Hydrodistillation Extraction of *Mariposa Christia Vespertilionis* (Butterfly Wing) Plant

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Abstract*— In this study, M. C. Vespertilionis (MCV) were extracted using hydrodistillation extraction method. Four types of extraction being used; fresh leaves (distillate), fresh leave (condensate), dry leave (distillate), and dry leave (condensate). The phytochemical tests were performed to identify different phytochemical compounds present in the MCV extract. Fourier transform infrared spectroscopy (FTIR) and Gas chromatography–mass spectrometry (GC-MS) analysis were done to identify the presence of functional groups and bioactive compounds. The FTIR results revealed the presence of phenol, alcohol, alkanes, aromatic carboxylic acid, halogen compound, alkyl halide, ester, and ether. The GC-MS results identified a total of 12 compounds in dry leaves (distillate), 15 in dry leaves (condensate), while 18 in fresh leaves (distillate), and 11 in fresh leaves (condensate) where all of the compound known to have a potent activity against cancer cells, anti-inflammatory, anticonvulsant, anti-bacterial and anti-oxidant. Overall, this study offer a platform by using CMV leaves as an option approaches to treat different illnesses.

***Keywords*— MCV (Mariposa Christia Vespertilionis), phytochemical, FTIR, GC-MS**

I. INTRODUCTION

Medicinal plant has been known for a very long time and are widely use in medicinal approaches, it has become another alternative ways to replace modern treatment. The genus *Christia* Moench is an ornamental legume in the Fabaceae family consisting 13 species found in tropical South East Asia, Malaysia and Australia [1]. This plant are also known in many names such as red butterfly wing, island peak, and 'rerama' which means butterfly in Malaysia. By referring to the National Park Singapore, this plant is an autotrophic, where they are producing its own food by photosynthesis which inorganic materials are converting into organic compound [2].

According to Mister Kamarul Rezan Lim Abdullah from University Putra Malaysia, the use of *Mariposa Christia Vespertilionis* in treating diseases such as cancer, stroke, migraine, dengue, diabetes, hypertension, intestinal problems, sinus, measles and asthma are effectively great [3]. The goodness of this plant has getting a lot of interest in Malaysia where not only the traditional pratitioners are attracted but also involved the researchers in Malaysia. Figure 1 shows the type of *Christia Vespertilionis* being used in this research.

Hydro distillation is the simplest method of extraction and it is the common technique approach to extract the essential oils from the plant. Regardless of many studies reported on the essential oils of Mariposa Christia Vespertilionis, there is no report on the extraction of essential oils form this medicinal plant by using hydro distillation. Therefore, hydro distillation method is being used for the extraction of essential oils from Mariposa Christia Vespertilionis leaves for this research. The essential oil obtain are then will be further analyze by using Fourier transform infrared spectroscopy (FTIR) and Gas Chromatography – Mass Spectrometry (GC-MS) analysis on four different types of extraction.

Fig. 1: *Christia Vespertilionis with* green colour leaves.

II. METHODOLOGY

A. Materials and methods

2.1 Collection of samples

The fresh sample of medicinal plant *Christia Mariposa Vespertillionis* (MCV) were obtained from Dr. Jeffri Jaapar garden in the Faculty of Chemical Engineering at level 10. Only the leaf parts are being focus for this research. The leaves were prepared with two ways, firstly, it was washed by using distilled water until it is clean and dried in open air for 2 days at room temperature and secondly fresh leaves were used straightaway.

2.2 Preparation of plant extract

2.2.1 Dry leaves plant

20 gram of dried *Christia Mariposa Vespertillionis* leaves were grounded to powder in blender. The sample were than sieved using mechanical sieve shaker to obtain 1-2 mm size and kept in zipper plastic bag to prevent moisture absorption before the extraction process. The leaves were extracted by using hydro-distillation Clevenger apparatus method. The powder (5g) were immersed in 150 ml distilled water in a round bottom flask on a heater. Heat was supplied by heating mantle with temperature of 100°C. The extracted of water-based extraction which was divided into two parts 50ml of distillate liquid and 50 ml of condensate liquid were collected. Essential oils were extracted by using rotary evaporator, methanol were added after the oil was obtained and were transferred into glass vials and kept at 4°C until being analyzed.

