Malays. Appl. Biol. (2019) 48(5): 11–17

PHYTOCHEMICAL COMPOSITION IN HEXANE AND METHANOLIC LEAF EXTRACT OF *Vernonia amygdalina*

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Accepted 28 August 2019, Published online 31 December 2019

ABSTRACT

Vernonia amygdalina or known as Pokok Bismillah in Malaysia is commonly used for medicinal purposes due to their pharmacological effects. The present study aimed to identify the chemical composition by GC-MS analysis of hexane and methanolic crude extract of *V. amygdalina*. Thirty-two and twenty-two components were identified from their fragmentation pattern in hexane and methanolic extract, respectively. Major components in hexane extract dominated by tetrapentacontane (21.56%), nonacosane (13.06%), squalene (12.77%), phytol (8.73%), δ-7,25-stigmastadienol (7.20%) and α-tocopherol (5.18%). Meanwhile, methanolic extract were linolenic acid (21.94%), hexadecanoic acid (15.51%), 2,3-hexanedione (12.40%), linoleic acid (10.45%), phytol (8.81%) and decanamide (5.92%). These compounds could be useful for biomedical activities and as a therapeutic agent.

Key words: *Vernonia amygdalina*, phytochemicals, GCMS, linolenic acid, tetrapentacontane

INTRODUCTION

Under stringent legislation and surveillance, herbal remedies have been adopted in several developed countries as essential and alternative medicine (Enioutina *et al*., 2017). Natural products, including plants contain higher chemical novelty which inspired today's modern medicine and contributes greater commercial drug. Approximately 25% of established drugs are derived from the plants worldwide (Wachtel-Galor & Benzie, 2011). Plants also provide a valuable source of secondary metabolites such as phenolics, quinones, flavonoids, saponins, tannins, coumarins, terpenoids, alkaloids, lectins and polypeptides (Pandey & Kumar, 2013). Thus, phytochemistry scientists are now focusing on plant-based bioactive compound for the pharmaceutical sector (Katz & Baltz, 2016). It is trending research to explore and develop new drugs by utilising natural products as fundamental.

Vernonia amygdalina is one of the herbs that come from the Asteraceae family and also known as African Bitter leaf or Bismillah Leaf in Africa and Asia (Alara *et al*., 2017). It is a woody plant that could reach 6 meters in height (Nwosu *et al*., 2013). This plant is efficiently propagated by stem cutting and could be found along the drainage as well as in the forest. Various plant parts of this plant are used as medicine for antioxidant, antiplasmodial, antidiabetic, anticancer, anti-inflammatory, anti-microbial, and hepatoprotective (Kadiri & Olawoye, 2016). There are a few extraction methods to be used in preparation of ethnomedicine such as infusion, decoction, boiling, maceration, squeezing or taking orally in powder form (Oyeyemi *et al*., 2018). Among the plant parts, researchers found that the leaf part has the highest bioactive compounds and nutritional compositions (Toyang & Verpoorte, 2013). However, a different geographical area would affect their chemical constituents and quantities.

Previous studies have reported that the chemical constituents of *V. amygdalina* leaves potentially significant as alternative medicine and a therapeutic agent. It has been proved by the presence of cardiac glycosides, alkaloids, saponins, and tannins that associated with antimicrobial activities (Yar'adua *et*

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al., 2015). The bitter taste of this plant is associated with the presence of these compounds. Other constituents are also found in this plant extract for instances vernoamyoside, 3'deoxyvernodalol and vernolide that responsible for anti-inflammatory (Quasi *et al*., Sinisi *et al*., 2016). Further purification of compounds from *V. amygdalina* extract has also led to the discovery of other potential active compounds including flavonoids, terpenes, phenolics, triterpenoids, steroidal glycosides and several types of sesquiterpene lactones (Alara *et al*., 2017). In previous research, most of the compounds are abundant in polar solvent extraction such as methanol and ethanol.

