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GC-MS Analysis of Phytochemical Compounds in Aqueous Leaf Extract of *Abrus Precatorius*

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ABSTRACT

Abrus precatorius is a flowering plant that belongs to the legume family, Fabaceae. In Malaysia, the leaves of *Abrus precatorius* are used traditionally to treat ailments such as fever, ulcer and mouth cancer. These traditional practices, however, have never been documented and usage of the plant is based on popular beliefs held by the local people. This work documented the phytocompounds that are present in the aqueous extract of *Abrus precatorius* leaves collected from a local area in Kota Bharu, Kelantan, Malaysia. The leaves were dried and then subjected to extraction using the decoction technique. The compounds were identified by gas chromatography with mass spectrometry analysis and characterised by comparison through the NIST02 and Wiley275 library search software. The GC-MS analysis showed that the classes of compounds identified in aqueous extracts of *Abrus precatorius* leaves were phenolic compounds, terpenoids and steroids.

Keywords: Abrus precatorius, aqueous extract, GC-MS

INTRODUCTION

Medicinal plants are widely used as food and medicine in traditional practice. In Malaysia, such plants are consumed as an alternative treatment for illness or to maintain a healthy lifestyle. A huge reservoir of bioactive compounds exists in over 400,000 species of plants on Earth, but only a small percentage of these compounds have been examined in research. In many developed countries,

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plant products used as Complementary and Alternative Medicine (CAM) are popular. In Malaysia, as reported in 2002, the use of CAM added up to US$500 million in cost, annually, compared to about the use of allopathic medicine, which added up to only US$300 million in cost (World Health Organisation, 2002). Medicinal plants are widely used in human and veterinary therapy, agriculture, scientific research and countless other areas. The local people generally use plants and herbs as an alternative for curing and treating various diseases and ailments.

Abrus precatorius is a flowering plant that belongs to the legume family, Fabaceae. The common names of *Abrus precatorius* include jequirity, Crab's eye, Rosary pea, precatory pea or bean, John Crow Bead, Indian licorice, 'Akar saga' and jumble bead. Phenotypically, this plant is characterised as a slender, perennial climber that twines around trees, shrubs and hedges.

Abrus precatorius is native to India and grows mostly in tropical and subtropical parts of the world. In traditional Hindu medicine, it has been used since ancient times in some regions to treat mouth ulcer by chewing the leaves. The plant was also used by other ancient cultures, including China. The leaves can also be used as a nerve tonic as well as to treat wounds and swellings due to its anti-inflammatory properties. Oil extracted from *Abrus precatorius* seeds can be used to promote hair growth, while the roots are used for treating jaundice, gonorrhoea and haemoglobinuria (Samy, Thwin, Gopalakrishnakone, & Ignacimuthu, 2008). In Malaysia, the leaves of *Abrus precatorius* are used traditionally to treat ailments such as fever, ulcer and mouth cancer. These traditional practices, however, have never been documented and so, usage of the plant is based only on popular beliefs held by the local people.

Decoction of the leaves is widely practised as a treatment for cold, coughs and colic. Juice from the leaves is applied to swellings after mixing with oil. Additionally, a mixture of rice starch and paste made from the leaves of this plant can be consumed orally to treat anthrax attacks (Pokharkar, Saraswat, Bhavare, & Kanawade, 2011). As a powdered leaf paste, the plant can also be used to treat conjunctivitis and convulsion in children (Joshi & Tyagi, 2011).

Studies undertaking phytochemical analysis of the leaves and roots of A*brus precatorius* have demonstrated the presence of glycyrrhizin (Karwasara, Jain, Tomar, & Dixit, 2010), an important compound of liquorice (Killacky, Ross, & Turner, 1976), which is widely used in the food and pharmaceutical industries. A known triterpenoid and three novel triterpenoids were identified from the acid hydrolysed methanol-soluble leaf extract (Kim, Kim, & Kinghorn, 2002) of *Abrus precatorius*. From the *n*-butanol leaves extract of *Abrus precatorius*, other compounds identified were abrusoside A (Choi, Hussain, Pezzuto, Kinghorn, & Morton, 1989), abrusosides B, C and D plus three other sweet glycosides based on the novel cycloartane-type aglycone, abrusogenin (Kinghorn & Soejarto, 2002).