2.2.2 Fresh leaves plant

20 gram of fresh *Christia Mariposa Vespertillionis* leaves were straightaway grounded to powder in blender after being plucked to ensure the freshness. The methods started from sieving, till keeping the extraction at 4°C for the dry leaves were repeated by using fresh leaves.

2.3 Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) spectrum were used in identifying the types of functional group present in CMV leaves. This equipment can be found in the instrumentation laboratory 2, at level 6, Faculty Chemical Engineering, UiTM Shah Alam. Four samples from previous method were prepared and subjected to FTIR with a scan range from 400 to 4000 cm^{-1}. FTIR machine (Perkin Elmer Spectrum 1) was ran for scanning and spectrum were showed at the computer provided.

2.4 Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

Four samples from previous method were prepared and subjected to GC-MS analysis. GC-MS equipment can be found in the instrumentation laboratory 1, room 1, at level 6, Faculty of Chemical Engineering, UiTM Shah Alam. GC-MS was equipped with a fused silica capillary column (30m x 0.25 mm i.d., film thickness 0.25 µm). To identify the compound in the sample, an ionization energy of 70 eV was used. Inert gas (helium) was applied as a carrier gas at constant flow rate of 1 ml/min. Mass transfer line was set at 220◦C while injector temperatures were set at 300◦C. The oven temperature was set from 50 to 150◦C at 3◦C/min, being held for 10 minutes and was proceed with increment of 300◦C at 10◦C/min. The particle-free diluted oil extracts (1µl) were placed in a syringe and injected into injector with split mode. The split ratio given was 1:120. The results in term of percentage composition of the oil extract compounds was determined as a percentage by peak area [4].

III. RESULTS AND DISCUSSION

A. FTIR spectral data interpretation of Mariposa Christia Vespertilionis Leaves

Four samples were prepared which were dry leaves (distillate), dry leaves (condensate), fresh leaves (distillate) and fresh leaves (condensate) with the additional of solvent (methanol). The functional groups of the bioactive compounds present in the four samples were identified by using FTIR spectrum and separated based on the peaks values in the region of IR radiation. The results of FT-IR analysis confirmed the presence of alcohol, alkanes, aromatic carboxylic acid, and halogen compound, alkyl halide, ester, ether and phenol.

Fig. 2 until **Fig. 5** shows the result of the four extraction, and the functional groups were tabulated in **Table-1** until **Table-4**. FTIR analysis of methanol extract of *M. C. Vespertilionis leaves* showed the presence of hydroxyl (-OH) group that was common in all phenolic constituents. The absorption band causes (OH) stretching vibrations from phenols (chemical) containing hydroxyl functional groups (-OH) being devoted to an aromatic hydrocarbon. This condition approved the presence of phenolic compounds (flavonoids) in all four methanol extract of *M. C. Vespertilionis*. In the meantime, carboxylic acids present in MCV leaves was said to be responsible to treat disease such as, fever, rheumatic joint paint and headache [5].

B. GC-MS of Mariposa Christia Vespertilionis Leaves

GC–MS chromatograms of methanol: from extraction of MCV for different retention time are given in **Fig. 6** until **Fig. 9**. The number and nature of phytochemical compound as represented by the peaks were characterized and identified by comparing the mass spectra of the compound with NIST library. Phytochemical analysis of dry leaves (distillate) CMV by GC–MS analysis revealed eight peaks corresponding to the presence of 12 compounds, **Table 5**. The compounds observed were Oxalic acid (0.00267%), Caffeine (0.0787%), Dihydrocodeine (1.01%), Papaverine (1.07%), Meptazinol (0.1356%), Pilocarpine (0.185%), Tramadol (0.707%), Theophylline (0.107%), Naproxen (0.0212%), Acyclovir (0.295%), Promethazine (0.188%), Flurazolidoneu (0.207%). All of this finding were said to have anticancer, and some, for example Naproxen had properties of non-steroidal anti-inflammatory [6]. Flurazolidoneu had been used to treat certain protozoan and bacterial infections in animals and humans for more than sixty years and studies also showed that it can increased the stability of tumor suppressor p53 protein in Acute Myeloid Leukimia cells [7].