The chemical fingerprint is one of the reliable methods to analyse the chemical constituents in herbal medicine. Referring to the World Health Organization (WHO), gas chromatography consisting of the chromatographic fingerprint is one of the acceptable analytical instrumental methods to detect and quantify the substance markers (Yongyu *et al*., 2011). The findings from the previous literature reported that most researches relating the phytochemical compounds in *V. amygdalina* leaves were extracted from the polar solvent. However, the report on phytochemical compounds of *V. amygdalina* leaves extract using non polar solvent is still scanty. For this reason, this study aimed to identify phytochemical constituents of *V. amygdalina* extracted from both polarities (hexane and methanol solvents) using GCMS.

MATERIALS AND METHODS

Sample collection and preparation

Fresh plants of *V. amygdalina* were collected in Puchong, Selangor. The whole plant was sent to Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia for verification. The specimen voucher was given as SK 3280/18. The leaves were rinsed with the running tap water to remove the dirt and sun-dried for two days and subsequently oven-dried at 40°C until obtained a constant weight. The dried leaves were blended into powder using a high-speed blender (Model HOF-500g-S1, Malaysia) in two minutes and stored in air-tight container for the required extraction.

Preparation of methanolic and hexane crude extracts

Five hundred grams powdered leaves were macerated for 48 hr in each 5 L organic solvents consists of hexane and methanol by sequential extraction according to the protocol adopted by Haron *et al*. (2013). Then, the extracts were filtered, and the flow-through was concentrated in a rotary evaporator (Buchi Rotary Evaporator R100, coupled to Buchi Vacuum pump V100 and Buchi Water Bath B100, Switzerland) until sticky dark green crude was formed.

Gas Chromatography-Mass Spectrometry (GCMS) analysis

Each crude sample of hexane and methanol was diluted in 20,000 ppm of hexane and methanol solvent, respectively, and analyzed using GC-MS QP2010 Ultra (Shimadzu, Japan) comprising gas chromatograph interfaced with a mass spectrometer. Compound partitioning was conducted using a Rxi-5MS fused silica capillary column (30 m length × 0.25 mm internal diameter × and 0.25 mm in thickness). The analysis was started by injecting 1 µL of the sample. The carrier gas, helium, was used at a flow rate of 1 mL/min. The analysis was conducted in the EI (electron impact) mode with 70 eV of ionization energy. The injector temperature was maintained at 250°C. The column oven temperature was set at 50°C (held for 3 min), raised at 10°C/min to 280°C (held for 3 min), and finally held at 300°C for 10 min.

Identification of phytochemical constituents

The phytochemical constituents were identified based on data libraries by analysing and comparing mass spectra and retention index (FFNSC1.3.lib, WILEY229.lib, and NIST11s.lib).

RESULTS AND DISCUSSION

The efficiency of compound separation from the plant tissue relies upon a few factors such as the nature of chemical constituents, the extraction method, the sample particle size, extraction duration, the ratio solute to solvent, and polarity of solvent (Do *et al*., 2014). During extraction, solvents are disseminated into plant tissue and solubilize compounds with the same polarity. Solvent polarity is very crucial to recover higher crude yield and bioactive composition. In this study, serial extraction method by increasing polarity of solvents has been applied from a non-polar to a polar solvent. Different solvents could extract different chemical compounds based on their polarity.

Extraction by using different polarity of solvents affected the crude yield of *Vernonia* sp. (Iwara *et al*., 2018). The methanolic and hexane crude extract from 500 g of powdered *V. amygdalina* leaves gave yield to 15.21 and 5.60%, respectively which was lower than in the previous report that obtained 19.95% for methanol extract and 6.25% for hexane extract using direct extraction (Asfere *et al*., 2018). This inconsistency might be due to pre-harvest factors including plant growth development, fertiliser application, harvesting time and agroclimatic condition. Besides that, postharvest factors such as drying process, extraction method, and storage temperature could also affect the yield and chemical composition in *V. amygdalina* extract (Ejike & Ndukwu, 2017).

