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To cite this article: Oluwaseun Ruth Alara, Nour Hamid Abdurahman, Chinonso Ishmael Ukaegbu & Nassereldeen Ahmed Kabbashi (2019) Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques, Journal of Taibah University for Science, 13:1, 414-422, DOI: [10.1080/16583655.2019.1582460](https://doi.org/10.1080/16583655.2019.1582460)

To link to this article: <https://doi.org/10.1080/16583655.2019.1582460>



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Published online: 22 Feb 2019.



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Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques

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ABSTRACT

The bioactive compounds in *Vernonia amygdalina* leaf were extracted using ethanol through Soxhlet and microwave-assisted extraction (MAE) techniques. The phytochemical analysis was carried out on the ethanolic extracts using the standard techniques. This analysis revealed the presence of flavonoids, alkaloids, steroids, terpenoids, glycosides, tannins, phenols, saponins, and the absence of anthraquinones. Furthermore, gas chromatography-mass spectroscopy (GC-MS) analysis results revealed the presence of more phytochemicals in the extract obtained through MAE compared to Soxhlet extraction technique, whereby phytol was present in a higher percentage. Fourier transform infrared spectroscopy analysis confirmed the presence of phytochemicals in the extracts. Moreover, the IC₅₀ values of the extracts antioxidant activities were evaluated using DPPH and ABTS assays. There exists a clear correlation between total phenolic content, total flavonoid content and antioxidant activity of the extracts. Therefore, this study suggested that higher phenolic compounds responsible for natural antioxidant could be obtained from *V. amygdalina* leaf using the microwave-assisted extraction technique.

ARTICLE HISTORY

Received 2 September 2017
Received 10 December 2018
Accepted 9 February 2019

KEYWORDS

Vernonia amygdalina leaf; extraction; phytochemicals; scanning electron microscopy; gas chromatography-mass spectroscopy

1. Introduction

The world is enriched with varieties of medicinal plants which have drawn the attention of researchers due to their myriad benefits to mankind, most especially their usage in pharmaceutical and food industries. Their medicinal and pharmacological properties were due to the presence of bioactive components that produce definite physiological action in the human body [1]. Over 80% of the world's population had been reported using herbal products as alternative medicine, food supplements and nutraceutical products [2]. Medicinal plants are associated with the presence of polyphenols, polysaccharides and hydrolyzable tannins [3]. The extracts from the plants not only contain primary metabolites and minerals but also a diverse array of secondary metabolites which possess antioxidant property [1].

Vernonia amygdalina is one of the promising medicinal plants that can be found in tropical Africa and Asia. It is commonly known to be "bitter leaf" in English due to its bitter taste. It was named after an English Botanist William Vernon [4]. It is a soft-wooded shrub that can regenerate rapidly, the shrub

typically grows to a height of 2–5 m. The elliptical leaves are green in colour, about 20 cm long, and 6 mm in diameter [5]. *V. amygdalina* leaves are rich in fats, proteins, fibres, minerals, amino acids, carbohydrate, and vitamins [6,7]. Moreover, the extracts from *V. amygdalina* leaves possess several secondary metabolites which include flavonoids, polyphenols, saponins, tannins, and terpenoids [7]. These phytochemicals might be responsible for its anti-diabetes, antimicrobial, anti-allergic, antibacterial, antimalarial, anti-fungi, anticancer, antileukemia, anti-inflammatory, analgesic, antipyretic, anti-oxidative, antihelminthic hepatoprotective, and hypolipidaemic physiological effects [8,9]. Thus, it is used in the formulation of traditional medicine for several ailments.

Over the years, different extraction methods which include Soxhlet, hydrodistillation, decoction, maceration, microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, and pressurized solvent extraction have been employed to isolate bioactive compounds from plant matrix [10,11]. Amongst these methods, microwave-assisted extraction (MAE) has gained significant research attention due

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to its shortened extraction time and reduced solvent consumption compared to conventional solvent liquid extraction [12,13]. Therefore, the aim of this study is to extract and characterize the bioactive compounds from ethanolic extracts from *V. amygdalina* leaf comparing Soxhlet and MAE techniques.

2. Materials and methods

2.1. Plant sample preparation

The fresh leaves of *V. amygdalina* were procured from a garden in Gambang (along Pahang capital), Malaysia. The fresh leaves were washed and shade-dried until constant weight was attained. The moisture content was recorded as 0.012 ± 0.15 g water/g dry sample. The sample was blended using a (Panasonic Blender PANA-MX-801S HG, Malaysia), sieved through standard 105 mm mesh size and stored in an airtight waterproof polyethylene bag until further use.