In comparison to dry leaves (condensate), GC–MS analysis of CMV extract exhibited 2 peaks predicting the presence of 15 compounds, **Table 6**. Out of these compounds, Hexadecanoic acid, methyl ester was the most significant compound representing 0.418% of the total percentage amount. Other major components observed were Pentacosane (0.35646%), Tetratetracontane (0.152%), Docosane, 10-ethyl-10-propyl- (0.1127%), Oxalic acid, isohexyl pentyl ester (0.0677%), Tetracosane, 11-decyl (0.0511%), Azetidine, 2-methyl (0.0402%), Octadecane, 5,14-dibutyl (0.0375%), Fluspirilen (0.0289%), and Heneicosane, 3-methyl (0.0259%). Studies showed that fluspirilen was used as antipsychotic drug, for the treatment of schizophrenia [8]. Others were to be said as antimicrobial, anti-tubercular, anticonvulsant, anti-cancer and anti-inflammatory [9].

On the other hand, eight peaks with an amount of 18 compounds in fresh leaves (distillate) CMV extract by GC-MS were identified, **Table 7**. The most percentage amount of the compound found were Pentacosane (1.1718%) followed by, Hexadecanoic acid, methyl ester (1.699%) Tetratetracontane (0.204%), Nonacosane (0.0802%), Cyclohexasiloxane, dodecamethyl (0.0501%), 1, 2, 3-Thiadiazole-4-carboxylic acid, 5-methyl (0.0434%), Pyrrolidine, 1-[8-(3-octyloxiranyl)-1-oxooctyl] (0.0395%), Heneicosane, 11-decyl (0.0361%), Nitrazepam (0.0361%), Eicosane, 3-methyl (0.0359%). Compound Nonacosane was composed of long-chain alkanes and fatty acids contains antioxidant and antimicrobial properties [10]. Cyclohexasiloxane, dodecamethyl act as antimicrobial [11]. Nitrazepam is a hypnotic drug used as sedative, as well as anticonvulsant, amnestic, and gives a skeletal muscle relaxant effects [12].

Apart of this, methanol extraction of fresh leaves MCV (condensate) that were extracted by GC-MS showed two peaks correlating with the presence of 11 compounds, **Table 8**. It was observed that the major compound hexadecanoic acid, methyl ester (0.775%), act as antioxidant, anti-inflammatory and can also decrease blood cholesterol [13] while, Pentadecanoic acid, 14-methyl-, methyl ester (0.212%) has an antioxidant properties [14]. Other compound found were 2-Hydroxy-3-methylanthraquinone,Otrimethylsilyl(0.00404%),Tetratetracontane (0.119%),Methabenzthiazuron(0.00849%),Dimethyl(allyl)silyloxy benzene(0.0338%),Tetradecaoic acid, 10,13-dimethyl-, methyl ester(0.00643%),Carprofen(0.000704%),2(2Hydroxyphenyl)benzot hiazole (0.000774%), and 1H-Indene, 2-decyloctahydro (0.0420%), were found in the extraction from fresh leave (condensate). The minor had the ability such as anti-proliferative, anticancer, antibiotic and nonsteroidal anti-inflammatory.

