



## Antibacterial, Antioxidant, Anti-inflammatory, and Anti-acetylcholinesterase Activity of *Mikania scandens* (L.) Willd (Climbing Hempvine)

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**Abstract:** *Mikania scandens*, known as Climbing hempvine has been used in many countries as traditional medicines. In this study, hexane, ethyl acetate and ethanol extracts from *Mikania scandens* (L.) Willd. tested its anti-cholinesterase, antioxidant, anti-inflammatory, and antibacterial activity. Anti-inflammatory activity is conducted by the 5-lipoxygenase inhibition activity, while the activity of anti-cholinesterase is determined by the TLC-bioautography test. Antioxidant activity was measured using FRAP, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and bleaching test of  $\beta$ -carotene methods. Antibacterial activity was evaluated with pour plate diffusion test, death kinetic assay, minimum bactericidal concentration, and minimum inhibitory concentration. The ethanol extract showed potential as antioxidant and anti-inflammatory activity. The hexane extract showed the highest bacterial growth inhibition, meanwhile hexane and ethanol extracts generate the best acetylcholinesterase inhibitory activity. The total value of the phenolic content was measured using Folin-Ciocalteu method and the value is in the range of 106.67 mg/g to 1066.67 mg/g equivalent gallic acid. The results show that *Mikania scandens* (L.) Willd. can provide a natural source of anti-cholinesterase, antioxidant, anti-inflammatory, and antibacterial agents.

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**Keywords:** antibacterial; antioxidant; anti-inflammatory; anti-cholinesterase; *Mikania scandens*; phenolics

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### INTRODUCTION

The Asteraceae family commonly referred to as the Sunflower family is a very large and widespread family of flowering plants that have medicine potential values. This family is known to consist of phytochemical compounds which the potential to have antipyretic, analgesic, antioxidant, antimicrobial, and anti-inflammatory properties (Odom et al., 2000). One of the valuable species in the Asteraceae family was *Mikania scandens*. The genus *Mikania* Willd., the invasive plant consists of 430 species distributed around the world. *Mikania scandens* is known as Climbing Hempvine normally found in tropical Asia (Sri Lanka, Indonesia, Malaysia, Mauritius,



Philippines, and Pakistan) (Latif et al., 1984). This vine origin from North America and is used as a vegetable in Malaysia and Indonesia. *Mikania scandens* is known as Climbing Hempvine normally found in tropical Asia (Sri Lanka, Indonesia, Malaysia, Mauritius, Philippines, and Pakistan (Parker, 1972; Sankaran, 2007). Climbing hempvine is elaborates some flavonoids, triterpenes, sesquiterpene lactones, kaurane diterpene, and phytosterols (Ghani, 2003). In India, a decoction of this plant used to treat stomach ulcers and other inflammatory diseases (Singh et al., 2010). Extracts of this plant exhibited antipyretic, vulnerary, and anticarcinogenic activities (Burkill, 1985; Ysrael and Croft, 1990; Bishayee and Chatterjee, 1994).

Antibiotic resistance is one of the major issues for the present days. Hence, the last decade researcher did the investigation of plants as a source of human disease management (Prashanth et al., 2001). Secondary metabolites, which synthesize from plants play a key role in the natural defense mechanism against microorganisms and insects (Cowan, 1999). Since, climbing hempvine known to have the potential to treat stomach ulcers and fever therefore this research aim to know the antibacterial potency of Climbing hexane extracts.

Inflammation is a body process to a response to bacterial attacks, wounds, or irritants (Khan et al., 2010). Inflammation itself plays an important role in degenerative diseases, therefore the finding of anti-inflammatory agents from natural sources in the prevention and treatment of degenerative diseases has been prioritized (Aggarwal and Harikumar, 2008). In this research, hexane, ethyl acetate and ethanol extracts of the aerial part of this plant were tested for their anti-cholinesterase, anti-inflammatory, antioxidant, and antibacterial activities, in an attempt to find the therapeutic value of climbing hempvine.