Several studies have been done in isolating and characterising some bioactive compounds from *V. amygdalina*. The phytochemical studies had resulted in the isolation of flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene (Luo *et al*., 2011; Quasie *et al*., 2016). The chromatogram from GCMS analyses of the hexane and methanolic crude extract showed the presence of 32 and 22 peaks, respectively (Figure 1). Each peak was representing different chemical constituents. The identified compounds are according to their elution order on a Rxi-5MS silica capillary column. Interestingly, the methanolic extract was commonly reported to contain higher in chemical constituents in *Vernonia* species (Iwara *et al*., 2018), but in this study, hexane extract was found to be higher in chemical compounds as compared to methanolic extract. This contradiction report of chemical constituents could be due to the plant species and method extraction.

Table 1 summarised the chemical composition in hexane and methanolic extract of *V. amygdalina* leaves. The hexane extract contains a complex mixture consisting of mainly terpenoid group, hydrocarbon, stigmastane and vitamin E. The major compounds which were composed more than 5% detected in this extract were tetrapentacontane (21.56%), nonacosane (13.06%), squalene (12.77%), phytol (8.73%), δ-7,25-stigmastadienol (7.20%) and α-tocopherol (5.18%). Tetrapentacontane showed the highest percentage of composition in hexane extract. According to Al-Madhagi *et al*. (2018), this compound was tested to possess anti-inflammatory and antioxidant. Meanwhile, nonacosane is an oily volatile component that has a role in plant metabolite and antibacterial activities (Thirumalaisaisamy *et*

