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PHYTOCHEMICAL, DPPH AND BIOASSAY STUDY OF *Mariposa christia vespertilionis* PLANT: A SHORT REVIEW

(Fitokimia, DPPH dan Kajian Bio Asai Terhadap Pokok *Mariposa christia vespertilionis*: Satu Ulasan Ringkas)

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Abstract

Mariposa christia vespertilionis (MCV), colloquially referred to as butterfly wing or Mariposa, has garnered attention in both traditional and modern medicine due to its purported therapeutic properties. This review aimed to consolidate existing knowledge regarding the phytochemical composition, antioxidant potential evaluated through DPPH assay, and bioassay studies of MCV. The plant, widely distributed across China and Southeast Asia, has been traditionally employed for various ailments, including cancer and malaria, with anecdotal evidence supporting its efficacy. In Malaysia, the usage of the green-leafed form of this plant has gained popularity because of customer testimonies about the effectiveness of the plant's water decoction in "curing cancer disease". However, the scientific validation of its medicinal claims against cancer remained unexplored. Current research has identified phytoconstituents such as alkaloids, flavonoids, and quinones in MCV, correlating with its reported pharmacological activities. This review underscores the need for comprehensive research to elucidate the chemical composition and biological activities of MCV, thus providing a foundation for future investigations and potential therapeutic applications.

Keywords: *Mariposa christia vespertilionis*, alkaloids, flavonoids, quinones, cytotoxicity

Abstrak

Mariposa christia vespertilionis (MCV), secara umumnya dirujuk sebagai sayap rama-rama atau Mariposa, telah menarik perhatian dalam perubatan tradisional dan moden disebabkan oleh sifat terapeutik yang diperkatakan. Kajian ini bertujuan untuk mengumpulkan pengetahuan sedia ada mengenai komposisi fitokimia, potensi antioksidan yang dinilai melalui ujian DPPH, dan kajian bioassay MCV. Tumbuhan ini, yang tersebar luas di China dan Asia Tenggara, telah digunakan secara tradisional untuk pelbagai penyakit, termasuk kanser dan malaria, dengan bukti anekdot menyokong keberkesanannya. Di Malaysia, penggunaan bentuk daun hijau tumbuhan ini telah mendapat populariti disebabkan oleh kesaksian pelanggan tentang keberkesanan dekoksi air tumbuhan ini dalam "mengubati penyakit kanser". Walau bagaimanapun, pengesahan saintifik terhadap dakwaan perubatan terhadap kanser masih belum dikaji. Kajian semasa telah mengenal pasti fitokimia seperti alkaloid, flavonoid, dan kuinon dalam MCV, yang berkaitan dengan aktiviti farmakologi yang dilaporkan. Ulasan ini menekankan keperluan penyelidikan menyeluruh untuk menghuraikan komposisi kimia dan aktiviti biologi MCV, seterusnya dapat menyediakan landasan asas untuk penyelidikan masa depan dan aplikasi terapeutik yang berpotensi.

Kata kunci: *Mariposa christia vespertilionis*, alkaloid, falanoid, kuinon, kesitotoksikan

Introduction

There has been a strong bond between plants and humans since ancient times. Plants have been used for decoration, as the primary source of food, and even for therapeutic purposes [1]. Though there is a vast advancement in the field of synthetic medicine, a large number of people as well as researchers throughout the world still rely on herbal medicines [2]. Plant consist of various phytoconstituents that has multiple pharmacological functions, due to which the demand for plant-based or plant-derived drugs has been increasing day by day [3]. Plants have been used as traditional medicine in Indian subcontinent as Ayurvedic medicine, in New Zealand as te rongoā Māori, in China as traditional Chinese medicine, in Australia as Aboriginal medicine, and several other parts of the world [4].

Mariposa christia vespertilionis (MCV) is one of the herbs that have been broadly used in traditional medicine practice [5]. The plant species originated in tropical Southeast Asia and is distributed particularly in Thailand, Vietnam, Cambodia, Indonesia, Malaysia, and Myanmar [6]. The MCV plant is found in two varieties: red-leafed (RMCV) and green-leafed (GMCV) (Figure 1). In Malaysia, GMCV is currently well-known for traditional medicine while the RMCV is another type of plant that is abundantly available. The MCV is an ornamental herb, commonly known as butterfly wing plant due to its vibrant features and unique shape. It is locally referred to as “Rerama” in Malay [7]. The plant can grow up to 60 - 120 cm tall and has 3 leaflets with purplish red leaves, red stripes, and green leaves [8, 9]. Apart from decorative usage, it has been documented in traditional medicine as a treatment for a variety of illnesses and injuries, including tuberculosis, bronchitis, cold, scabies, poor blood circulation, snake bites, bone fractures, and muscular weakness [10, 11]. Several studies have shown that the plant is, anti-inflammatory, antiproliferative, cytotoxic, antimalarial, antidiabetic, and antioxidant [5, 12-16]. The dried leaves can be used to make tea drinks. Recently, in Malaysia, the usage of the green-leafed form of this plant has gained popularity because of customer testimonies about the effectiveness of the plant's water decoction in "curing cancer disease”. There are several in vitro studies exploring the plant extract’s anticancer effects [7, 17-19]. The Bunong people in the Mondulkiri province of Northeast Cambodia used MCV in several methods, the most common way is by crushing the plant with water and smearing it as topical medicine [13].