## MATERIALS AND METHODS

*Sample preparation:* The aerial parts of climbing hempvine were collected from Broga, Selangor Malaysia. Dried powder (300 g) was extracted sequentially using nonpolar solvent (hexane), semi-polar (ethyl acetate), and polar solvent (ethanol). Extracts concentrated using a rotary evaporator (Buchi, USA). Extracts were store at -20°C before the further test was conducted. Hundred mg/ml of each extract was dissolved in DMSO (dimethyl sulfoxide) for stock solutions.

*Antibacterial assays - Pour Plate Disc Diffusion Assay:* *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Bacillus cereus* ATCC 11778, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 11632, and *Citrobacter freundii* ATCC 8090 were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Soft Mueller Hinton Agar (MHA) inoculated with 100 µl of inocula (1 x 10<sup>8</sup> microorganism/ml) was spread on solidified MHA. Sample (1 mg) was impregnated on sterile filter paper discs (6 mm) and placed on top of the inoculated agar plates. The plates kept in an incubator at 37°C for 24 hours (Mayachiew and Devahastin, 2008). The experiment was done in duplicate in three independent experiments. The diameter zone of inhibition ± SD was expressed as antibacterial results. Streptomycin (5 µg), tetracycline (5 µg), and ampicillin (5 µg) were used as antibiotic controls because of their broad spectrum of actions.

*Antibacterial assays - Determination of minimum inhibition concentration (MIC):* This assay was conducted according to Otzurk and Ercisli (2006). The extracts were prepared at concentrations ranging from 23 to 3000 µg/ml in 96-well microplates. The microplate wells were incubated in an incubator shaker at 180 rpm for 24 hours at 37°C. The reading of the absorbance at 600 nm was taken with a spectrophotometer (Dynex MRX-Revelation, USA). The MIC values were identified as the lowest concentration of sample inhibits bacteria growth. Duplicate tests were performed for



each microorganism to appreciate the reproducibility of the results. Serial dilution of streptomycin (0.78125 -100 µg/ml), ampicillin (0.78125 -100 µg/ml) and tetracycline (0.15625 -20 µg/ml) were used as standard drugs for positive controls.

*Antibacterial assays - Death kinetic assay:* *Escherichia coli* and *Staphylococcus aureus* were used for this assay. From a stock solution of 100 mg/ml, a required volume was pipetted into MHB to obtain a concentration range of 0.01 up to 10 mg/ml. Readings of the absorbance were taken at 0 min and every 30 minutes for 16 hours using a spectrophotometer (Dynex MRX-Revelation, USA). EC<sub>50</sub> values of the plant extracts, which are the concentration of samples (plant extracts or antibiotics) that kills 50% of the population of microorganism, were determined from the graph of dose against a percentage of survival index (SI) (Noga, 1994).

*Antioxidant assays - 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay:* The assay was done in 96 well plates. The sample was prepared at 1 mg/ml to 0.003 mg/ml and 20 µL pipetted into each well. A total of 180 µL DPPH (0.1 mM) were added into each well. The plates were kept in the dark at room temperature for 30 minutes. The absorbance was measured at 550 nm. The antioxidant potency was measured using the % Scavenging effect (Juan-Badaturuge et al., 2011).

*Antioxidant assays - Ferric Reducing Power Antioxidant (FRAP) assay:* FRAP reagent was prepared freshly by mixing 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 300 mM acetate buffer (pH 3.6) in a 1:10:1 ratio. FRAP reagent (180 µL) was added with the sample (20 µL). The absorbance was read at 593 nm using a spectrophotometer (Dynex MRX-Revelation, USA) after the sample was allowed to stand for 90 minutes. Ferrous sulfate was used for the standard with concentrations in the range 1 µM to 125 µM meanwhile Trolox and quercetin were used as positive controls. Antioxidant activity was expressed as a FRAP value, the concentration of sample which produced an absorbance value equal to 1 mM of FeSO<sub>4</sub> (Benzie and Strain, 1996).

*Antioxidant assays - β-Carotene Bleaching Assay:* Two mg of β-carotene was dissolved in 10 mL of chloroform and two mL of this solution was pipetted into a 100 mL round-bottom flask. Tween 80 (400 mg), linoleic acid (40 mg), and distilled water (100 mL) were added to the flask with vigorous shaking, after removal of chloroform. The absorbance was recorded every 20 min for 240 minutes at 490 nm. The antioxidant potency of the sample was expressed as the concentration with exhibit 50% of the antioxidant activity (EC<sub>50</sub>) (Miller, 1971).