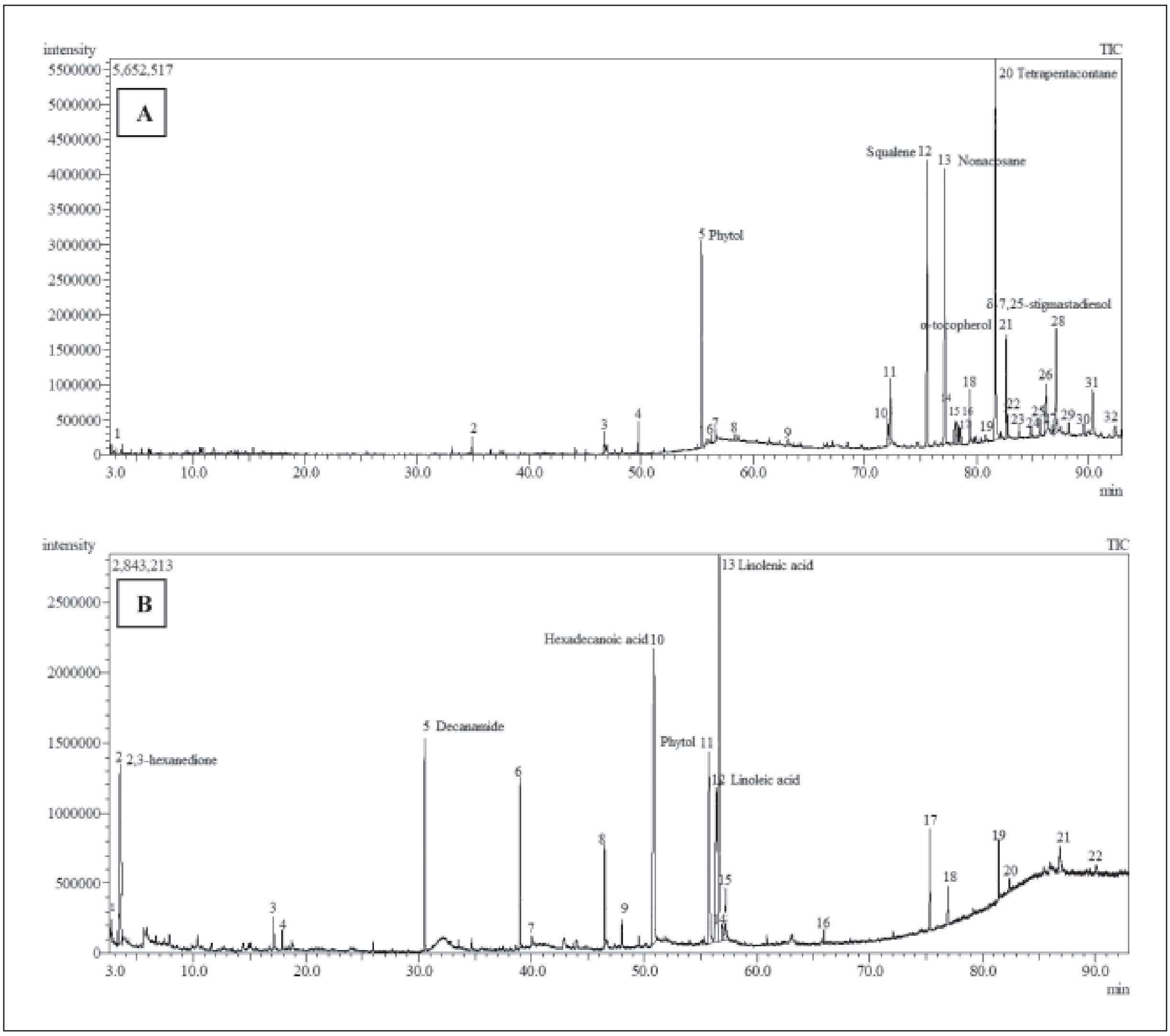

Fig. 1. Ion chromatogram of hexane (A) and methanolic (B) crude extract using GCMS.

Table 1. Chemical composition in hexane and methanolic extract of *V. amygdalina*

Retention index*	Compound name	Compound class**	Hexane extract (%)	Methanolic extract (%)
691	Propionic acid	Carboxylic acid	0.21	–
729	Glycerin	Sugar alcohol	–	0.47
739	2,3-hexanedione	Diketone	–	12.40
1142	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	Dihydropyranones	–	0.97
1158	3,4-dimethyl-2-pentanone	Ketone	–	0.55
1433	Decanamide	Fatty acid	–	5.92
1530	Actinidiolide	Benzofurans	0.68	–
1638	Dodecanamide	Fatty acid	–	4.89
1665	Tetramethylchromanol	Vitamin E derivative	–	0.35
1839	Neophytadiene	Sesquiterpene	0.79	3.35
1882	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Acyclic diterpene	–	0.80
1966	Hexadecanoic acid	Fatty acid	1.51	15.51
2119	Phytol	Diterpene	8.73	8.81
2140	Linoleic acid	Fatty acid	0.37	10.45
2147	Linolenic acid	Fatty acid	0.66	21.94
2153	Ethyl linoleate	Fatty acid	–	0.87
2164	Octadecanoic acid	Fatty acid	–	1.83
2191	Eicosanol	Fatty alcohol	0.22	–
2353	4,8,12,16-tetramethylheptadecan-4-olide	Isoprenoid lactone	0.24	–
2463	Hexanoic acid	Fatty acid	–	0.47
2685	Linolein	Fatty acid	1.76	–
2695	2-methylhexacosane	Hydrocarbon	3.35	–
2830	Squalene	Triterpenoid	12.77	3.22
2897	Nonacosane	Hydrocarbon	13.06	1.27
2934	Solanesol	Fatty alcohol	0.99	–
2946	Oxirane	Epoxides	1.33	–
2953	Geranyl linalool	Monoterpenoid	0.67	–
2958	Tetraprenol	Diterpenoid	0.70	–
2993	Tetratriacontane	Hydrocarbon	2.25	–
3055	α-tocopherol	Vitamin E	0.26	–
3095	Tetrapentacontane	Hydrocarbon	21.56	–
3097	Triacontane	Hydrocarbon	–	1.78
3139	α-tocopherol	Vitamin E	5.18	0.47
3146	Unknown	–	0.91	–
3192	Tetracontane	Hydrocarbon	0.66	–
3238	1,30-triacontanediol	Fatty alcohol	0.47	–
3272	Stigmasterol	Stigmastane	1.04	–
3295	Chondrillasterol	Stigmastane	3.89	1.84
3320	2-nonadecanone	Hydrocarbon	0.52	–
3334	δ-7,25-stigmastadienol	Stigmastane	7.20	–
3381	β-amyrin	Triterpenoid	0.78	–
3437	α-amyrin	Triterpenoid	0.85	–
3472	α-tocopherol acetate	Vitamin E	3.39	0.49
3557	17-pentatriacontene	Hydrocarbon	0.63	–