Most of the studies done on *Abrus precatorius* were carried out in India (Solanki & Zaveri, 2012). Though Malaysia has an abundance of useful herbs and medicinal plants, less is known about *Abrus precatorius* in Malaysia. The aim of this study was to identify the phytochemical compounds of the aqueous leaf extract of *Abrus precatorius* by gas chromatography-mass spectrometry analysis.

METHODOLOGY

Plant Collection

Abrus precatorius leaves were collected from Kampung Sabak, Pengkalan Chepa, Kelantan, Malaysia and authenticated by Dr. Rahmad Zakaria from the Herbarium Unit, School of Biological Sciences, Universiti Sains Malaysia. The voucher specimen (USM 11730) was submitted for future reference.

Preparation of Extract

The leaves of the *Abrus precatorius* were oven-dried at 50°C and macerated to fine powder using a mechanical grinder. The decoction method, which is used in traditional medicine, was applied in this experiment. An amount of 11 g of dried leaves ground to a fine powder was soaked in 450 ml water at 50°C until the water reduced to one third the initial volume. The extract was then freeze-dried for subsequent analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)

A Hewlett Packard 6890 Gas Chromatograph with a 5973N Mass Selective Detector was used to carry out the GC-MS. The column was fused silica capillary, HP-5 column (30 m x 0.25 mm i.d x 0.25 µm film thickness) (Agilent Technologies, USA). The carrier gas was helium with a flow rate of 1.0 ml/min with the oven temperature programmed from 50°C (held for 2 min) to 280°C (held for 10 min) at a rate of 20°C/min. The injection and interface temperatures were set at 250°C and 280°C, respectively. A 1-ml sample was injected in splitless mode and was analysed in MS full scan mode (*m/z* 40-650). The electron ionisation was fixed at 70eV. Acquisition of data was performed using the Chemsation software.

Identification of Phytochemical Compounds

The mass spectrum of the GC-MS was interpreted based on the database of the National Institute of Standards and Technology (NIST02) and Wiley275 libraries with matches of ≥80 % to identify the phytochemical compounds.

RESULTS

The GC-MS analysis showed that the classes of compounds identified in aqueous extracts of *Abrus precatorius* leaves were phenolic compounds, terpenoids and steroids. The

GC-MS chromatogram obtained is given in Figure 1. Seventeen chemical compounds were identified, as shown in Table 1. The main class of compounds identified was phenolic compounds (2.82%). Four phenolic compounds were identified, with the major phenolic compound being 4-vinylphenol (1.17%). Other major compounds that were found in the Abrus precatorius leaves were methyl jasmonate (1.89%), decylenic alcohol (1.46%) and cis-11-Tetradecen-1-ol (1.41%). Methyl jasmonate is categorised as a fragrance that belongs to the structural groups, ketones cyclopentanones and cyclopentenones (Scognamiglio, Jones, Letizia, & Api, 2012). Decylenic alcohol also belongs to the fragrance group; it is also known as Rosalva. The chemical structure and molecular weight of each identified compound are listed in Table 2.

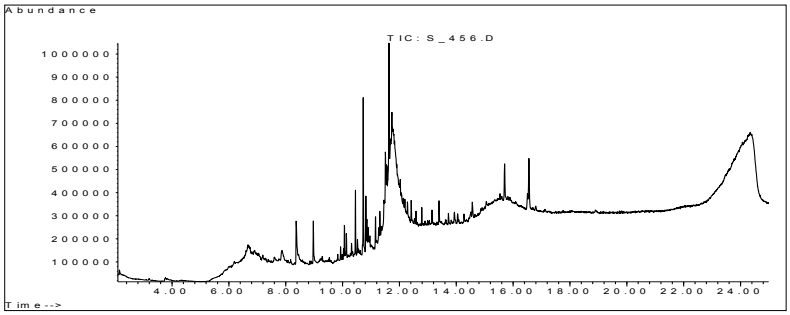


Figure 1. GC-MS Chromatogram of aqueous leaf extract of *Abrus precatorius*

Table 1
Compounds found present in the aqueous leaf extract of Abrus Precatorius using GC-MS

