  | Polydatin                              | C_{16}H_{16}O_{4} | 9.76     | Glycoside          | 0.79       | 435.13   |
| 40  | Feroxinid                              | C_{16}H_{16}O_{4} | 9.78     | Simple phenol      | 2.06       |          |
| 41  | 2,3,5,4-Tetrahydroxystilbene-2-O-β-D-glucopyranoside | C_{16}H_{20}O_{14} | 9.94     | Glucoside          | 0.37       | 405.12   |
| 42  | 1-O-Methyl-3,5-O-dicaffeoylquinic acid | C_{12}H_{18}O_{12}| 9.96     | Phenolic acid      | 0.47       | 543.15   |
| 43  | 2,4,7-Trihydroxy-9,10-dihydrophenanthrene | C_{16}H_{20}O_{14} | 10.20    | Phenanthrene phenol | 0.87       | 273.08   |
| 44  | Polydatin                              | C_{16}H_{16}O_{4} | 10.20    | Glycoside          | 0.47       | 435.13   |
### Phytochemical compounds in F2ASP using LC-MS.

| No. | Compound | Molecular Formula | RT (min) | Chemical Classes | Responses % |
|-----|----------|-------------------|----------|------------------|-------------|
| 1   | 1-Galloyl-glucose | C_{13}H_{16}O_{10} | 0.43      | Gallotannin      | 8.34        | 331.07 |
| 2   | 1-Galloyl-glucose | C_{13}H_{16}O_{10} | 0.80      | Gallotannin      | 4.44        | 331.07 |
| 3   | 1-Galloyl-glucose | C_{13}H_{16}O_{10} | 1.06      | Gallotannin      | 6.27        | 331.07 |
| 4   | 1-Galloyl-glucose | C_{13}H_{16}O_{10} | 1.33      | Gallotannin      | 20.24       | 331.07 |
| 5   | 1-Galloyl-glucose | C_{13}H_{16}O_{10} | 1.57      | Gallotannin      | 17.05       | 331.07 |
| 6   | 1-Galloyl-glucose | C_{13}H_{16}O_{10} | 2.21      | Gallotannin      | 2.41        | 331.07 |
| 7   | Sesamin   | C_{15}H_{13}O_{3} | 3.47      | Hydroquinone derivative | 7.73 | 183.03 |
| 8   | Feroxin A  | C_{14}H_{12}O_{8} | 5.56      | 3-O Glucoside    | 5.60        | 401.14 |
| 9   | Feroxin A  | C_{14}H_{12}O_{8} | 5.75      | 3-O Glucoside    | 11.84       | 401.14 |
| 10  | Sinapaldehyde | C_{14}H_{12}O_{8} | 7.03      | Lignin intermediate | 4.90 | 253.07 |
| 11  | Sinapaldehyde | C_{14}H_{12}O_{8} | 9.00      | Lignin intermediate | 3.49 | 253.07 |
| 12  | 7,2′,3′-Trihydroxy-4′-methoxy-isoflavan | C_{16}H_{15}O_{5} | 12.48      | Isoflavane | 5.37 | 287.09 |
| 13  | Kukoamine A | C_{13}H_{12}N_{4}O_{3} | 16.97 | Benzenoid | 2.32 | 529.30 |

Note. RT: Retention Time, [M+]: Molecular ion mass (m/z)
intensity compounds were feroxin A (11.84 %) and sesamol (7.73 %). Apart from that, the compound with the lowest intensity in F2ASP was kukoamine A (2.32 %), eluted at 16.97 min.

F3ASP eluted the minimum number of compounds as compared to ASP, F1ASP, and F3ASP. Only 5 peaks eluted out including some that existed in redundant as can be seen in Figure 10 while Table 7 listed all the eluted compounds. To be exact, only 3 compounds were identified in F3ASP with negative mode ionization of LC-MS. Only the phenolic groups of gallotannin and simple phenols were identified in this fraction. Again, as in F2ASP, 1-galloyl-glucose was observed with the highest intensity of 34.45 % at 4.14 min. Another highest intensity compound was polydatin (30.51 %). The compound with the lowest intensity was feroxin A, by 16.39 %.