2.2. Chemical procurement

The ethanol, methanol, ferric chloride (FeCl_3), hydrochloric acid, Dragendorff's reagent, Mayer reagent, lead acetate, chloroform, sulphuric acid, ammonia, sodium nitroprusside, sodium hydroxide, acetate, sodium carbonate (Na_2CO_3), potassium persulfate salt, 2,2-diphenyl-picrylhydrazyl (DPPH), 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($\text{ABTS}^{+\bullet}$), gallic acid, quercetin, aluminium chloride salt, and Folin-Ciocalteu phenol reagent were obtained from Sigma-Aldrich (M) Sdn Bhd, Selangor, Malaysia. All the chemical used were of analytical grade.

2.3. Extraction processes

The extraction of *V. amygdalina* leaves was carried out using two different extraction methods which include Soxhlet and microwave-assisted extraction techniques. An aqueous ethanol solution (60% v/v) was used as the extracting solvent.

2.3.1. Soxhlet extraction

One hundred grams (100 g) of powdered *V. amygdalina* leaves were placed in a Soxhlet apparatus. The extraction was carried out using 1000 mL of 60% v/v ethanol concentration for 4 h. After the extraction process, the suspension was filtered and concentrated to dryness using a rotary evaporator (Buchi Rotavapor R-200 coupled with Buchi Vac V-500 pump, Switzerland). The extractions were performed in triplicate and the results were expressed as mean \pm SD.

2.3.2. Microwave-assisted extraction (MAE)

The MAE was conducted by adding a 10 g of powdered *V. amygdalina* leaf to 100 mL of ethanol in a

250 mL round bottom flask. The mixture was irradiated for 2 min at 600 W and 78°C in an enclosed ethos reflux microwave extractor (1000 W, Frequency 2450 MHz, Milestone, Italy) using 3-level of heating: 2 min of preheating to boiling temperature of the solvent, irradiation for 5 min at solvent boiling point, and 2 min of cooling to 30°C. The mixture was filtered and concentrated to dryness using a rotary evaporator (Buchi Rotavapor R-200 coupled with Buchi Vac V-500 pump, Switzerland). The extractions were performed in triplicate and the results were expressed as mean \pm SD.

2.4. Preliminary phytochemical analysis

2.4.1. Flavonoid test

The presence of flavonoids in the extract was determined following the procedure described by Bargah [14]. Formation of a yellow precipitate was taken as an indication for the presence of flavonoids.

2.4.2. Terpenoids test

Terpenoids were tested following the procedure described by Bargah [14]. Formation of a greyish colour indicates the presence of terpenoids.

2.4.3. Alkaloids test

This test was carried out followed the procedure described by Bargah [14].

2.4.4. Tannins test

This test was carried out followed the procedure described by Bargah [14].

2.4.5. Saponin glycoside test

A 10 mL of distilled water was shaken vigorously for 30 s with 10 mL of the extract in a tube. The tube in a vertical position was observed for 30 min. The formation of honeycomb froth persisted for 10–15 min indicated the presence of saponins.

2.4.6. Glycosides test

Glycoside test was carried out followed the method explained by Bargah [14].

2.4.7. Test for free anthraquinones derivatives

A 0.5 mg of the extract was shaken with 5 mL chloroform for about 5 min. The mixture was filtered and an equal volume of 100% ammonia solution was added. A pink violet or red colour in the ammoniacal layer (lower layer) indicates the presence of free anthraquinones (Bontrager's test).

2.4.8. Steroids screening

A 50 mg of *V. amygdalina* leaf extract was dissolved in 1 mL of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface showed the presence of steroidal ring [14].

2.4.9. Phenols test

Phenol screening was carried out using the procedure described by Bargah [14].

2.5. Total phenolic content determination

The total phenol content in the extract was evaluated using the procedure used by Saravanan and Parime-lazhagan [15] and Dahmoune et al. [17]. The standard calibration curve was prepared, using a standard solution of gallic acid (50-500 mg/mL). TPC was expressed as mg gallic acid equivalents per gram of dried weight sample (mg GAE/g d.w.). The TPC in the extracts were calculated using Equation (1). Ethanol was used as the blank. The analyses were done in triplicate.

$$\text{TPC} = \frac{c \times V}{m} \quad (1)$$

where, c is the sample concentration from the calibration curve (mg/mL), V is the volume (mL) of the solvent used in the extraction, and m represents the weight (g) of the dried sample used.

2.6. Total flavonoid content determination

The total flavonoid content in the *V. amygdalina* leaf extract was determined following the method used by Oriakhi et al. [18]. Quercetin (50-500 mg/mL) was used to prepare a standard calibration curve ($y = 0.112x + 0.178$, $R^2 = 0.9945$), where y is the absorbance at 420 nm and x is the sample concentration in mg/mL. TFC was expressed as mg quercetin equivalents per gram of dried weight sample (mg QE/g d.w.). The TFC of the extracts were calculated using Eq. (2). Ethanol was used as the blank. The analyses were carried out in triplicate.

$$\text{TFC} = \frac{c \times V}{m} \quad (2)$$

where c is the sample concentration in from the calibration curve (mg/mL), V is the volume (mL) of the solvent used in the extraction, and m represents the weight (g) of the dried sample used.