*Acetylcholinesterase Inhibitory– TLC Bioautography Assay:* Fresh spray reagent was prepared by mixing forty mL of fast blue B salt and ten mL of naphthyl acetate. Samples were prepared at the concentration of 10 mg/ml then 10 µL were applied to silica thin layer chromatography (TLC) plates. Plates were eluted with proper solvent and let it dry. The TLC plate was sprayed with the enzyme solution (acetylcholinesterase 500 U) and kept in a water bath (37 °C) for 20 minutes. This condition allowed the completion of the enzymatic reaction. After the plate kept in a water bath for 20 minutes, the spray reagent was then sprayed onto the TLC plate. White spots against a purple background occurred as an acetylcholinesterase inhibitor (Baylac and Racine, 2003).

*Antiinflammatory Assay – 5-Lipoxygenase Assay:* Extract with the concentration of 50 mg/mL were prepared. Extract (5 µL ) was mixed with phosphate buffer (pH 9; 570 µL) and linoleic acid (17 µL) in a 1 ml cuvette maintained at 25 °C. The initiation of the enzyme reaction was induced after adding 4 µL of the aliquot enzyme and 4 µL of the phosphate buffer (4 °C). Absorbance was measured at 234 nm over a period of 10 minutes (Noga, 1994). Nordihydroguaiaretic acid (NDGA), as positive control was used in this method. The percentage activity of the enzyme was



determined using the slopes of the straight-line portions of the sample and the control curves (Rhee et al., 2001).

*Total phenolic contents:* Twenty  $\mu\text{L}$  of diluted extracts were mixed with water (1.58 mL) and Folin-Ciocalteu reagent (100  $\mu\text{L}$ ) and 300  $\mu\text{L}$  of sodium carbonate (20% w/v) were added after the mixed sample was standing for 5 minutes at room temperature. The absorbance was determined at 765 nm after the solutions were mixed and stand for 30 minutes at 40°C. Gallic acid with the ranging concentration of 50, 100, 150, 250 mg/mL was used for a calibration standard curve. Results were expressed on a fresh weight basis of mg gallic acid equivalents/g of sample (Slinkard and Singleton 1977).

*Data Analysis:* Data were calculated and showed as mean  $\pm$  standard deviation. The significant difference was analyzed using one way ANOVA and followed by the Tukey test ( $P < 0.05$ ).

## RESULTS

Table 1 summarizes the diameter inhibition zones of climbing hempvine against six bacteria tested. Hexane, ethyl acetate, and ethanol fractions showed activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. All the extracts tested gave MIC and MBC value in the range of 0.38 mg/mL – 6 mg/mL (Table 2). The ethanol extract abrogated the survival of 5 of the 6 bacterial strain tested and was most active against *Bacillus cereus*. In the death kinetic assay, the lowest value of EC50 means the highest antibacterial properties. The hexane extract gave the lowest EC50 value of 1.32 mg/mL against *Staphylococcus aureus* and 1.57 mg/mL against *Escherichia coli*, respectively (Table 3).

The antioxidant activities of climbing hempvine extract were tested using 3 antioxidant methods. The ethanol extract exhibited the strongest antioxidant properties amongst 3 fractions (Table 4). The rank order of antioxidant potency observed was ethanol > ethyl acetate > hexane extract. Total phenolic content provides linier correlation result with antioxidant properties (Figure 1).