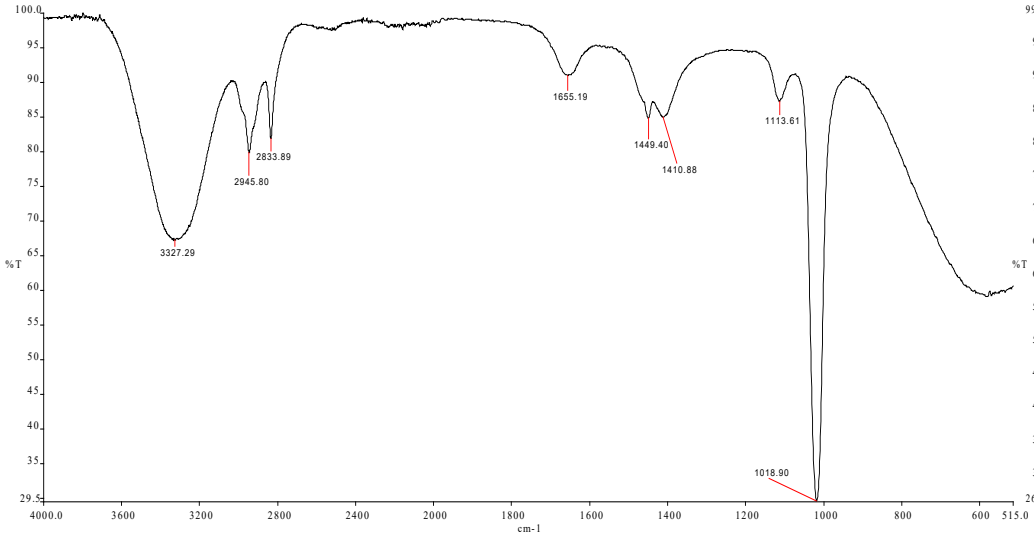

Fig. 2: FTIR spectrum for methanolic extract from dry MCV leaves (distillate)

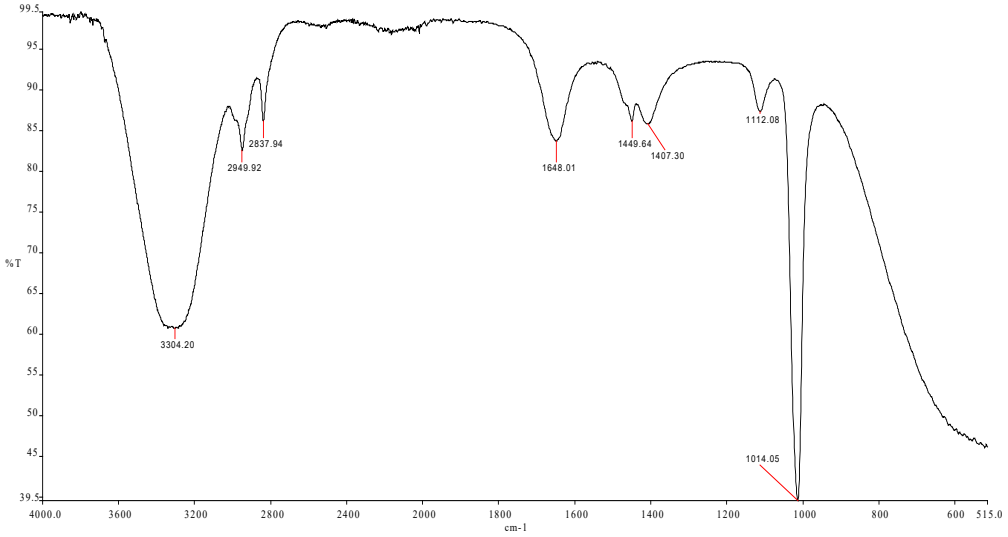

Fig. 3: FTIR spectrum for methanolic extract from dry MCV leaves (condensate)