No	Retention Time (min)	Name of Compound	Area	*Therapeutic activity
		Phenolic Compound		
1	8.4	4-vinylphenol	1.17%	No activity recorded
2	8.9	p-Vinylguaiacol	0.68%	No activity recorded
3	10.1	β-Phenoxyethyl iso-butyrate	0.47%	No activity recorded
4	11.2	Cinnamaldehyde, β-hexyl-	0.50%	No activity recorded
		Terpenoids		
5	12.6	Phytol	0.39%	Anti-microbial, anti-cancer, anti-inflammatory
		Steroids		
6	16.5	Stigmasterol	0.31%	Anti-hepatotoxic, anti-inflammatory, anti-nociceptive, anti-ophidic, anti-viral, cancer preventive, ovulant, sedative
		Others		
7	7.8	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	0.66%	No activity recorded
8	9.8	6-Tridecene	0.13%	No activity recorded

Table 1 *(continue)*

9	9.9	β-lonone	0.17%	Anti-bacterial, anti-tumour, fungicide, pesticide, trichomonicide
10	10.7	Methyl dihydrojasmonate	1.89%	No activity recorded
11	10.9	Propanoic acid,2-methyl-3-[4-t-butyl]phenyl-	0.68%	No activity recorded
12	11.5	Decylenic alcohol	1.46%	No activity recorded
13	11.5	3-Decen-1-ol, (E)-	1.07%	No activity recorded
14	11.6	cis-11-Tetradecen-1-ol	1.41%	No activity recorded
15	13.7	Palmitic acid á-monoglyceride	0.19%	No activity recorded
16	15.7	1-Heneicosanol	0.72%	No activity recorded
17	16.6	1-Heptacosanol	1.17%	No activity recorded

**Source of reference:* Dr. Duke's Phytochemical and Ethnobotanical Databases, 1992-2016

Table 2
Compounds found present in the aqueous leaf extract of Abrus Precatorius using GC-Ms

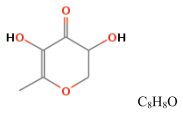
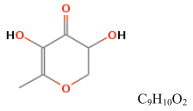
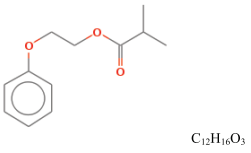
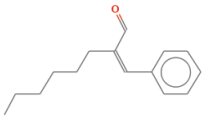
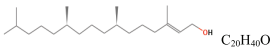
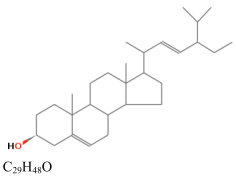
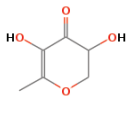
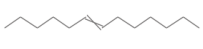
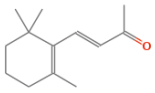
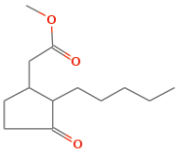
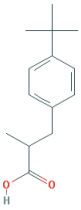
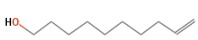
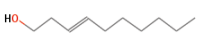
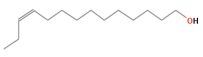
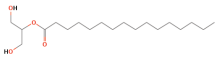
No	Name of Compound	Molecular Weight (g/mol)	Chemical Structure
1	4-vinylphenol	120.15	 C_8H_8O
2	p-Vinylguaiacol	150.17	 $C_9H_{10}O_2$
3	β-Phenoxyethyl iso-butyrate	208.25	 $C_{12}H_{16}O_3$
4	Cinnamaldehyde, β-hexyl-	216.32	 $C_{15}H_{20}O$
5	Phytol	296.53	 $C_{20}H_{40}O$
6	Stigmasterol	412.69	 $C_{29}H_{48}O$