Table 1. Diameter of inhibition zone of climbing hempvine extracts

Sample		<sup>a</sup> Inhibition zone (mm)					
		Ec**	Cf**	Sa**	Se**	Bs**	Bc**
<i>Mikania scandens</i> (L.) Willd.	H*	6.00 $\pm$ 0.00	6.28 $\pm$ 0.34	9.95 $\pm$ 1.60	6.80 $\pm$ 0.57	6.65 $\pm$ 1.30	7.60 $\pm$ 0.71
	EA*	6.00 $\pm$ 0.00	6.20 $\pm$ 0.18	8.66 $\pm$ 1.19	6.68 $\pm$ 0.44	7.33 $\pm$ 1.65	9.37 $\pm$ 0.36
	E*	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00	9.00 $\pm$ 1.67	6.63 $\pm$ 0.43	6.00 $\pm$ 0.00	7.42 $\pm$ 0.70
Streptomycin		11.4 $\pm$ 0.66	12.70 $\pm$ 0.26	6.60 $\pm$ 1.41	6.00 $\pm$ 0.00	16.44 $\pm$ 0.54	14.47 $\pm$ 1.25
Ampicillin		7.02 $\pm$ 0.62	6.00 $\pm$ 0.00	10.45 $\pm$ 0.69	9.42 $\pm$ 0.45	10.77 $\pm$ 1.24	6.00 $\pm$ 0.00
Tetracycline		15.89 $\pm$ 1.68	21.21 $\pm$ 1.38	20.64 $\pm$ 1.39	6.00 $\pm$ 0.00	24.15 $\pm$ 0.97	35.5 $\pm$ 1.85

\*H: Hexane; EA: Ethyl acetate; E: Ethanol

\*\*Ec: *Escherichia coli* ATCC 8739; Cf: *Citrobacter freundii* ATCC 8090; Sa: *Staphylococcus aureus* ATCC 11632; Se: *Staphylococcus epidermidis* ATCC 12228; Bs: *Bacillus subtilis* ATCC 6633; Bc: *Bacillus cereus* ATCC 11778.

<sup>a</sup>Diameter of paper disc 6 mm.



Table 2. Minimum inhibition concentration (MIC) and Minimum bactericidal concentration (MBC) values of climbing hempvine (L.) Willd. extracts

Sample	MIC and MBC values (mg/ml) <sup>a</sup>						
		Ec**	Cf**	Sa**	Se**	Bs**	Bc**
<i>Mikania scandens</i> (L.) Willd.	H*	n.a	n.a	6	n.a	3	0.75
	EA*	n.a	n.a	6	6	3	1.5
	E*	6	6	6	6	n.a	0.38
Streptomycin		6.25 x 10 <sup>-3</sup>	3.13 x 10 <sup>-3</sup>	2.5 x 10 <sup>-2</sup>	0.1	3.13 x 10 <sup>-3</sup>	3.13 x 10 <sup>-3</sup>
Ampicillin		0.1	0.1	3.13 x 10 <sup>-3</sup>	0.05	6.25 x 10 <sup>-3</sup>	0.1
Tetracycline		2.5 x 10 <sup>-3</sup>	2.5 x 10 <sup>-3</sup>	1.25 x 10 <sup>-3</sup>	2 x 10 <sup>-2</sup>	2.5 x 10 <sup>-2</sup>	3.1 x 10 <sup>-4</sup>

\*H: Hexane; EA: Ethyl acetate; E: Ethanol

\*\*Ec: *Escherichia coli* ATCC 8739; Cf: *Citrobacter freundii* ATCC 8090; Sa: *Staphylococcus aureus* ATCC 11632; Se: *Staphylococcus epidermidis* ATCC 12228; Bs: *Bacillus subtilis* ATCC 6633; Bc: *Bacillus cereus* ATCC 11778.

<sup>a</sup> MIC refers to the minimum concentration of an antimicrobial agent that inhibits the growth of the microorganism and MBC is the lowest concentration of antimicrobial that results in ≥ 99.9% killing of the microorganism  
 n.a: not active

Table 3. EC<sub>50</sub> of antibacterial activity of climbing hempvine extracts against *Escherichia coli* and *Staphylococcus aureus*

Sample	*EC <sub>50</sub> (mg/ml)		
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	
<i>Mikania scandens</i> (L.) Willd.	Hexane	1.57 <sup>a</sup>	1.32 <sup>a</sup>
	Ethyl acetate	1.94 <sup>b</sup>	1.54 <sup>b</sup>
	Ethanol	6.90 <sup>c</sup>	2.61 <sup>c</sup>
Streptomycin	1.15 x 10 <sup>-3d</sup>	3.13 x 10 <sup>-3e</sup>	
Ampicillin	2.25 x 10 <sup>-3d</sup>	95.23 x 10 <sup>-3f</sup>	
Tetracycline	0.34 x 10 <sup>-3d</sup>	1.35 x 10 <sup>-3e</sup>	

Values in the same column with the same superscript letter are not significantly different (P<0.05) according to Tukey multiple comparison test.