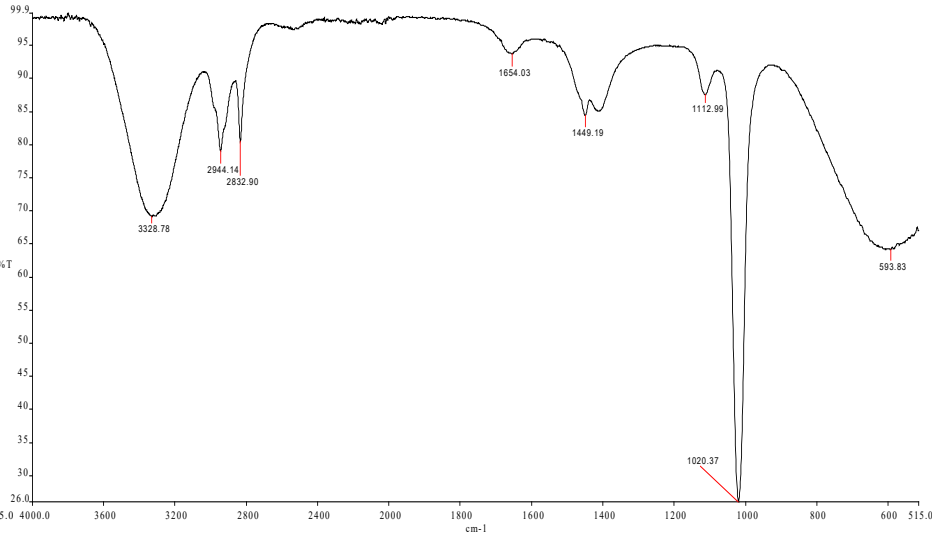

Fig. 4: FTIR spectrum for methanolic extract from fresh MCV leaves (distillate)

Fig. 5: FTIR spectrum for methanolic extract from fresh MCV leaves (condensate)

Table 1: FTIR peaks values and functional groups of dry MCV leaves (distillate) [15,16]

Extract	Peak Values	Type of Stretching	Functional Group
Methanol	3327.29	O-H Stretching N-H Stretching	Alcohol, Phenol Amine, Amide
	2945.80	C-H Stretching O-H Stretching	Alkyl, Alkane, Carboxylic Acid
	2833.89	O-H Stretching =C-H Stretching	Carboxylic Acid Aldehyde
	1655.19	C=C Stretching C=O Stretching C=C Bending	Alkene, Alkenyl Amide Aromatic
	1449.40	C=C Stretching -C-H Bending	Aromatic Alkane
	1410.88	-C-H Bending C=C Stretching	Alkane Aromatic
	1113.61	C-O Stretching C-N Stretching C-F Stretching	Ester, Ether, Alcohol Amine Alkyl Halide
	1018.90	C-F stretching C-O Stretching	Alkyl Halide Ether, Ester

Table 2: FTIR peaks values and functional groups of dry MCV leaves (condensate) [15,16]

Extract	Peak Values	Type of Stretching	Functional Group
Methanol	3304.20	O-H Stretching N-H Stretching	Alcohol, Phenol Amine, Amide
	2949.92	C-H Stretching O-H Stretching	Alkyl, Alkane, Carboxylic Acid
	2837.94	O-H Stretching =C-H Strecthing	Carboxylic Acid Aldehyde
	1648.01	C=O Stretching C=C Stretching C=C Bending	Amide Alkene, Alkenyl Aromatic
	1449.64	C=C Stretching -C-H Bending	Aromatic Alkane
	1407.30	-C-H Bending C=C Stretching	Alkane Aromatic
	1112.08	C-O Stretching C-N Stretching C-F Stretching	Ester, Ether, Alcohol Amine Alkyl Halide
	1014.05	C-F stretching C-O Stretching	Alkyl Halide Ether, Ester

Table 3: FTIR peaks values and functional groups of fresh MCV leaves (distillate) [15,16]

Extract	Peak Values	Type of Stretching	Functional Group
Methanol	3328.78	O-H Stretching N-H Stretching	Alcohol, Phenol Amine, Amide
	2944.14	C-H Stretching O-H Stretching	Alkyl, Alkane, Carboxylic Acid
	2832.90	O-H Stretching =C-H Stretching	Carboxylic Acid Aldehyde
	1654.03	C=C Stretching C=O Stretching C=C Bending	Alkene, Alkenyl Amide Aromatic
	1449.19	C=C Stretching -C-H Bending	Aromatic Alkane
	1112.99	C-O Stretching C-N Stretching C-F Stretching	Ester, Ether, Alcohol Amine Alkyl Halide
	1020.37	C-F stretching C-O Stretching	Alkyl Halide Ether, Ester
	593.83	C-Br stretching	Alkyl Halide

Table 4: FTIR peaks values and functional groups of fresh MCV leaves (condensate) [15,16]