Table 2 *(continue)*

No	Name of Compound	Molecular Weight (g/mol)	Chemical Structure
7	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	144.13	 $C_6H_8O_4$
8	6-Tridecene	182.35	 $C_{13}H_{26}$
9	β-lonone	192.30	 $C_{13}H_{20}O$
10	Methyl dihydrojasmonate	226.31	 $C_{13}H_{22}O_3$
11	Propanoic acid,2-methyl-3-[4-t-butyl]phenyl-	220.31	 $C_{14}H_{20}O_2$
12	Decylenic alcohol	156.27	 $C_{10}H_{20}O$
13	3-Decen-1-ol, (E)-	156.27	 $C_{10}H_{20}O$
14	cis-11-Tetradecen-1-ol	212.37	 $C_{14}H_{28}O$
15	Palmitic acid á-monoglyceride	330.50	 $C_{19}H_{38}O_4$
16	1-Heneicosanol	312.57	 $C_{21}H_{44}O$
17	1-Heptacosanol	396.73	 $C_{27}H_{56}O$

DISCUSSION

Phytochemical studies of the aqueous leaf extract of *Abrus precatorius* are still limited as they have only been able to identify the polar compounds. The composition of the compounds identified in the aqueous extract of *Abrus precatorius* leaves comprises a complex mixture of several classes of components, mainly phenolic compounds, terpenoids and steroids. A

study by Hussain and Kumaresan (2014) showed that only phenolic compounds and steroids were present in aqueous leaves extract of *Abrus precatorius*. Other studies have indicated that the solvent extract from *Abrus precatorius* leaves is rich in alkaloids, carbohydrates, steroids, phenolic compounds and terpenoids (Gul, Ahmad, Kondapi, Qureshi, & Ghazi, 2013; Hussain & Kumaresan, 2014; Yonemoto, Shimada, Gunawan-Puteri, Kato, & Kawabata, 2014).

One study of *Abrus precatorius* leaves showed that their biological activities were related to their active compounds such as terpenoid and phenolic compounds. Yonemoto et al. (2014) found that terpenoids isolated from *Abrus precatorius* leaves had an α amylase inhibitory effect, which is one of the therapeutic approaches for preventing diabetes mellitus. Phenolic compounds such as flavanoids and phenolic acids are well known to have antioxidant and anti-proliferative activities (Gul et al., 2013)

The main phenolic compound identified in the aqueous extract of *Abrus precatorius* leaves was 4-vinylphenol (Table 1). A recent study of 4-vinylphenol showed that this compound has anti-angiogenic activities (Yue et al., 2015). Other identified compounds that have some therapeutic activities were β-lonone, phytol and stigmasterol, as listed in Table 1. The compound β-lonone has been shown to have anti-proliferative (Faezizadeh, Gharib, & Godarzee, 2016), anti-bacterial (Kubo, Muroi, & Himejima, 1993; Patra, Das, & Baek, 2015) and anti-tumour activities (Cho, So, Chun, & Jeon, 2016; Liu et al., 2008; Sharma, Chaudhary, Arora, Saxena, & Ishar, 2013; Yu, Anderson, & Elson, 1995). In addition, it can also be used as a fungicide, pesticide and trichomonicide. Phytol has anti-microbial (Pejin et al., 2014), anti-cancer (Song & Cho, 2015) and anti-inflammatory activities (Silva et al., 2014). Stigmasterol is listed to be an anti-hepatotoxic (El-Domiaty, Wink, Abdel Aal, Abou-Hashem, & Abd-Alla, 2009), anti-inflammatory (Gabay et al., 2010), anti-nociceptive (Kamurthy, Sumalatha, Rao, & Sudhakar, 2013), anti-ophidic, anti-viral and cancer-preventive agent (Ali et al., 2015; Kasahara et al., 1994), a hypocholesterolemic agent (Barriuso, Ansorena, Poyato, & Astiasarán, 2015), an ovulant agent (Zaman, Parvez, Ali, & Sayeed, 2015) and a sedative agent (Habib, Nikkon, Rahman, Haque, & Karim, 2007).

The therapeutic activity of the aqueous leaf extract of *Abrus precatorius*, which in traditional practice is obtained by decoction, might be due to the presence of phytol, stigmasterol and β-lonone. Individually, these phytochemical compounds are known for their bioactivities; however, this does not answer how the plant works as a whole crude extract as used in traditional practice. Further experiments are needed in order to prove that this extract does have therapeutic effects when used as a whole crude extract and that its efficacy does not result from the beneficial effects of any of its individual phytochemical compounds.

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