\*EC<sub>50</sub> represents the effective concentration at 50% of total antibacterial activity.

Table 4. Antioxidant properties and total phenolic contents (TPC) of climbing hempvine extracts.

Sample		FRAP assay	DPPH assay	B-carotene bleaching assay	TPC
		<sup>a</sup> FRAP value (mg/ml)	<sup>b</sup> IC <sub>50</sub> (mg/ml)	<sup>c</sup> EC <sub>50</sub> (mg/ml)	[Gallic acid equivalent (mg/g)]
<i>Mikania scandens</i> (L.) Willd.	H*	13.00 ± 0.04A	0.50 ± 0.01A	96.48 ± 0.64A	106.67 ± 0.03A
	EA*	2.57 ± 0.01B	0.28 ± 0.01B	26.67 ± 0.29B	441.56 ± 0.03B
	E*	1.41 ± 0.03C	0.14 ± 0.02C	5.87 ± 0.59C	1066.67 ± 0.01C
Quercetin		1.31 ± 0.03E	11 x 10 <sup>5</sup> ± 0.00E	0.13 ± 0.17E	
Trolox		1.14 ± 0.02F	9 x 10 <sup>5</sup> ± 0.00E	0.05 ± 0.17E	

Data were obtained from three independent experiments, each performed in triplicates (n=9) and represented as mean ± SD.

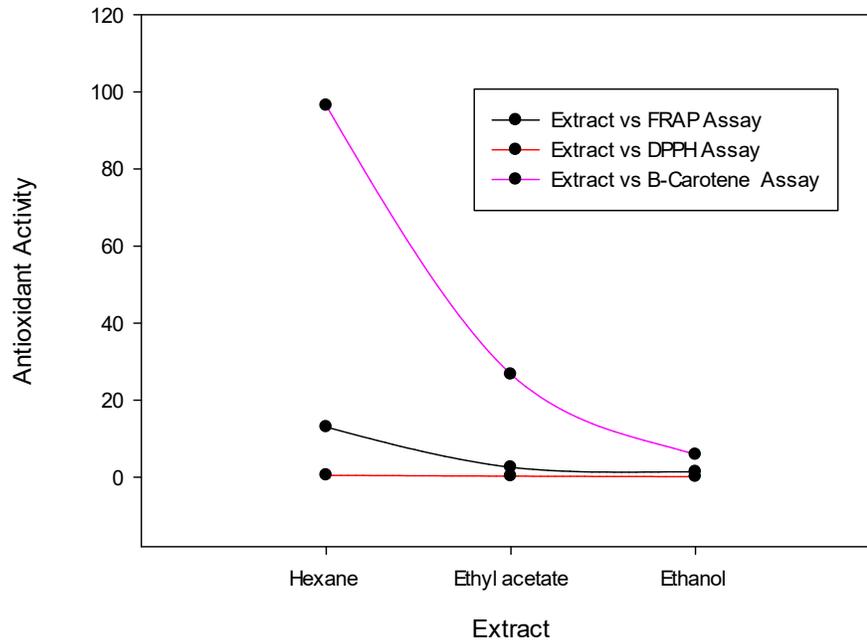
Values with the same letter are not significantly different (P<0.05) according to Tukey multiple comparison test.

<sup>a</sup>FRAP value was calculated as Ferrous Equivalents, the concentration of trolox/queretin or extracts which produced an absorbance value equal to that of 1 mM Fe<sub>2</sub>SO<sub>4</sub>.

<sup>b</sup>IC<sub>50</sub> was the concentration of substance that provides 50% inhibition

<sup>c</sup>EC<sub>50</sub> represents the effective concentration at 50% of total antioxidant activity.

\*H: Hexane; EA: Ethyl acetate; E: Ethanol



The *in vitro* anti-inflammatory activity of climbing hempvine extract are shown in Table 5. Lower IC<sub>50</sub> 5-LOX values indicate greater anti-inflammatory activities. The ethanol extract displayed the highest inhibitory activity, with an IC<sub>50</sub> value of 23.87 µg/ml. Hexane and ethanol extracts showed the presence of potential compound as acetylcholinesterase inhibitor (Table 5).