Extract	Peak Values	Type of Stretching	Functional Group
Methanol	3328.16	O-H Stretching N-H Stretching	Alcohol, Phenol Amine, Amide
	2949.01	C-H Stretching O-H Stretching	Alkyl, Alkane, Carboxylic Acid
	2837.72	O-H Stretching =C-H Stretching	Carboxylic Acid Aldehyde
	1646.96	C=O Stretching C=C Stretching C=C Bending	Amide Alkene, Alkenyl Aromatic
	1449.83	C=C Stretching -C-H Bending	Aromatic Alkane
	1409.22	-C-H Bending C=C Stretching	Alkane Aromatic
	1113.82	C-O Stretching C-N Stretching C-F Stretching	Ester, Ether, Alcohol Amine Alkyl Halide
	1014.43	C-F stretching C-O Stretching	Alkyl Halide Ether, Ester

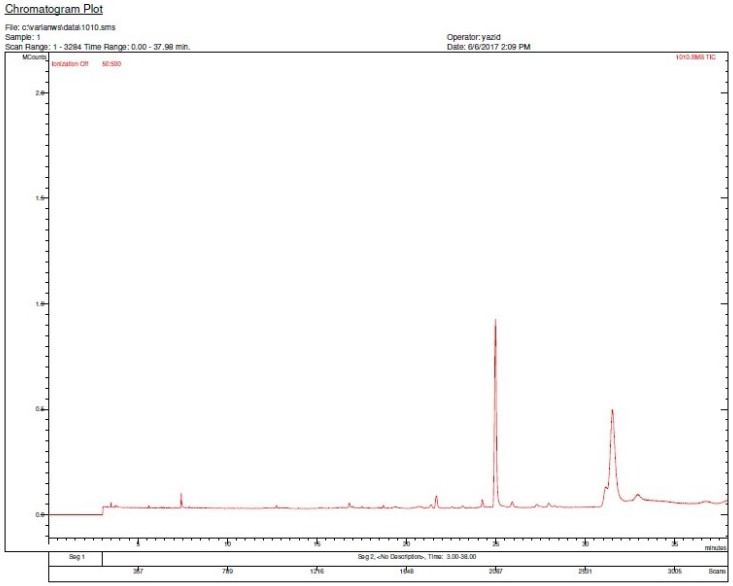

Fig. 6: GC-MS analysis of methanolic extraction from dry leaves CMV (distillate)

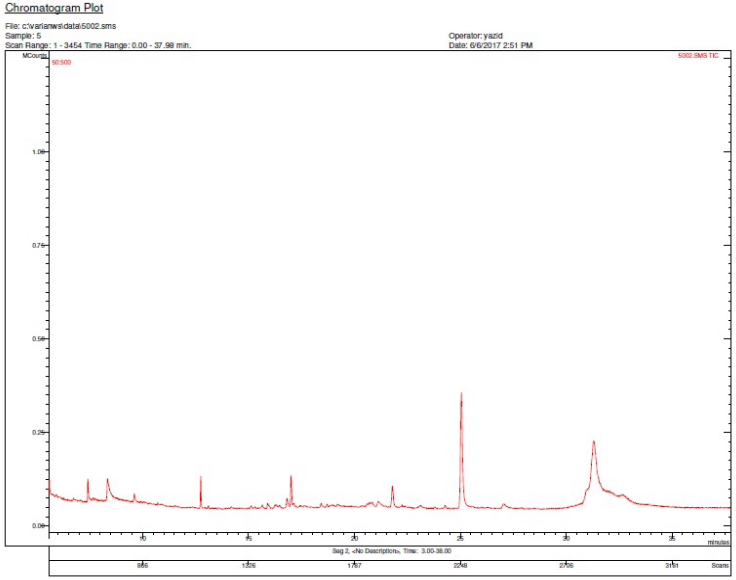

Fig. 7: GC-MS analysis of methanolic extraction from fresh leaves CMV (distillate)