Over time, numerous researchers have delved into the pharmacological characteristics of this plant, conducting both in vitro and in vivo experiments to unveil its potential applications in contemporary medicine [1]. Despite its widespread usage; little is known about the phytochemical components of this plant's leaf metabolome and the bioassay studies that have been carried out. This review will provide knowledge and information on the chemicals and biological activities of this plant that would be beneficial for future research. Thus, given the medical significance of the plant leaves and the importance of quality control, whether for product manufacturing or regulatory purposes, a better understanding of the leaf metabolome is a logical and critical extension of the research and development activities that should be conducted on this plant [20]. Therefore, in this study, the detailed literature that revealed the use of MCV leaf extract in phytochemical study will be known such as, phenolic content, DPPH, and bioassay.

Figure 1. Morphology of leaf of (a) RMCV (b) GMCV [6, 7]

Sample extraction

Prior to the phytochemical analysis sample extraction was carried out using diverse solvents like (Water (Aq), Ethanol, Methanol (M), Ethyl acetate: Methanol (EM), Hexane (H), Hexane: Ethyl acetate (HE), and Ethyl acetate (E)) [5-7, 15, 21, 22]. For example, Zambari et al. [21] extracted a total of 1g of dried powdered sample of each type of MCV leaves with 200 mL of solvent (ethanol) using the Soxhlet apparatus for 8 h which was then filtered and evaporated using a rotary evaporator. The dry weight was determined from 1 mL of the plant extract and the extraction yield of each extract was calculated by following the formula for extraction yield.

$$\text{Extraction yield (\%)} = (m^2/\mathrm{m}^1) \times 100 \quad (1)$$

Where m^1 is the mass of dry weight of MCV leaf (g); and m^2 is the mass of crude extract of MCV leaf (g).

In a recent study by Yasin and his co-workers [16] exhibited that usage of different solvent polarity lead to variation in the yield of extraction, with a tendency toward EM > M > HE > H > E. Nawaz et al. [23] stated that the extraction yield and the antioxidant activity of phenolic compounds in plant material is remarkably affected by the solvent polarity. The polarity-dependent increase in extraction yield, antioxidant activity, reducing properties and free radical scavenging activity perhaps due to the high affinity of antioxidant compounds towards more polar solvents as compared to nonpolar ones. It is also reported that the phytochemical compounds extracted in polar solvents are pharmaceutically more important due to comparatively higher values of antioxidant activity, reducing properties and free radical scavenging activity [23]. However, the higher values of TPC and TFC in non-polar solvents could be assigned to the non-polar nature of phenolic compounds. Govardhan et al. [24] highlighted the importance of the solvent used to extract phytochemical compounds in producing a high yield of phytochemical compounds. Apart from that, the extraction method also influences the analytical quality of phytochemical compounds. Hence selecting a proper solvent for the extraction process plays a key role in providing better results.

Phytochemical Study

The metabolites present in the plant are generally classified as primary and secondary metabolites [25]. Primary metabolites include carbohydrates, fatty acids, amino acids, and similar compounds which support the plant's growth [26]. In contrast, secondary metabolites such as alkaloids, flavonoids, and terpenoids impart specific characteristics to plants, like coloration or defense against predators [27, 28]. The MCV plant has been gaining tremendous attention because of its therapeutic usage (e.g. anticancer, antimalarial, anti-inflammatory, antidiabetic etc.) [5, 7]. The medicinal plants are known to be fascinating source of novel drugs which are the key ingredients in traditional medicine, pharmaceutical intermediates, modern medicines, and prime compounds in synthetic drugs. The actual reason behind their use as medicine is that they hold chemical components with therapeutic properties that contain defense mechanism and guard against diverse illnesses. Usually, the medicinal value of plants lies in the secondary metabolites which leads to definite physiological action in the human body [29]. Hence, there is a need to determine the phytochemical constituents especially, secondary metabolites present in the plant. Therefore, in the present study, we focused on reviewing the phytochemical studies that has been done against the MCV plant. Phytochemical studies can be carried out as qualitative and quantitative determination.

Qualitative phytochemical analysis

The preliminary phytochemical analysis of the medicinal plant extract by using different types of solvents can be carried out by using the standard protocols available to identify the presence and absence of metabolites such as flavonoid (ammonia test), Alkaloid (Mayer's test), saponin (foam test), tannin ($FeCl_3$ test), cardenolide (sodium picrate test), cardiac glycoside (Keller-Kiliani test), phenolic ($FeCl_3$ test), terpenoid (Salkowski test), steroid (Libermann-Burchard test) etc. [21, 30, 31]. From Table 1 it can be deduced that MCV leaf contains secondary metabolites like alkaloids, flavonoids, proteins, glycosides, tannins, phenols, saponins, diterpenes, coumarins, steroids, and quinones. Based on studies conducted by Lee et al. [22] the H extract of MCV did not include any phenol or tannins. Only traces of flavonoids were found in HE and E extracts, as well as M extracts. However, moderate levels of flavonoids were present in the H and EM extracts. The presence of coumarin was moderate in all the extracts, except for the M extract, which had a low abundance of the compound. The MCV plant extracts from the E, EM, and M had measurable amounts of tannins. On the other hand, there were none observed in H and HE extracts. Meanwhile, the glycoside concentration of the M extract was the highest, followed by E and EM extracts and the lowest are H and HE extracts [22].