Table 5. Anti-inflammatory and acetylcholinesterase activities of climbing hempvine crude extracts.

Sample		5-Lipoxygenase assay	Acetylcholinesterase assay
		<sup>a</sup> 5-LOX IC <sub>50</sub> (µg/ml)	Presence of the white spot
<i>Mikania scandens</i> (L.) Willd.	Hexane	50.16 ± 0.02	+
	Ethyl acetate	449.4 ± 0.03	-
	Ethanol	23.87 ± 0.12	+
	NDGA	5.33 ± 0.05	

<sup>a</sup> 5-LOX IC<sub>50</sub> was the concentration of substance that provides 50% inhibition of 5-lipoxygenase

Yields of different extracts obtained from 300 g of dried milled plant materials were 0.92 % (hexane), 0.94 % (ethyl acetate), and 2.32 % (ethanol). The antibacterial result showed that Gram-positive bacteria were more susceptible to the extracts compare to the Gram-negative bacteria. This may be attributed to the peculiar ultra-structure of the Gram-negative bacteria wall (Negi and Jayaprakasha, 2003), but the uniformly diffuse ability of the inhibition of the antibacterial compounds through the agar should also be considered (Rauha et al., 2000). The MIC and MBC values of climbing hempvine extracts against the six bacteria tested were in the range of 0.38 mg/mL to 6 mg/mL (Table 2). According to previous report, a plant extract which give MIC values lower than 0.5 mg/ml considered as a strong inhibitor, while moderate inhibitors give MIC values between 0.6 - 1.5 mg/mL, and weak inhibitors give MIC values above 1.6 mg/mL (Duarte, 2007; Wang et al., 2008; Sartoratto et al., 2004). The hexane and ethyl acetate extracts presented some level of antibacterial activities and the ethanol extract demonstrated strong antibacterial activities against *Bacillus cereus*, which is a causative agent of food poisoning (Lund et al., 2008). An antibacterial study conducted by Cannillac and Mourey (Cannillac and Mourey,



2001) suggested that, the strain is considered to be susceptible if the MBC/MIC ratio is less than or equal to 4 and the strain is considered to be tolerant if this ratio is greater than 4. In our study, the MBC/MIC ratios of samples were less than 4 (Table 2). In the death kinetic assay, the hexane extract of climbing hempvine showed remarkable EC<sub>50</sub> values against *Escherichia coli* and *Staphylococcus aureus* which are causative agents of foodborne poisoning and abscesses, respectively (Table 3) (Lund et al., 2008).

Antioxidant properties of climbing hempvine extracts were determined as reducing power (FRAP assay), ability to prevent oxidation of linoleic acid ( $\beta$ -carotene bleaching assay), and free radical scavenging ability (DPPH assay). The results are presented in Table 4. FRAP assay is commonly used to determine the ferric reducing ability of biological fluids and active compounds from plants (Cruz et al., 1996; Alothman et al., 2009). Expressed in FRAP value, a low FRAP value indicates a high antioxidant activity. The principal of the  $\beta$ -Carotene bleaching method is based on the fact that  $\beta$ -Carotene will act as a scavenger for a free-radical which produces by linoleic acid which makes  $\beta$ -Carotene becomes colorless (Krinsky, 1989). This assay is usually employed to test for lipophilic antioxidants. The EC<sub>50</sub> values represent the concentration at which 50% of  $\beta$ -carotene is reduced and low EC<sub>50</sub> value indicates the greater antioxidant activity. In this assay, the ethanol extract displayed the highest antioxidant activity with an EC<sub>50</sub> value equal to 5.87 mg/mL. According to the "polar paradox" the more hydrophilic of the sample would have shown a lower antioxidant activity. This phenomenon occurred because lipid oxidation occurs at the water/oil interface, where lipophilic antioxidants are located. However, in our study, using  $\beta$ -carotene bleaching assays the crude ethanol extract exhibited higher antioxidant activity than the ethyl acetate and hexane extracts. This might be due to the value of total phenolic contents in the ethanol extract which is higher than the hexane and ethyl acetate extracts and ethanol was a universal solvent that dissolves almost all secondary metabolites with low molecular weight. The DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical with two major mechanisms: by reduction via electron transfer or by hydrogen atom transfer. In our study, the ethanol extract showed the highest antioxidant activity shown as the lowest IC<sub>50</sub> value (0.14 mg/mL). Overall, the ethanol extract of climbing hempvine displayed profound antioxidant properties in vitro (Figure 1).