2.7. Antioxidant activity

2.7.1. DPPH assay

The DPPH assay was carried out using the procedure used in the previous study [19]. Briefly, 0.1 mM solution of DPPH was prepared by dissolving 0.004 g of DPPH crystalline solid in 100 mL of analytical grade methanol and stored at 4°C. A 0.2 mL of DPPH solution was added to 2 mL of the extract. After 30 min of incubation in the dark, the absorbance at 517 nm using a UV-Vis Spectrophotometer (Hitachi U-1800, Japan) was recorded. Methanol was used as the blank. The analysis was performed thrice. The percentage of DPPH radical inhibition was determined using Eq. (3). The DPPH inhibitory

activity of *V. amygdalina* leaf extract was expressed as IC_{50} (concentration in $\mu\text{g/mL}$ of the extracts required to inhibit 50% of the DPPH radical formation) by preparing four different concentrations 100, 200, 300, and 500 $\mu\text{g/mL}$ of the extracts. The IC_{50} values were calculated from the linear regression between the percentage of inhibition and concentrations.

$$\text{DPPH inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (3)$$

where A_{control} is the mixture of methanol and DPPH solution; and A_{sample} is the mixture of sample extract and DPPH solution.

2.7.2. ABTS assay

ABTS assay was carried out using the procedure used in the previous study [19]. In concise, ABTS radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate in equal quantities, the mixture was left in dark at room temperature for 12 h until the reaction was completed and the absorbance was stabled. The fresh working solution of ABTS radical cation was prepared by diluting 2 mL $\text{ABTS}^{+\bullet}$ solution with 120 mL methanol to obtain an absorbance of 1.1 ± 0.02 at 734 nm using UV-Vis Spectrophotometer (Hitachi U-1800, Japan). A 0.15 mL of *V. amygdalina* leaves extract (at concentrations 100, 200, 400 and 500 $\mu\text{g/mL}$) was mixed with 2.85 mL of $\text{ABTS}^{+\bullet}$ solution and allowed to stand in the dark for 2 h at room temperature. Thereafter, the absorbance was determined at 734 nm using a UV-Vis Spectrophotometer (Hitachi U-1800, Japan). Methanol was used as the blank. The results were expressed in milligram gallic acid per gram dry weight (mg GAE/g d.w.). Eq. (4) was used for determining the percentage of inhibition. The $\text{ABTS}^{+\bullet}$ inhibitory activity of *V. amygdalina* leaf extract was expressed as IC_{50} (concentration in $\mu\text{g/mL}$ of the extracts required to inhibit 50% of the $\text{ABTS}^{+\bullet}$ radical formation) by preparing four different concentrations 100, 200, 300, and 500 $\mu\text{g/mL}$ of the extracts. The IC_{50} values were calculated from the linear regression between the percentage of inhibition and concentrations.

$$\% \text{ABTS}^{+\bullet} \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} * 100\% \quad (4)$$

where A_{control} is the mixture of methanol and $\text{ABTS}^{+\bullet}$ solution; and A_{sample} is the mixture of sample extract and $\text{ABTS}^{+\bullet}$ solution.

2.8. Gas chromatography-mass spectroscopy (GC-MS) analysis

The chemical components and their percentage of abundance in the extracts were evaluated using a gas chromatography-mass spectrometry analysis. Alara

et al. [19] procedure with some modification was followed. Briefly, the *V. amygdalina* leaf extracts were analysed using an Agilent GC-MS equipment (Agilent 5975C inert, USA) coupled with a mass spectrometer MS 597C and equipped with a DB-1MS capillary column (30 m tubular column length \times 0.25 mm i.d. \times 0.25 μ m film thickness) and an autosampler. The operating conditions for the analysis were as follows: initial temperature of 35°C which was raised to 95°C at the rate of 3°C/min for 10 min, then raised to 270°C at the rate 10°C/min and finally to 300°C at the rate 3°C/min which was maintained for 3 min; column flow rate of 0.8 mL/min; carrier helium at a flow rate of 1.0 mL/min; split ratio 10:1; the ionization voltage of 70 eV; run time 67.5 min; and sample injection volume was 1 μ L solution of extract (5 mg/mL). The components were identified by comparing the obtained spectra with those on the NIST 05a library database, and the percentages of abundance were determined with the total ion chromatogram. 1 mL sample was prepared by diluting the extract with analytical absolute ethanol at a ratio of 1:20 (w/v).

2.9. Fourier transform infrared spectroscopy analysis

The *V. amygdalina* leaf extracts were subjected to FTIR analysis for the determination of present functional groups. Fourier transform infrared spectrometry (Nicolet iS5 iD7 ATR; Thermo Scientific, Germany) equipped with OMNIC software was used in the analysis. The plant samples were analysed to obtain IR spectra in the scanning wave number ranging from 500–4000 cm^{-1} with a resolution of 4 cm^{-1} [21]. The spectra of the observed compounds, group frequencies and characteristics bonds from the extracts were compared with the table of expected absorption bands for the molecule's various groups and bonds.