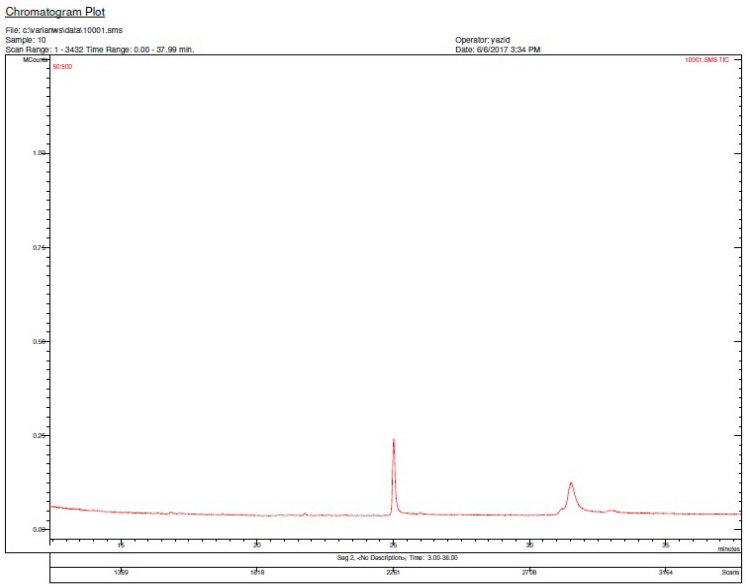

Fig. 8: GC-MS analysis f of methanolic extraction from dry leaves CMV (condensate)

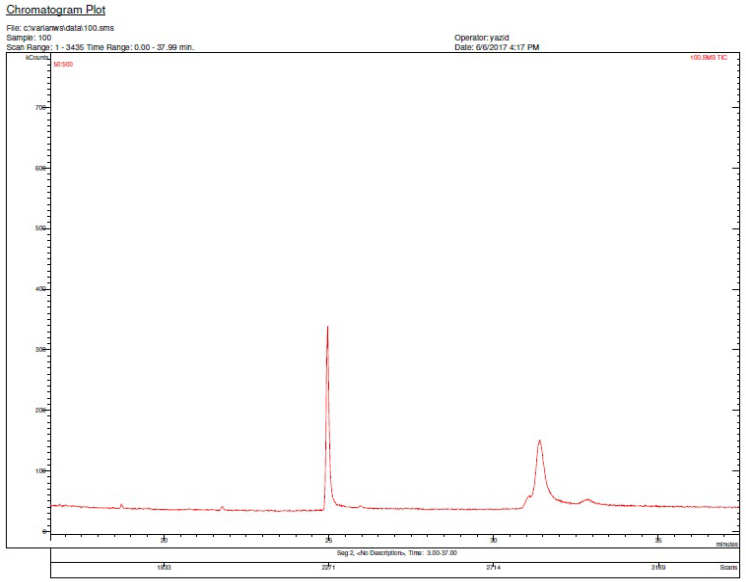

Fig. 9: GC-MS analysis of methanolic extraction from fresh leaves CMV (condensate)

Table 5: Chemical compound identified from GC-MS analysis of dry leaves CMV (distillate)

No.	Retention Time (min)	Chemical Name	Amount (%)
1.	3.2012	Oxalic acid	0.00267
2.	21.3758	Caffeine	0.0787
3.	24.9871	Dihydrocodeine	1.01
4.	24.9938	Papaverine	1.07
5.	25.9125	Meptazinol	0.1356
6.	31.1253	Pilocarpine	0.185
7.	31.4323	Tramadol	0.707
8.	31.4848	Theophylline	0.107
9.	31.4925	Naproxen	0.0212
10.	31.5703	Acyclovir	0.295
11.	31.5800	Promethazine	0.188
12.	31.6083	Flurazolidoneu	0.207

Table 6: Chemical compound identified from GC-MS analysis of dry leaves CMV (condensate)