TLC bioautography method, one of the methods which are rapid and simple to show the presence of acetylcholinesterase inhibitors on plant extracts. The method relies on the reaction of 1-naphthol (forming from the cleavage of 1-naphthyl acetate by acetylcholinesterase) with fast blue B salt to give a purple-colored diazonium dye. The white spot against purple background determines the potential of plant extract to inhibited acetylcholinesterase. In this study the hexane and ethanol extracts showed the presence of acetylcholinesterase inhibitor. The previous study on *Achyrocline tomentosa*, *Eupatorium viscidum*, and *Trichocline reptans* from the family Asteraceae also afforded anti-acetylcholinesterase materials (Ammon et al., 1993). This is proved species from the Asteraceae family could be promising agents for acetylcholinesterase inhibitor, especially to treat neurodegenerative disease.

Climbing hempvine has been used for inflammatory diseases in India and this study climbing hempvine showed the potential to inhibit the 5-lipoxygenase enzyme. The ethanol extract of climbing hempvine inhibited the enzymatic activity of 5-lipoxygenase (5-Lox) with an IC<sub>50</sub> equal to 23.87  $\mu$ g/ml (Table 5). This remarkable value due to the ability of the phenolic compound to catalyze the oxidation of unsaturated fatty acids containing 1-4 diene structures radical substances produce by linoleic acid. Antioxidants are known to inhibit lipoxygenases (Trouillas et al., 2003). Phenolic compounds have shown the ability to block the cascade process of arachidonic acid metabolism by inhibiting lipoxygenase activities, and also act as a scavenger of reactive free



radicals which are produced during arachidonic acid metabolism (Sreejayan and Rao, 1996; Benzie and Stezo, 1999; Robards et al., 1999).

The polyphenol content was measured using Folin-Ciocalteu assay with the basic mechanism of electron transfer and reducing ability. Gallic acid was used as a standard ( $y = 0.0011x + 0.0018$ ,  $R^2 = 0.9996$ ) and the results were expressed in Gallic Acid Equivalent (mg/g). The ranks of total phenolic contents in Climbing hempvine extracts were ethanol > ethyl acetate > hexane (Table 4). Generally, phenolic compounds inactivate lipid free radicals and prevent the decomposition of hydroperoxides into free radicals. Several reports emphasize the fact that there is a positive relationship between total phenols and antioxidant activity (Oktay et al., 2003; Ferreira et al., 2007). In agreement to previous reports, our study revealed the existence of a positive correlation between FRAP values and the total phenolic contents ( $y = 388.66x + 135.36$ ,  $r^2 = 0.8567$ ); total phenolic contents and  $\beta$ -carotene bleaching (EC50) values ( $y = 1.3793x + 378.17$ ,  $r^2 = 0.8980$ ) and total phenolic contents and DPPH (IC50) values ( $y = -2564.7x + 1324.8$ ,  $r^2 = 0.9124$ ). The presence of electron-withdrawing and electron-donating substituent in the ring structure arrangement, the number of the hydroxyl groups, and the extent of structural conjugation normally influenced their antioxidant potential (Lapornik et al., 2005). Studies have compromised that phenolic compounds also possess the inhibition of lipoxygenase due to their antioxidant properties (Carpinella, 2010). These activities are indeed key pharmacological targets in the pathophysiology of neurodegenerative diseases. Oxidative stress, neuroinflammation, and lack of acetylcholine are the main key factors in Alzheimer's disease. The present study warrants further phytochemical analysis, which may lead to the discovery of phenolic neuroprotective agents.

## CONCLUSION

The hexane extract of climbing hempvine exhibited antibacterial activities against causative agents of foodborne poisoning and abscesses and could be a potential source of antibiotics for the treatment of bacterial infections. Besides, the evidence obtained in this study clearly demonstrates that the ethanol extract of climbing hempvine which abounds with phenolic substances, has strong antioxidant, anticholinesterase, and anti-inflammatory activities.

## DECLARATION OF CONFLICT OF INTEREST

No conflict of interest associated with this work.

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