2.10. Scanning electron microscopy and elemental analysis

The scanning electron microscopy (SEM) was used to observe the changes in the morphology of *V. amygdalina* leaves before and after Soxhlet and MAE techniques. The samples were mounted on an SEM stub and the morphologies were observed in SEM (HITACHI TM3030Plus, Japan). The analyses were examined under the high vacuum condition at an accelerated voltage of 15 keV, 100x magnification and an analytical working distance of 2 mm. Likewise, the elemental analysis of the extracts was analysed using Energy-Dispersive X-ray Spectroscopy (EDX). The detection of the elements in the *V. amygdalina* leaf extract was carried out using silicon (lithium) detector cooled with liquid nitrogen. EDX Shimadzu software package was used to determine the intensity of each element counts per second from the sample X-ray spectrum deconvolution.

2.11. Statistical analysis

All the experiments were carried out thrice and the average results were selected. The statistical analyses were performed using one-way ANOVA and the results were expressed as mean \pm SD.

3. Results and discussion

3.1. Phytochemical analysis

The pharmacological effects of *V. amygdalina* leaf may perhaps be due to the presence of several secondary metabolites such as flavonoids, terpenoids, alkaloids, tannins, saponins, glycosides, steroids, and phenols. The preliminary phytochemical analyses of the ethanolic extracts from *V. amygdalina* leaf using Soxhlet and MAE techniques are shown in Table 1.

The presences of flavonoids, terpenoids, alkaloids, tannins, saponins, glycosides, steroids, and phenols were observed in both extracts (Table 1). These secondary metabolites had been studied to have many biological properties [19,8]. Moreover, anthraquinones were absent in the *V. amygdalina* leaf extracts. The presence of flavonoids had been reported to be associated with the naturally occurring phenolic compounds that possess antioxidant properties in the human diet [20,21]. In different studies, the presence of terpenoids and steroids in *V. amygdalina* possess different biological activities which include anti-inflammatory, anti-cancer, anti-diabetes, antibacterial, analgesic, hepatoprotective, and antioxidant [8,22]. The presence of alkaloids could be well correlated with antimicrobial activities. Thus, this species is expected to have many medicinal uses.

3.2. TPC, TFC and antioxidant activities of the extracts

Phenolics and flavonoids are the plant secondary metabolites that possess several pharmacological activities which include antioxidant, anti-inflammatory, antimicrobial, anticancer, and among biological activities [23]. The TPC, TFC and antioxidant activities of *V. amygdalina* leaf using Soxhlet and MAE techniques are presented in Table 2. The highest TPC (114.03 ± 1.25 mg

Table 1. Preliminary phytochemicals screening of the *V. amygdalina* leaf extracts.

| Number | Constituents | Soxhlet | MAE |
|--------|----------------|---------|-----|
| 1 | Flavonoids | + | + |
| 2 | Terpenoids | + | + |
| 3 | Alkaloids | + | + |
| 4 | Tannins | + | + |
| 5 | Saponins | + | + |
| 6 | glycosides | + | + |
| 7 | Anthraquinones | – | – |
| 8 | Steroids | + | + |
| 9 | Phenols | + | + |

Present (+) and absent (–)

Table 2. The TFC, TPC and antioxidant activity of *V. amygdalina* leaf using Soxhlet and MAE techniques.

| Extraction technique | TPC yield (mg GAE/g d.w.) | TFC yield (mg QE/g d.w.) | IC ₅₀ for DPPH (µg/mL) | IC ₅₀ for ABTS ⁺ (µg/mL) |
|----------------------|----------------------------|---------------------------|-----------------------------------|--|
| Soxhlet | 70.33 ± 2.11 ^a | 63.82 ± 0.99 ^a | 321.37 ± 3.19 ^a | 198.87 ± 1.76 ^a |
| MAE | 114.03 ± 1.25 ^b | 96.29 ± 1.70 ^b | 286.00 ± 2.20 ^b | 162.38 ± 2.01 ^b |

Data marked with different letters along the columns are significantly different at $p < 0.05$.