From Table 1, it can be highlighted that the M extract comprises maximum number of phytochemical compounds followed by E extract and ethyl acetate: methanol EM. It is noteworthy that flavonoids are found in the MCV leaf extract derived from all types of solvents and saponins are found in aqueous extract. On other hand, it should be noticed that highest number of phytochemicals are found in methanolic extract although, chemical constituents like carbohydrates, amino-acids, anthraquinone, Phytosterols, resins, saponins, and phlobatannins have not been identified through this extract. The phytochemical screening results for aqueous MCV leaf extracts from the previous study show similar results for the similar extract used in the phytochemical test [5-7, 22]. Simple phytochemical screening can only expose the chemical class of the compound in a broad sense. However, further advancement in the qualitative phytochemical analysis include identification and separation of the compounds from different MCV extracts using high end techniques like TLC, HPLC, LCMS, and GCMS (Table 2) [29]. Additionally, the compound identification with exact chemical structure can be elucidated using techniques like UV, IR, ^{1}H-, ^{13}C-NMR, and MS [15]. The previous findings related to the phytochemical constituents of MCV plant extracts along with their role in therapeutic application are shown in Table 2. [32].

Table 1. Preliminary phytochemical screening of MCV extract using different solvents

	Water (Aq)	**Ethanol**	**Methanol (M)**	**Ethyl acetate: Methanol (EM)**	**Hexane (H)**	**Hexane: Ethyl acetate (HE)**	**Ethyl acetate (E)**
Alkaloids	+	+	+	NA	NA	NA	NA
Flavonoids	+	+	+	+	+	+	+
Carbohydrates	-	NA	-	NA	NA	NA	NA
Proteins	NA	NA	+	NA	NA	NA	NA
Amino acids	NA	NA	-	NA	NA	NA	NA
Glycosides	NA	+	+	+	+	+	+
Tannins	-	+	+	+	-	-	+
Phenols	+	+	+	+	-	+	+
Saponins	+	+	-	NA	NA	NA	NA
Anthraquinone	NA	NA	-	NA	NA	NA	NA
Phytosterol	NA	NA	-	NA	NA	NA	NA
Resin	NA	NA	-	NA	NA	NA	NA
Diterpene	NA	NA	+	NA	NA	NA	NA

	Water (Aq)	**Ethanol**	**Methanol (M)**	**Ethyl acetate: Methanol (EM)**	**Hexane (H)**	**Hexane: Ethyl acetate (HE)**	**Ethyl acetate (E)**
Coumarins	NA	NA	+	+	+	+	+
Quinone	NA	NA	+	NA	NA	NA	+
Steroids	-	+	+	NA	NA	NA	NA
Phlobatannins	-	NA	NA	NA	NA	NA	NA
Terpenoid	NA	-	NA	NA	NA	NA	NA
Cardenolide	NA	+	NA	NA	NA	NA	NA

(+) = presence of phyto-constituents in MCV; (−) = no phyto-constituents in MCV; (NA)= data not available

Table 2. Phytochemical compounds found in MCV plant and their activity

Chemical Class	**Name of the Compound**	**Chemical Formula**	**Analytical Technique**	**Source/ Solvent**	**Activity**	**Ref.**
Alkene hydrocarbon	1-octadecene	$C_{18}H_{36}$	GCMS	RMCV (leaf)/ ethanol	Antibacterial, antioxidant, anticancer	[32]
aliphatic ether alcohol	4-O-methylmannose	$C_7H_{14}O_6$		RMCV (leaf)/ ethanol and methanol	Antibacterial	[32]
Phenolic ester	Phenol, 3,5-bis(1,1-dimethyl ethyl)	$C_{14}H_{22}O$	GCMS	RMCV (leaf)/methanol	Antiseptic, disinfectant, flavoring, antibacterial	[32]
Carboxylic acid ester	Acetic acid, butyl ester	$C_{16}H_{12}O_2$	GCMS	RMCV (leaf)/ methanol	Antifungal, Antitumor	[32, 33]
	1-butanol, 3-methyl-, acetate	$C_7H_{14}O_2$			Antimicrobial	
Fatty acid	n-hexadecanoic acid	$C_{16}H_{32}O_2$	GCMS	RMCV (leaf)/ ethanol and methanol GMCV (leaf)/ ethanol	Anti-inflammatory, antibacterial, antioxidant, and antiandrogenic	[21, 32]
Polyunsaturated omega-6 fatty acid	Linoleate	$C_{18}H_{31}O_2$	LC-MS	GMCV and RMCV (leaf)/ 5% ethanol	Anti-inflammatory, acne reductive, and skin moisturizer	[9]
	Olivetol	$C_{11}H_{16}O$		RMCV (leaf)/ 5% ethanol	Anti-septic and repellent	

Chemical Class	Name of the Compound	Chemical Formula	Analytical Technique	Source/ Solvent	Activity	Ref.
	17β-estradiol	$C_{18}H_{24}O_2$			Help in the female reproductive tissues' development and maintenance	
	Mycophenolate	$C_{17}H_{19}O_6$			Immunosuppressant used to prevent rejection in organ transplantation	
Flavonoid	Quercetin	$C_{15}H_{10}O_7$	LC-MS/MS	MCV (root)/ ethyl acetate	Anticancer, Antidiabetic, antioxidant	[22, 34]
	Kaempferol	$C_{20}H_{18}O_6$				
	Sternbin	$C_{16}H_{14}O_6$			-	
	Kaempferol	$C_{15}H_{10}O_6$			-	
	Kuchecarpins C	$C_{17}H_{16}O_7$			-	
	8-C-Prenyl Kuwanon L	$C_{35}H_{30}O_{11}$			-	
	3,4-Dihydro-4-(40-hydroxyphenyl) -5,7-dihydroxy coumarin	$C_{15}H_{12}O_5$			-	
Flavonoid glycosides	quercetin-3-O-glucoside	-	NMR, ESI-MS, Hyphenated LC-PDA-MS, HRMS	RMCV (root, stem and leaf)/ ethyl acetate	Antioxidant, antidiabetic	[15]
	catechin-3-O-ß-D-glucopyranoside	-		RMCV (root, stem and leaf)/n-butanol		
Isoflavonoids	2'-hydroxygenestin	-	NMR, ESI-MS, Hyphenated LC-PDA-MS, HRMS	RMCV (Root, stem, and leaf)/ ethyl acetate	Immunosuppressive, antiinflammatory, anticancer	[15, 35]
	Orobol	-				
	2,3-dihydro-2'-hydroxygenestin	-				
Quinones	Fallacinol	$C_{16}H_{12}O_6$	LC-MS/MS	MCV (root)/ ethyl acetate	-	[22]
	Alizarin	$C_{14}H_8O_4$			-	
	Purpurin	$C_{14}H_8O_5$			-	
	Rhein	$C_{15}H_8O_6$			Anticancer	