Table 8 summarizes the phytochemical compounds related to antihypertensive activity which were present in the ASP crude extract and the three derived fractions (F1ASP, F2ASP, and F3ASP). The possible phenolic compounds that have potential in contributing to the antihypertensive effect by S. polyanthum are 1-galloyl glucose, polydatin, sesamol, brazillian, eugenol, ellagic acid, kukoamine A and cyclocurcurmin. 1-galloyl glucose or glucogallin is a compound that is present across all fractions as well as in the crude ASP extract. In fact, it becomes the major compound in F2ASP and F3ASP, whereby the concentration of this compound intensified by 30 times as compared to its original crude extract. Previously, this compound was shown to inhibit the angiotensin-converting enzyme 1 (ACE-1) activity by the formation of chelate complexes within the active site of ACE-1.\textsuperscript{47} Inhibition of this enzyme indicates huge potential in reducing blood pressure and this is actually the mechanism of action of captopril, the positive control drug used in this study. Polydatin is a major compound found in F3ASP, while it is also present in smaller amounts in ASP and F1ASP. Polydatin, a glucoside of resveratrol can upregulate the level of nitric oxide (NO) and it also decreases the levels of endothelin (ET) and angiotensin II and thus depresses blood pressure in pressure-overload rats.\textsuperscript{48} Sesamol which was found in ASP crude extract, F1ASP, and F2ASP was found to exhibit an antihypertensive effect in uninephrectomized deoxycorticosterone acetate (DOCA)-salt-induced hypertensive rats at a specific dosage of 50 mg/kg.\textsuperscript{49} Other than sesamol, brazillian which was found only in ASP crude extract was previously reported to induce vasorelaxation in rat aortic rings through both endothelium-dependent and independent pathway\textsuperscript{50} by activating calcium dependent nitric oxide synthesis.\textsuperscript{51} Vasorelaxation is one of the main mechanisms of actions that may result in an antihypertensive effect.

Not only these, eugenol which was also found in ASP crude extract was previously reported to relax mesenteric arteries, and thus reducing systemic blood pressure by activating endothelial cell TRPV4 channels.\textsuperscript{52} It was also reported to have significant inhibition on ACE activity by 28 % in the serum of untreated diabetic rats.\textsuperscript{53} Ellagic acid, another phenolic acid compound found in ASP crude extract was

Table 7: Phytochemical compounds in F3ASP using LC-MS.

| No. | Compound          | Molecular Formula | RT (min) | Chemical Classes | Response % | [M+]     |
|-----|-------------------|-------------------|----------|------------------|------------|----------|
| 1   | 1-Galloyl-glucose | C\textsubscript{10}H\textsubscript{16}O\textsubscript{10} | 0.43     | Gallotannin      | 9.37       | 331.07   |
| 2   | 1-Galloyl-glucose | C\textsubscript{10}H\textsubscript{16}O\textsubscript{10} | 0.80     | Gallotannin      | 9.28       | 331.07   |
| 3   | 1-Galloyl-glucose | C\textsubscript{10}H\textsubscript{16}O\textsubscript{10} | 1.43     | Gallotannin      | 34.45      | 331.07   |
| 4   | Polydatin         | C\textsubscript{20}H\textsubscript{22}O\textsubscript{8} | 4.14     | Glucoside        | 30.51      | 435.13   |
| 5   | Feroxin A         | C\textsubscript{20}H\textsubscript{22}O\textsubscript{8} | 5.74     | 3-O Glucoside    | 16.39      | 401.14   |

Note. RT: Retention Time, [M\textsuperscript{+}]: Molecular ion mass (m/z)
Table 8. Bioactive phenolic compounds in the crude aqueous extract of *S. polyanthum* leaves and its derived fractions with previous reported activities related to antihypertensive effect.

| Compound          | Chemical Structure | Highest intensity (%) | ASP   | F1ASP  | F2ASP  | F3ASP  |
|-------------------|--------------------|------------------------|-------|--------|--------|--------|
| 1-galloyl-glucose |                    | 0.67 %                 | 6.90 %| 20.24 %| 34.35 %| (major compound) |
| Polydatin         |                    | 0.29 %                 | 2.57 %| -      | -      | 30.51 %| (major compound) |
| Sesamol           |                    | 0.24 %                 | 2.75 %| 7.73 % | -      |
| Brazilin          |                    | 1.19 %                 | -     | -      | -      |
| Eugenol           |                    | 0.17 %                 | -     | -      | -      |
| Ellagic acid      |                    | 0.08 %                 | -     | -      | -      |
| Kukoamine A       |                    | -                      | -     | -      | 2.32 % |
| Cyclo-curcumin    |                    | 0.10 %                 | 0.38 %| -      | -      |
able to attenuate β-nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit p47phox expression which is responsible for increased vascular oxygen radical, and this can eventually prevent any oxidative stress and reinstate nitric oxide bioavailability.34 Nitric oxide is an important endothelium-derived relaxing factor that might cause vasorelaxation, reducing the total peripheral resistance, and this might have contributed to the antihypertensive effect. Furthermore, kukoamine A which was found only in F2ASP was shown to induce hypotension in rats at a dose of 5 mg/kg when administered intravenously.35 Other than that, cyclocurcumin that was found in ASP crude extract and F1ASP in the current study, were previously shown to significantly inhibit the contraction of the vascular muscle of isolated rat aorta ring.36

CONCLUSION

This study found 1-galloyl glucose as the major compound with several other phenolic compounds such as polydatin, sesamol, brazilin, eugenol, ellagic acid, kukoamine A, and cyclocurcumin in the active antihypertensive crude extract and fractions of Syzygium polyanthum leaves. These phenolic compounds have proven biological activities related to the antihypertensive effect, thus they may be in part, responsible for the antihypertensive effect by Syzygium polyanthum leaves and thus further isolation is recommended.

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