Table 3. Chemical composition of *V. amygdalina* leaf using MAE and Soxhlet techniques.

| MAE | | | | Soxhlet | | | |
|---------|--|---|--------------|---------|--|--|--------------|
| RT(min) | Compound name | MF | Abundance(%) | RT(min) | Compound name | MF | Abundance(%) |
| 3.986 | 2,3-pentanedione | C ₅ H ₈ O ₂ | 3.08 | 7.617 | 2-methyl-3-hexanol | C ₇ H ₁₆ O | 6.83 |
| 4.023 | 2,3-hexanedione | C ₆ H ₁₀ O ₂ | 3.86 | 7.617 | 2,4-dimethyl-3-pentanol | C ₇ H ₁₆ O | 3.42 |
| 4.125 | 4-methyl-vinyl butyrate | CH ₃ CH ₂ CH ₂ COOCH = CH ₂ | 7.00 | 41.998 | Ethyl ester hexadecanoic acid | C ₁₈ H ₃₆ O ₂ | 5.06 |
| 4.290 | Oxalic acid | C ₂ H ₂ O ₄ | 0.77 | 43.140 | Phytol | C ₂₀ H ₄₀ O | 50.57 |
| 7.318 | 1,3-butanediol | C ₄ H ₁₀ O ₂ | 0.77 | 43.498 | Ethyl ester-9,12-octadecadienoic acid | C ₂₀ H ₃₆ O ₂ | 8.33 |
| 7.516 | 2-pentanol | C ₅ H ₁₂ O | 5.14 | 43.501 | Ethyl ester linoleic acid | C ₂₀ H ₃₄ O ₂ | 8.19 |
| 7.655 | Pentanoic acid | C ₅ H ₁₀ O ₂ | 1.03 | 43.523 | (z,z,z)-methyl ester-9,12,15-octadecatrienoic acid | C ₁₉ H ₃₂ O ₂ | 17.60 |
| 7.933 | 2-methyl-3-hexanol | C ₇ H ₁₆ O | 5.66 | | | | |
| 14.640 | 2-methyl azetidine | C ₄ H ₉ N | 1.03 | | | | |
| 15.169 | 2-oxoacetic acid | C ₂ H ₂ O ₃ | 0.26 | | | | |
| 40.602 | 1,9-nonanediol | C ₉ H ₂₀ O ₂ | 0.51 | | | | |
| 40.602 | 2-tridecen-1-ol | C ₁₃ H ₂₆ O | 0.51 | | | | |
| 41.030 | 1-tridecyne | C ₁₄ H ₂₈ | 0.26 | | | | |
| 41.030 | 8-hexadecyne | C ₁₅ H ₂₆ O | 0.26 | | | | |
| 42.009 | Ethyl ester hexadecanoic acid | C ₁₈ H ₃₆ O ₂ | 3.86 | | | | |
| 43.142 | Phytol | C ₂₀ H ₄₀ O | 51.10 | | | | |
| 43.304 | 4,8,12,16-octadetetraen-1-ol | C ₂₁ H ₄₀ O ₂ | 0.26 | | | | |
| 43.501 | Ethyl ester-9,12-octadecadienoic acid | C ₂₀ H ₃₆ O ₂ | 1.54 | | | | |
| 43.521 | Ethyl ester linoleic acid | C ₂₀ H ₃₄ O ₂ | 1.54 | | | | |
| 43.533 | (z,z,z)-methyl ester-9,12,15-octadecatrienoic acid | C ₁₉ H ₃₂ O ₂ | 6.94 | | | | |
| 48.850 | Squalene | C ₃₀ H ₅₀ | 0.77 | | | | |
| 48.869 | 2,6,10,14,18,22-tetracohexaene | C ₃₀ H ₆₂ | 1.54 | | | | |
| 51.610 | Vitamin E | C ₂₉ H ₅₀ O ₂ | 2.31 | | | | |

GAE/g d.w.) and TFC (96.29 ± 1.70 mg QE/g d.w.) were obtained for extracts from MAE. This might be due to the shorter extraction time with a higher yield in the MAE technique [24]. Furthermore, there exists a clear correlation between TPC, TFC and antioxidant activity of the extracts whereby a higher phenolic and flavonoid contents resulted in stronger antioxidant activity. The IC₅₀ values of DPPH and ABTS inhibitions were higher in Soxhlet than MAE, implying a lesser antioxidant activity of the extracts from Soxhlet extraction techniques. This implies that the method of extracting phenolic compounds from the plant matrix plays an important role in determining the antioxidant activity of the extracts.

3.3. Components identification from the extracts of *V. amygdalina* leaf

The identified chemical components from GC-MS chromatogram analysis of the ethanolic extract of

V. amygdalina leaf using Soxhlet and MAE techniques are shown in Table 3. A total number of 23 and 9 chemical components belonging to hydrocarbons, alcohols, esters, fatty acids, and ketones were identified from *V. amygdalina* leaf extracts through MAE and Soxhlet extraction, respectively. For MAE, the most prevailing compounds were phytol, a terpenoids compound (56.30%), 4-methyl-vinyl butyrate (7%), and (z,z,z)-methyl ester-9,12,15-octadecatrienoic acid (6.94%) which belongs to unsaturated fatty acids. Similarly, the most abundant chemical compounds in extract using the Soxhlet technique were phytol (50.57%) and (z,z,z)-methyl ester-9,12,15-octadecatrienoic acid (17.60%). Phytol was found to be a strong antioxidant, possessing anti-microbial, anti-inflammatory, and anti-proliferative activity. Phytol, a precursor of synthetic vitamin E and vitamin K, was found to be cytotoxic against breast cancer cell lines MCF7 [25]. This chemical component had previously been reported as components of