Chemical Class	Name of the Compound	Chemical Formula	Analytical Technique	Source/ Solvent	Activity	Ref.
	Denbinobin	$C_{16}H_{12}O_5$			Anticancer	
Coumarins	5,7-Dihydroxy chromone	$C_9H_6O_4$	LC-MS/MS	MCV (root)/ ethyl acetate	Anticancer	[22]
	Wedelolacetone	$C_{15}H_{12}O_5$	LC-MS/MS	MCV (root)/ ethyl acetate		
Sterols	Stigmasterol	$C_{29}H_{48}O$	UV, IR, ^{1}H-, ^{13}C-NMR, MS, Co-TLC	MCV (root, stem and leaf)/ n-hexane	Antidiabetic, antiinflammatory, and anticancer	[15, 33]
	ß-sitosterol acetate	-				
	ß-sitosterol	-				
Phenolic acids	Sanleng acid	$C_{18}H_{34}O_5$	LC-MS/MS	MCV (root)/ ethyl acetate	Anticancer	[22]
	2'-Hydroxydecanylpentadec-5,8,10,12-tetraenoate (christanoate)	$C_{25}H_{42}O_3$	NMR, ESI-MS, Hyphenated LC-PDA-MS, HRMS	RMCV (root, stem, leaf)/ *n*-hexane	-	[15]
Pentacyclic triterpenes	D:C-friedoolean-8-en-29α-ol	-	UV, IR, ^{1}H-, ^{13}C-NMR, MS	RMCV (root, stem, leaf)/ *n*-hexane	Antiinflammatory, anticancer, antidiabetic, antioxidant, antibacterial effects	[15, 36]
	Ursolic acid methyl ester	-				
	Oleanolic acid methyl ester	-				
	Erythrodiol	-				
Monoterpene	Geraniol	-	UV, IR, ^{1}H-, ^{13}C-NMR, MS, co-TLC	RMCV (root, stem, leaf)/ *n*-hexane	Antimicrobial, antioxidant, anti-inflammatory	[15, 37]
Sesquiterpene	Zerumbone	$C_{15}H_{22}O_1$	LC-MS	GMCV (leaf)/ 5% ethanol RMCV (leaf)/ 5% ethanol	Anticancer particularly leukemia	[9]
	7-isopropylidene-1-methyl-1, 2, 6, 7, 8,	$C_{14}H_{20}$	NMR, ESI-MS, Hyphenated	RMCV (root, stem,	Anti-plasmodial	[15]

Chemical Class	Name of the Compound	Chemical Formula	Analytical Technique	Source/ Solvent	Activity	Ref.
	9-hexahydronaphthalene (christene)		LC-PDA-MS, HRMS	leaf)/ *n*-hexane		
Nonsteroidal anti-inflammatory drug (NSAID)	Ibuprofen	$C_{13}H_{17}O_2$	LC-MS	GMCV (leaf)/ 5% ethanol	Anti-inflammatory	[9]
Others	Nicotinamide	$C_6H_6N_2O_1$	LC-MS	GMCV (leaf)/ 5% ethanol	Vitamin (food supplement)	[9]
	Psoralen	$C_{11}H_6O_3$			Ultraviolet light therapy for eczema, graft-versus-host disease, psoriasis, vitiligo, large-plaque parapsoriasis, mycosis fungoides, and cutaneous T-cell lymphoma	
	phytol	$C_{20}H_{40}O$	GCMS	RMCV and GMCV (leaf)/ ethanol	Anti-cancer, antinociceptive, antioxidant, and antiarthritic	[22]
	9, 12, 15-octadecatrienoic acid, (Z, Z, Z)-	$C_{18}H_{30}O_2$			Anti-inflammatory, cancer preventive, and antiarthritic	
	squalene	$C_{30}H_{50}$			Antioxidant, antitumor, chemopreventive effect, and Anti-inflammatory	

Quantitative phytochemical analysis

Quantification of phytochemical constituents of the MCV plants has been carried out to check the differences in the concentrations of Alkaloids, Flavonoids, Phenol, Saponin, Steroids and Tannins. The total phytochemical content can be quantified in the MCV plant extract prepared using suitable solvent. According to Mokhtar et al. [38], standard anti-inflammatory agents like Gallic acid and quercetin were used as standards with some modifications to the Folin-Ciocalteu method. whereas, tannic acid was used as a standard anti-inflammatory agent according to the method described by Folin-Ciocalteu, slightly altered by Mesfin and Won [39]. Shimadzu UV-1700 spectrophotometer (Tokyo, Japan) was used to measure the absorbance of extracts.