– not detected.
* Retention index on the Rxi-5MS silica capillary column.
** Search using ClassyFire web-based application developed by Feunang *et al.* (2016).

al., 2015). Authors have documented that squalene is a non-polar substance and structurally similar to beta-carotene. It was observed to help in the synthesis of cholesterol, effective as skin emollient, scavenging free radicals, and inhibit the fungal growth (Huang *et al*., 2009; Li *et al*., 2015). Phytol is an acyclic diterpene alcohol and commonly detected in medicinal plants. It could act as cytotoxic, antinociceptive, antimicrobial effects, antioxidant, anti-inflammatory (Islam *et al*., 2018). Unexpectedly, a very peculiar compound which is δ-7,25-stigmastadienol is detected in this extract. This stigmastane-type steroid glucoside is reported to have antimicrobial effects (Zeb *et al*., 2017). Particularly, α-tocopherol is categorized as fat-soluble vitamin which exhibit antioxidant activities, promoting immune system, keeping the endothelial cell integrity, and balancing normal coagulation (Shils *et al*., 2006).

In the methanolic extract, the main groups identified were fatty acid and diketone such as linolenic acid (21.94%), hexadecanoic acid (15.51%), 2,3- hexanedione (12.40%), linoleic acid (10.45%), phytol (8.81%) and decanamide (5.92%). Linoleic and linolenic acids are grouped in a polyunsaturated fatty acid. Linoleic acid in a sufficient amount is essential in human nutritional aspect. This fatty acid is collaborated with the presence of linolenic acid to avoid any deficiency symptoms by lowering many disorders including cancer, visual impairment, cardiovascular and neurological diseases (Kaur *et al*., 2014). Hexadecanoic acid is also known as palmitic acid which is a saturated fatty acid and was profound in *V. amygdalina* fraction (Oche *et al*., 2017) with antioxidant activity and hypocholesterolemic effect. According to the previous report, 2,3- hexanedione from alpha-diketone is important in antimicrobial activities (Al-Madhagi *et al*., 2018). Some scientist suggested that decanamide could induce the plant immunity (Méndez-Bravo *et al*., 2011), reduced plant pathogen necrosis and inhibit fungal proliferation. In spite being as antifungal substance, this compound also represents a new group of plant growth-promoting substances (Ramírez-Chávez *et al*., 2004).

In the present study, sequential extraction has been applied in order to extract different polarity of chemical compounds from hexane and methanol. Based on the analysis, ten analogous compounds were identified in both extracts. There were five identical phytocomponents specifically neophytadiene, hexadecenoic acid, phytol, linoleic acid, and linolenic acid which contain higher in methanolic extract as compared to hexane extract. These compounds were more favourable in the polar solvent and diffuse higher in methanolic extract. On the other hand, squalene, nonacosane, α-tocopherol, chondrillasterol, and α-tocopherol acetate were found at later elution order, at a retention index 2830 onwards which were higher in molecular weight with a carbon range of 29 to 31. This is supported by Vilegas *et al*. (1997) who found that hexane plant extract comprised of numerous low-polarity compounds.

CONCLUSION

The present study showed that the crude extract of hexane from *V. amygdalina* exhibited many bioactive compounds that have diversified function in biological activities. However, most of the compounds identified from methanolic extract are useful as antioxidant, antimicrobial and decreasing disorders such as cancer, visual impairment, cardiovascular and neurological diseases. Though, further investigation is needed to enlighten the mechanism of action and to quantify the primary phytochemicals in this *V. amygdalina* that can serve as a basis for health benefits leading to extension biological as well as pharmacological studies.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the funding from Mrs. Zainura Yusoff (Director of Konsortium Memandu Sejati Erat), technical support from Mrs. Mazina Mohd. Yusoff (Laboratory of Natural Products, Institute Bioscience, Universiti Putra Malaysia) and Pathology Laboratory, Pest and Disease Management Programme, Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI).

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