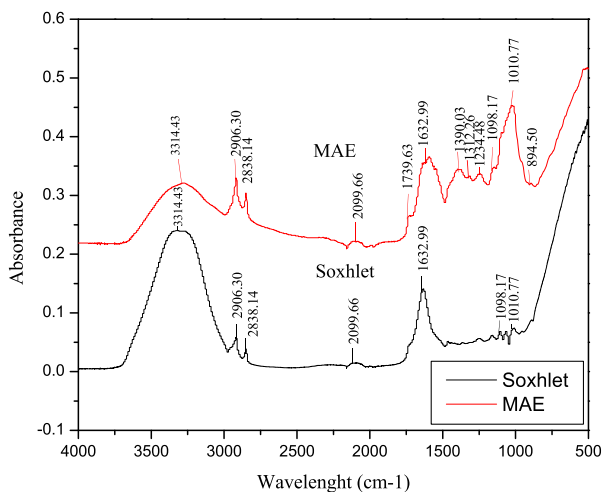


Figure 1. FTIR spectra of ethanolic extracts from *V. amygdalina* leaf using Soxhlet and MAE.

V. amygdalina leaf but interesting, the identified phyto from this study was in abundance compared to the previous studies [17,19,24]. This may be attributed to the geographical location where the plant samples were harvested and the extraction methods. Moreover, the presence of higher percentages of terpenoids and unsaturated fatty acids showed the anti-inflammatory and antioxidant potential of *V. amygdalina* leaf [17,19].

In addition to antimicrobial activity, the squalene was also reported to have anticancer, antioxidant, chemopreventive, pesticide, anti-tumor, and sunscreen properties. The coumarin compound (8-methyloctahydrocoumarin) had antioxidant and anti-inflammatory properties [17,19]. The presence of aromatics and fatty acids in the ethanolic extracts revealed the pharmacological properties of *V. amygdalina* leaf. Thus, this study has revealed the presence of major bioactive