The total phenolic content was identified using the standard gallic acid solution in the dilutions from 0.0-1.0 mg/mL. Nearly 600 µL of Folin's reagent with 1.0 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) was added into 200 µL of the MCV extract. After the incubating the mixture for 2 h in the dark, the absorbance was measured at 765 nm against the different dilutions of the standard solution which gives the total phenolic content that is expressed in terms of gallic acid in mg GAE/mL of extract. Figure 1 shows the total phenolic content of MCV in free phenolic, soluble bound, and insoluble bound extracts [40]. According to the results, the insoluble bound extract (4.94 0.042 mg GAE/g sample) contained the most phenolic extracts, followed by soluble bound extracts (3.16 0.28 mg GAE/g sample) and free phenolic extracts (0.98 0.07 mg GAE/g sample). There was a significant difference ($p < 0.05$) between free phenolic and soluble bound extracts, free phenolic and insoluble bound extracts, and between soluble bound extracts and insoluble bound extracts within group [40].

The total flavonoid content was determined by mixing 600 µL of methanol (CH_3OH), 40 µL of 10% (w/v) aluminum chloride ($AlCl_3$), 40 µL of 1 M potassium acetate (CH_3COOK), and 1.12 mL of Milli-Q water with 200 µL of the extract. The mixture was then incubated for 30 minutes at room temperature. Absorbance was measured at 420 nm and compared to a standard solution of quercetin dilutions ranging from 0.0 to 1.0 mg/mL. To estimate the total tannin content, 250 µL of Folin's reagent, 500 µL of 35% (w/v) Na_2CO_3, and 3.75 mL of distilled water were combined with 500 µL of the extract, followed by a 30-minute incubation at room temperature. The absorbance was then measured at 725 nm using tannic acid dilutions (0.0-1.0 mg/mL) as a standard solution. The total tannin content was quantified in terms of tannic acid equivalent (TAE) concentration in mg/mL of extract [21]. Table 3 indicates the total phenolic content, total flavonoid content, and total tannin content by Zambari et al. [21]. From Table 3 it can be noticed that the total phenolic content in ethanolic extracts of GMCV and RMCV were found to be 29.25 ± 0.50^a and 24.16 ± 0.50^a respectively. The total flavonoid content (mg QE/mL) values of ethanolic extract of GMCV was 1.57 ± 0.03^a while for RMCV was 1.31 ± 0.04^a. for respectively which clearly indicate that the flavonoid content in GMCV is higher than RMCV. The total tannin content in mg TAE/mL was found to be 22.70 ± 3.15ns in GMCV and 17.19 ± 0.32ns in RMCV extract. Overall, it can be deduced that in the phytoconstituent values for ethanolic extracts of GMCV were higher than the RMCV extract.

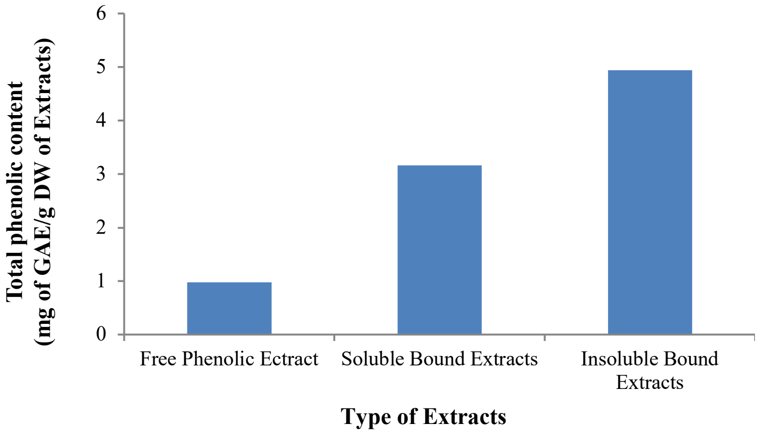

Figure 1. The total phenolic content (TPC) of C. vespertilionis in free phenolic, soluble bound and insoluble bound extracts [40]

Table 3. Total phenolic, flavonoid and tannin content in GMCV and RMCV ethanolic leaf extracts

MCV Leaf Extract	Total Phenolic Content (mg GAE/mL)	Total Flavonoid Content (mg QE/mL)	Total Tannin Content (mg TAE/mL)
Green	29.25 ± 0.50^a	1.57 ± 0.03^a	22.70 ± 3.15^{ns}
Red	24.16 ± 0.50^a	1.31 ± 0.04^a	17.19 ± 0.32^{ns}

All values are triplicate with mean ± SD. a, refer to *p* <0.05, a significant difference between green and red leaf extract, using a two-way ANOVA test, while ns is a non-significant

Bioassay Studies

2, 2-diphenyl-1-picrylhydrazyl (DPPH) study

Antioxidant activity is often quantified and evaluated using stable DPPH scavenging activity. This method is beneficial over others because it requires less time to reduce the DPPH form to the DPPH non-radical state through hydrogen atom donation [41]. Reduced DPPH can be evaluated by the antioxidants' ability to reduce the absorbance at 517nm. The physical change that occurs during DPPH assessment is the shift in color from purple to yellow [24]. Polyphenols with hydroxyl groups have antioxidant capabilities that scavenge the DPPH radical. The hydroxyl group, specifically, functioned as the main antioxidant. Another screening investigation was conducted using several solvent extracts (hexane, hexane: ethyl acetate, ethyl acetate, and ethyl acetate: methanol), with the ethyl acetate: methanol extract showed the greatest DPPH scavenging activity and the lowest IC_{50} value of 0.549±0.02 mg/mL [5]. Table 4 summaries the antioxidant activity of the MCV extracts and fractions as determined by the DPPH radical scavenging activity based on the IC_{50} values for the DPPH radical scavenging activity [22]. The ethyl acetate root extract and the F6 fraction were found to have the greatest levels of free radical scavenging activity [22]. By comparing the results of the test to previous studies, ascorbic acid (vitamin C) and tocopherol (vitamin E) were used as natural standards to verify the analysis [42].