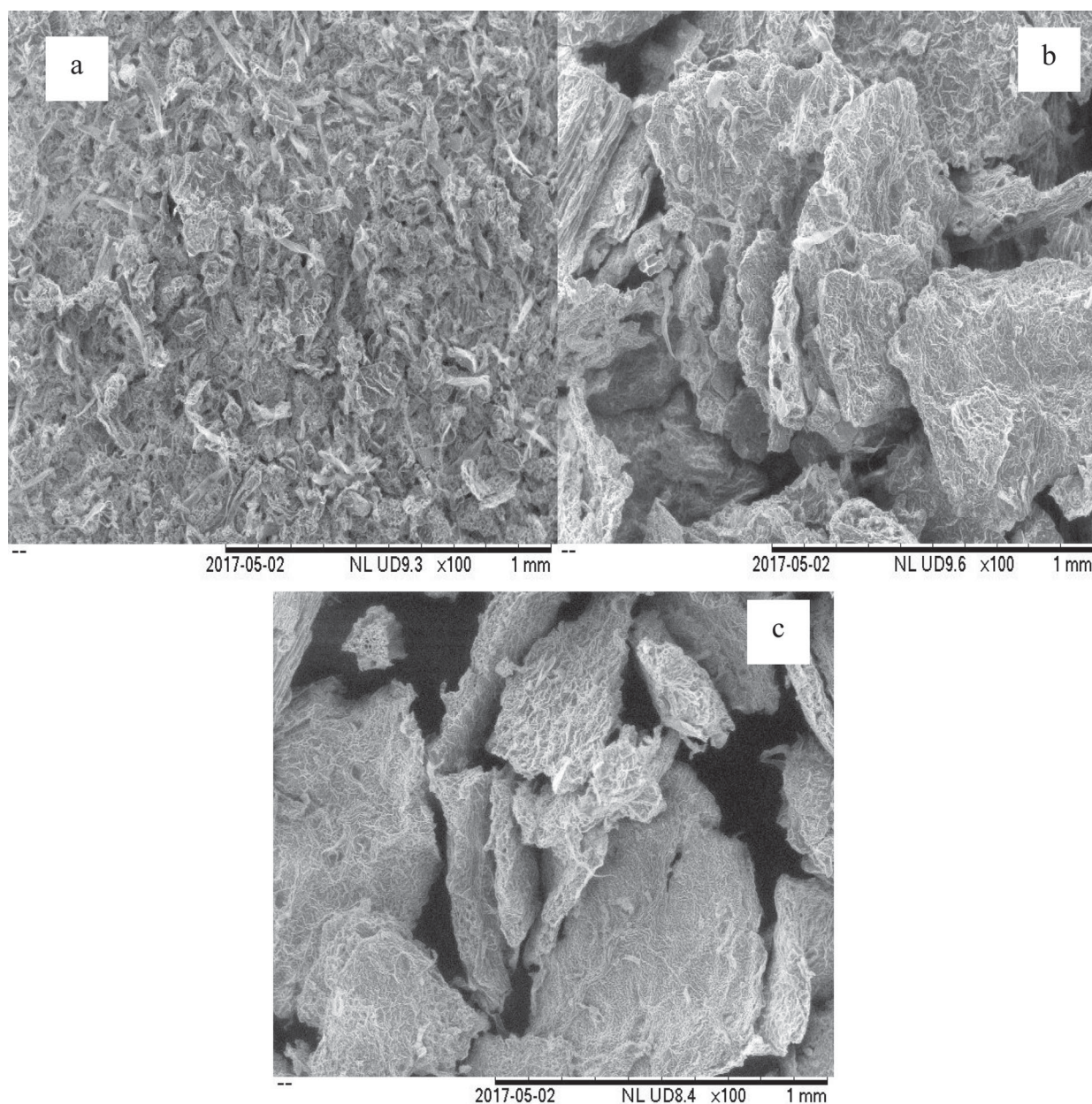


Figure 2. SEM analysis of *V. amygdalina* leaf before extraction (a); after MAE (b); after Soxhlet extraction (c).

compounds that can serve as a basis for determining the possible health benefits of this plant leading to further biological and pharmacological studies.

3.4. Structural analysis of *V. amygdalina* leaf extracts

Figure 1 shows the characteristic fingerprinting of ethanolic extracts from *V. amygdalina* leaf using Soxhlet and MAE techniques. In general, each band in the spectra corresponds to a functional group responsible for IR absorption [26]. There are 7 and 12 peaks identified from the extracts using Soxhlet and MAE, respectively. The bands between the wavelengths of $1800\text{--}750\text{ cm}^{-1}$ showed the biochemical composition, especially the compounds associated with lipid, carbohydrate, protein secondary structures, lipid, and phenolic compounds in the plant extract. The band at 894.50 cm^{-1} in MAE might be caused by ring C–H deformation which could reflect structural information on phenolic compounds [27]. Moreover, the stretching peak at 1010.17 cm^{-1} is associated with the presence of an O–C–C stretch of esters. A group of bands appearing in the wavelength region of $1300\text{--}1000\text{ cm}^{-1}$ is due to C–O stretching vibration of esters, free fatty acids of alcohols. The presence of a minor peak at 1739 cm^{-1} revealed the presence of C = O stretching vibration in carbonyl groups that characterized abundance of the terpenoids and the stretched C–H absorption band at 2838.14 and 2906.30 cm^{-1} show the presence of methylene compounds. The distinguished peak at 1312.26 cm^{-1} is due to the plane C–O stretching vibration combined with the ring stretch of phenyl [28]. The presence of higher wavelength (lower frequency) at peak of 3314.43 cm^{-1} is due to N–H stretching of proteins and O–H stretching of carbohydrate and water. In addition, the peak at 2906.30 cm^{-1} is due to the CH_2 anti-symmetric stretch of methyl groups mainly from lipids [28]. Thus, it can be deduced that MAE has higher potential comparing to Soxhlet in the extraction of bioactive compounds from *V. amygdalina* leaf.

3.5. Morphological and elemental analyses of *V. amygdalina* leaf before and after extraction

The morphological analyses of the powdered sample of *V. amygdalina* leaf before extraction and the residues after MAE and Soxhlet extraction are shown in Figure 2. Before extraction, the cell wall of the plant was firmly packed (Figure 2(a)). In contrast, the fractural changes in the physical structure of the plant sample were obvious after the extraction whereby the physical morphology of the *V. amygdalina* leaf after MAE was destroyed, showing that the microwave irradiation played an important role in breaking the vegetal cell wall (Figure 2(b)). Therefore, it can be deduced that the molecular movement and rotation of liquids associated with a permanent dipole resulted in rapid heating of the extracting solvent [16]. Moreover, the physical structure of the sample after Soxhlet extraction was severely damaged (Figure 2(c)). This might be due to the effect caused by localized heating from the long period of extraction [10].

The results of the elemental analysis of *V. amygdalina* leaf of the extracts using MAE and Soxhlet are shown in Figure 3 and Table 4. Carbon, oxygen, aluminium, silicon, phosphorus, chlorine, potassium, calcium, sulphur, and iron are present in the extracts (Figure 3). The presence of these elements in the plant extracts made them responsible for curing several diseases [29]. Amongst all these element, carbon and oxygen were present in a higher amount while silicon and potassium in moderate concentrations. On the contrary, aluminium, phosphorus, calcium, chlorine, iron, and sulphur were present in trace quantities. It was observed that toxic elements such as Hg, Cd and Sb were not detected in the extracts. Iron was not detected in the extract from Soxhlet. Moreover, the concentration of carbon in the Soxhlet extraction was higher than that of MAE, this might be because of localized heating generated from Soxhlet extraction that may lead to the degradation of the plant sample and produced more carbon residue. Other elements detected in MAE extract were higher than extract from

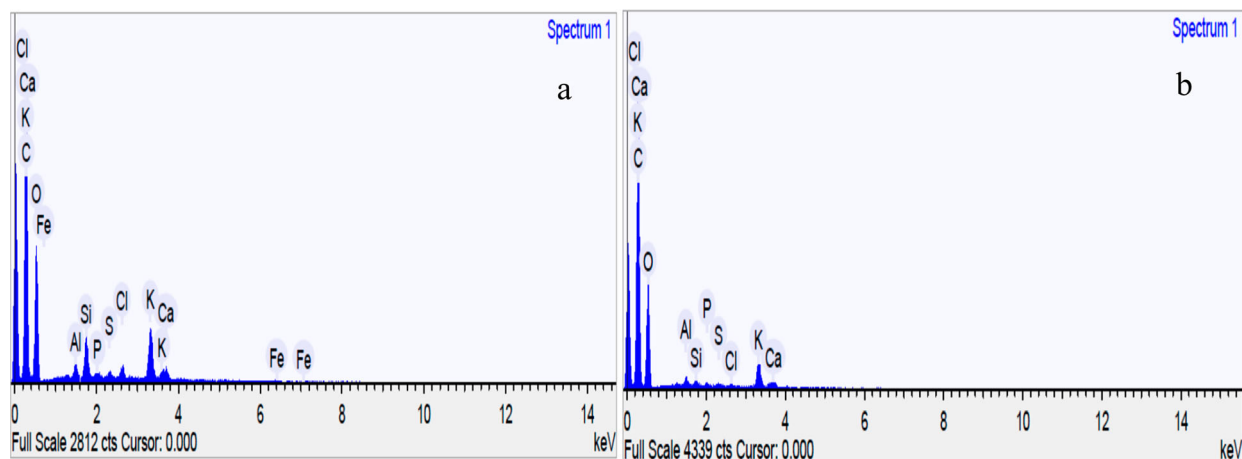


Figure 3. EDS spectra of ethanolic extract from *V. amygdalina* leaf using MAE (a) and Soxhlet extraction (b).

Table 4. Nutrient composition (%) in *V. amygdalina* leaf after MAE and Soxhlet extraction.

| Element | MAE | | | Soxhlet | | |
|---------|------------|---------------------|------------|------------|---------------------|------------|
| | Weight (%) | Weight (%) σ | Atomic (%) | Weight (%) | Weight (%) σ | Atomic (%) |
| C | 57.123 | 0.775 | 66.232 | 65.372 | 0.660 | 68.539 |
| O | 35.521 | 0.709 | 30.799 | 30.479 | 0.634 | 30.006 |
| Al | 0.459 | 0.040 | 0.237 | 0.376 | 0.034 | 0.189 |
| Si | 1.500 | 0.061 | 0.744 | 0.236 | 0.032 | 0.114 |
| P | 0.240 | 0.045 | 0.108 | 0.189 | 0.035 | 0.083 |
| S | 0.252 | 0.042 | 0.109 | 0.155 | 0.034 | 0.066 |
| Cl | 0.523 | 0.053 | 0.261 | 0.470 | 0.036 | 0.065 |
| K | 3.289 | 0.103 | 1.171 | 2.211 | 0.075 | 0.766 |
| Ca | 0.680 | 0.065 | 0.236 | 0.512 | 0.052 | 0.173 |
| Fe | 0.413 | 0.109 | 0.103 | – | – | – |

Soxhlet. Therefore, it can be deduced that the MAE technique possesses a higher capacity to extract more nutrients compared to Soxhlet extraction technique.

The calcium and potassium found in *V. amygdalina* leaf extracts showed it potent in muscle contraction and synthesis of some protein. In addition, calcium helps in teeth and bone development, blood clotting, regulate heart rhythm, and lower blood pressure [30]. Furthermore, chlorine acts as an electrical charge when dissolving body fluids and the regulation of body pH. Thus, *V. amygdalina* leaf can be used as pharmaceutical and therapeutic agents.

4. Conclusions

This study clearly revealed that phytochemical and elemental composition of *V. amygdalina* leaf varied with the method of extraction whereby extract through MAE contained more extractable metabolites compared to Soxhlet extraction technique. Moreover, both methods of extraction showed a considerable antioxidant activity with a varying amount due to differences in their phytochemical compositions from the GC-MS analysis. Thus, this study suggests the use of MAE in extracting higher yields of bioactive compounds from *V. amygdalina* leaf which may possibly be utilized as a therapeutical source for managing several diseases.

Acknowledgement

We acknowledge the support of Universiti Malaysia Pahang, Malaysia through the grant number RDU180329.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

We acknowledge the support of Universiti Malaysia Pahang, Malaysia through the [grant number RDU180329].

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