Table 4. Scavenging activity of C. Vespertilionis crude Extracts, fraction, and standards DPPH free radicals [23]

DPPH IC_{50} (µg/mL)			
	Root Extract		**Leaf Extract**
Hexane	>2000		>2000
Chloroform	338.07 ± 3.32^{b}		$679.43 \pm 4.72^{a,A}$
Ethyl acetate	70.16 ± 1.49^{a}		$644.90 \pm 20.09^{a,A}$
Methanol	421.73 ± 5.40^{c}		$716.37 \pm 16.46^{a,B}$
Ethyl Acetate Root Fraction			
F1	>2000	F5	85.28 ± 1.13^{a}
F2	1786 ± 14.73^{e}	F6	76.71 ± 0.29^{a}
F3	169.63 ± 4.89^{b}	F7	235.63 ± 11.75^{c}
F4	101.53 ± 1.47^{a}	F8	1157.33 ± 22.50^{d}
Standards			
Ascorbic	16.99 ± 1.22	α-tocopherol	10.49 ± 0.98

Antiplasmodial and Antimalerial activity

The *in vitro* anti-plasmodial assays are normally assessed to evaluate the antimalarial activity (Table 5). Upadhyay et al. [15] performed anti-plasmodial assays of MCV root, leaf, and stem extracts (prepared using methanol and aqueous-methanol in 1:4, v/v) against *P. falciparum* (NF-54 strain) that was cultured in human type O-positive red blood cells while the dose response curves prepared from percentage inhibition values versus concentration (Graph Pad Prism (Version 4.0)) revealed the IC_{50} values. It is found out that the aqueous-methanolic (1:4, v/v) extract of the stem was the most powerful [15]. The *in vitro* anti-plasmodial activity exhibited relatively similar TLC profiles even when different extracts were used. Thus, *in vivo* assessment of antimalarial activity of the combined extracts of MCV plant was carried out using *Plasmodium berghei* K173 (sensitive to chloroquine at 10 mg/Kg) [15] that showed suppressed parasitaemia by 87.8%, compared to chloroquine, which suppressed parasitaemia completely on day 8 [15]. Another study by Nguyen-Pouplin et al. [13], MCV plant exhibited anti-plasmodial activity with IC_{50} values ranging from 10 to 20 µg/ml [13].

Malarial SYBR Green I fluorescence-based (MSF) assay is another method was used to determine the antimalarial activity of MCV. MCV methanol extract (CVME) was used in the subsequent antimalarial and anticancer activity assays [16]. NF-54 strains of *P. falciparum* used against CVME for inhibition activity. A graph plotted as the logarithm of CVME concentration versus the percentage of parasite inhibition was generated and the half-maximal inhibitory concentration (IC_{50}) of CVME was determined by using probit regression analysis with GraphPad Prism software (Version 6) represented in Figure 2. The antimalarial activity of a plant extract can be classified as promising (IC_{50} < 15 µg/mL), moderate (15 < IC_{50} < 50 µg/mL) and inactive activity (IC_{50} > 50 µg/mL). CVME has a moderate antimalarial activity (IC_{50} = 43.87 ± 2.04 µg/mL). Artemisinin as a standard antimalarial drug was potent in inhibited the parasite growth with an IC_{50} value of 4.0 ± 0.22 µg/mL. The methanolic leaf extract has been proven to yield antimalarial activity [16].

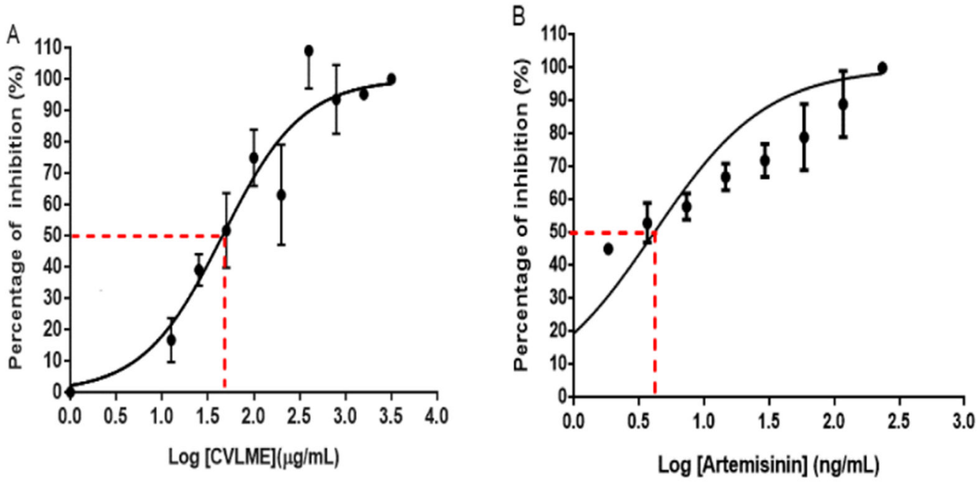

Figure 2. In vitro susceptibility of P. falciparum to (A) CVME and (B) artemisinin [16]

Table 5. Anti-plasmodial activity against NF-54 Plasmodium falciparum strain [15]

SI No.	Extract/compound	IC_{50} *(µg/mL)
1	Methanolic extract of roots (MR)	60.5
2	Methanolic extract of leaf (ML)	32.0
3	Methanolic extract of stem (MS)	62.0
4	Aqueous-methanolic extract of roots (AR)	78.5
5	Aqueous-methanolic extract of leaf (AL)	63.0
6	Aqueous-methanolic extract of stem (AS)	7.5
7	Compound 1	9.0
8	Compound 4	72.0
9	Compound 13	84.0
10	Chloroquine (Standard)	0.024
11	Artemisinin (Standard)	0.0088

Cytotoxicity

As helpful as medicinal plants are, some of them may also show harmful effects depending on the type of toxicant present and its abundance. As a result, it is critical to assess the toxicity of the therapeutic plant extracts being investigated for its pharmacological activities. In a 28-day subacute oral toxicity trial, the researchers used an ethanolic extract of MCV leaf at doses of 75, 125, and 250 mg/kg. Both treated and control rats had no significant changes in serum biochemical and hematological markers. Moreover, histological examination of the kidney and liver revealed no substantial lesions in the kidney tissues of any of the treatment groups. However, mild to severe hepatic necrosis was detected in all three doses, respectively. Apart from that, all three groups showed moderate hepatic deterioration and eventual hepatitis. The plant may include toxicant(s) that contributed to these observations, or it may contain chemicals that interact antagonistically [16]. A study by Lee et al. [22] suggested that the F3 fraction of the ethyl acetate root extract of MCV consist of flavonoids, quinones, and coumarins that were responsible toward the cytotoxicity specifically against MDA-MB-231 cells, indicating anti-breast cancer activity (Figure 3) [22].

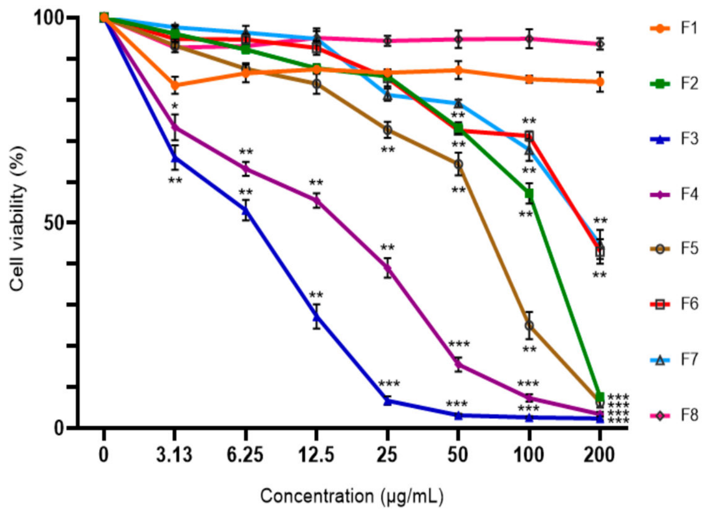

Figure 3. Cytotoxicity of MCV ethyl acetate root fractions (F1–F8) determined using MTT assay on MDA-MB-231 (All values are expressed as mean ± SD and data with asterisks are significantly different at $p < 0.05$ when compared with the untreated group)

Antidiabetic activity

The inhibition of the intestinal enzyme, a-glucosidase will reduce postprandial hyperglycemia which is a common problem for diabetic people. The a-glucosidase inhibitory assay test was adapted from Murugesu et al. and carried out as an improved method [5] (Table 6). Quercetin, a natural a-glucosidase enzyme inhibitor was used as the positive control while PNPG (4-Nitrophenyl-β-D-glucopyranoside) used as the substrate. The linear regression analysis method was used to determine the half maximal inhibition concentration (IC_{50}), in which the concentration of the extract required to inhibit 50% of a-glucosidase activity under the assay conditions was calculated [43]. The HE extracts of MCV shows the highest a-glucosidase inhibitory with IC_{50}: 0.195 mg/mL while M extract expressed the least potential with IC_{50}: 0.416 mg/mL). These results are influenced by the compound present in the extract most probably terpenoids. Outwardly, polarity of solvent at different level may attract various class of compounds that tend to exhibit their distinctive pharmacological properties. Less interference of antagonistic occur between the phytoconstituents in the matrix [5]. Hence, the MCV leaf extracts can be recommended as potential medicine against antidiabetic.

Table 6. AGI IC_{50} value of α-glucosidase inhibitory activity of MCV leaves extracts [5]

SI No.	Extract	AGI IC_{50} (mg/mL) ± SD
1	Hexane (H)	0.201 ± 0.024^{c}
2	Hexane- Ethyl Acetate (HE)	0.195 ± 0.005^{c}
3	Ethyl Acetate (E)	0.211 ± 0.011^{c}
4	Ethyl Acetate- Methanol (EM)	0.370 ± 0.015^{b}
5	Methanol (M)	0.416 ± 0.015^{a}
6	Ascorbic Acid (AA)	nd
7	Quercetin	0.005 ± 0.001^{d}

nd – not detected

Ferric reducing antioxidant activity

The antioxidant activity of the MCV leaf extracts was determined using FRAP (Ferric Reducing Antioxidant Power) assay method adapted Alam et al. [43] with some improvements. The FRAP reagent consist of 0.001M of TPTZ dissolved in 0.004 M HCl, 0.002 M $FeCl_3$ (2.5 mL) and 0.1 M acetate buffer (25 mL) with pH of 3.6, incubated for 10 min at 37 °C [5]. Ascorbic acid was used as the positive control. The highest ferric reducing antioxidant power exhibited by the hexane: ethyl acetate extract. The FRAP value measured to be ranged from 190.60 to 271.67 AAE/g with the HE extracts (271.67 AAE/g) showing the potential ferric reducing antioxidant power. Thus, it can be proved that MCV leaves contain potent antioxidant's ability.

Table 7. FRAP value of MCV leaf extracts [5]

SI No.	Extract	FRAP (AAE/g) ± SEM
1	Hexane (H)	190.60 ± 1.94^{e}
2	Hexane- Ethyl Acetate (HE)	271.67 ± 0.93^{a}
3	Ethyl Acetate (E)	234.05 ± 0.89^{c}
4	Ethyl Acetate- Methanol (EM)	245.55 ± 1.67^{b}
5	Methanol (M)	205.16 ± 1.67^{d}
6	Ascorbic Acid (AA)	194.32 ± 1.39^{e}

Anticancer activity

Research on MCV examines its anticancer potential against various cell lines such as HaCaT (keratinocyte), HepG2 (liver carcinoma), MCF-7 (breast cancer), WRL68 (normal liver), and CRL 2522 (fibroblast). Ethanolic leaf extract showed viability against multiple cell lines, with promising results in combination with cyclophosphamide. Isoorientin, a prominent compound, contributes to its activity, inducing apoptosis and altering cell pathways. Similarly, ethyl acetate and chloroform root extracts demonstrated dose-dependent cytotoxicity against breast cancer cell lines, possibly through different apoptosis mechanisms involving flavonoids and triterpenes. The anticancer potential of MCV root extracts, specifically ethyl acetate and chloroform extracts, was assessed on hormone receptor-positive (MCF-7) and triple-negative (MDA-MB-231) breast adenocarcinoma cell lines. Both extracts exhibited dose-dependent cytotoxic effects, with IC_{50} values of 11.34 ± 1.20 and 29.58 ± 3.80 µg/mL against MDA-MB-231, and 44.65 ± 5.78 and 54.55 ± 9.51 µg/mL against MCF-7, respectively. The differential efficacy observed may stem from varying apoptosis mechanisms attributed to bioactive compounds in the extracts. Flavonoids likely undergo metabolism primarily via the CYP1A1 pathway in MCF-7, while MDA-MB-231 metabolizes them through the CYP1B1 pathway. Additionally, previous research suggests triterpenes induce apoptosis in breast cancer cells through P53-dependent and P53-independent signaling in MCF-7 and MDA-MB-231, respectively. Overall, these findings suggest MCV extracts as potential anticancer agents [34].

Anti-Inflammatory activity

MCV leaf extract has been reported to have phytoconstituents responsible for anti-inflammatory activity. Oyedapo [44] also stated that phenolic compounds, such as flavonoids and tannins, are primarily responsible for anti-inflammatory activity. As protease plays key a role in inflammation and is one of the biological mediators exhibited during inflammation thereby regulating the inflammation complex system via cleaving chemokines, cytokines, and immune components. Therefore, the anti-inflammatory activity can be deduced by correlating the protease inhibition assay with the total phenolic content. According to Zambari et al [21] out of the 2 plant variations of MCV (GMCV and RMCV), GMCV leaf extract has known to contains a greater number of phytoconstituent that lead to suppress inflammation. To test the anti-inflammatory activity the 2 varieties of MCV extract was subjected to *in vitro* protease inhibition activity assay. Table 8 shows the comparative values for GMCV and RMCV against protease inhibition. High protease inhibition value indicates the capability of the substance to treat inflammation. From Table 8, it is clear that the GMCV showed a higher inhibition of 66.55 ± 2.59% and IC50 value of 284.59 ± 10.63 µg/mL, compared to RMCV with 62.63 ± 2.49% inhibition and IC50 value of 226.59

± 10.35 µg/mL. The correlation analysis of the protease inhibition assay with total phenolic content has also revealed positive relationships [21]. Thus, it can be inferred that the phenolic compounds in MCV leaf extract particularly the GMCV extract, contribute to the anti-inflammatory activities.

Table 8. Protease inhibition activity assay in GMCV and RMCV extract with aspirin as a control [21]

Sample	Inhibition (%)	IC_{50} values (µg/mL)
GMCV	66.55 ± 2.59	284.59 ± 10.63^{a}
RMCV	62.63 ± 2.49	226.59 ± 10.35^{a,b}
Aspirin	75.66 ± 0.99	275.72 ± 6.93^{b}

All values are triplicate with mean ± SD. a and b; each refers to $p < 0.05$, a significant difference between different samples, using a two-way ANOVA test

Conclusion

MCV, a commonly available herb in Malaysia is found in 2 varieties namely GMCV and RMCV. Both varieties show comparable beneficial phytoconstituents like alkaloids, flavonoids, proteins, glycosides, tannins, phenols, saponins, diterpenes, coumarins, steroids, and quinones that can be effectively contributed to pharmacological actions. The maximum phytoconstituents were found using methanol followed by ethyl acetate. However, their activity varies based on the solvent utilized for extraction. The MCV plant extract has proven its anticancer, antioxidative, antimalarial, anti-inflammatory, and antidiabetic activities through various bioassays. Although, current review states several therapeutic properties of chemical compounds present in MCV plants, there is a still lot of scope to study several other chemical compounds particularly the flavonoids groups whose pharmacological activities has not been reported. Hence, further studies on this plant can be carried out for the bio-guided fractionation, structural elucidation of the active compounds and need to be extended in every aspect for future pharmaceutical applications.

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