No.	Retention Time (min)	Chemical Name	Amount (%)
1.	3.0454	Tetratetracontane	0.152
2.	3.3033	Oxalic acid, pentyl propyl ester	0.0229
3.	3.4441	Oxalic acid, monoamide, N-(2-chlorobenzyl)-N-(2-thienylmethyl)-, butyl ester	0.00108
4.	3.8236	Pentacosane	0.35646
5.	3.8717	Uleine, dihydro	0.0130
6.	4.2650	Tetracosane, 11-decyl-	0.0511
7.	4.5168	Azetidine, 2-methyl-	0.0402
8.	4.0929	Docosane, 10-ethyl-10-propyl-	0.1127
9.	4.5832	Aspidofractinine, 1-acetyl-17-methoxy-	0.0192
10.	4.6024	Fluspirilen	0.0289
11.	4.7161	Heptadecane, 9-hexyl-	0.0228
12.	4.7576	Octadecane, 5,14-dibutyl-	0.0375
13.	4.8040	Oxalic acid, isohexyl pentyl ester	0.0677
14.	4.8630	Heneicosane, 3-methyl-	0.0259
15.	25.0089	Hexadecanoic acid, methyl ester	0.418

Table 7: Chemical compound identified from GC-MS analysis of fresh leaves CMV (distillate)

No.	Retention Time (min)	Chemical Name	Amount (%)
1.	3.0464	Tetratetracontane	0.204
2.	3.2062	Furfural	0.0221
3.	3.7469	Nonacosane	0.0802
4.	3.7864	Tetracosane, 5-ethyl-5-methyl-	0.0266
5.	3.8259	Heneicosane, 11-decyl-	0.0361
6.	3.9300	Pyrrolidine, 1-[8-(3-octyloxiranyl)-1-oxooctyl]-	0.0395
7.	4.0580	Nitrazepam	0.0361
8.	4.0860	Pentacosane	1.1718
9.	4.1235	1,2,3-Thiadiazole-4-carboxylic acid, 5-methyl-	0.0434
10.	4.1331	Pyrrolizidine-1-one, 7-acetylmethyl-	0.0278
11.	4.2959	Eicosane, 3-methyl-	0.0359
12.	4.4030	5-O-Methyllupiwighteone	0.0300
13.	4.4891	Vinpocetine	0.00242
14.	5.6034	2-Bromo-6-(3,5-dimethyl-7,8-dihydro-6H-isoxazolo[4,5-b]azepin-7-yl)phenol	0.0165
15.	16.2513	2,4-Dimethyl-1-penten-3-ol	0.00259
16.	21.7979	Cyclohexasiloxane, dodecamethyl-	0.0501
17.	25.0447	Hexadecanoic acid, methyl ester	1.699
18.	27.0297	Imipramine	0.02887

Table 8: Chemical compound identified from GC-MS analysis of fresh leaves CMV (condensate)

No.	Retention Time (min)	Chemical name	Amount (%)
1.	3.0294	2-Hydroxy-3-methylanthraquinone, O-trimethylsilyl	0.00404
2.	3.0455	Tetratetracontane	0.119
3.	3.5106	Methabenzthiazuron	0.00849
4.	3.8701	Dimethyl-(allyl)-silyloxybenzene	0.0338
5.	18.7128	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	0.00643
6.	21.7562	Carprofen	0.000704
7.	21.7762	2-(2-Hydroxyphenyl)benzothiazole	0.000774
8.	24.9486	Pentadecanoic acid, 14-methyl-, methyl ester	0.212
9.	24.9672	Hexadecanoic acid, methyl ester	0.775
10.	31.3921	1H-Indene, 2-decyloctahydro-	0.0420
11.	31.4102	2,4-Azetidinedione, 3,3-diethyl-1-methyl	0.00863

I. Conclusion

The leaves of Mariposa Christia Vespertilionis were collected and being extracted by using hydro-distillation method. The extract were then subjected to FTIR and GC-MS analysis. The presence of various bioactive chemical compounds indicate the benefits of the MCV leaves to treat various diseases. However, further studies will need to be done to conform its toxicity profile, effect on the ecosystem and agricultural products.

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References

[1] Dash, G. K. (2016). An Appraisal of Christia vespertilionis (L. F.) Bakh. F.: A Promising. *International Journal of Pharmacognosy and Phytochemical Research, 8*(6:June 2016), 1037-1039.

[2] Board, N. P. (2013). *Christia Vespertilionis*. Retrieved from Nparks Flora & Fauna Web: https://florafaunaweb.nparks.gov.sg/Special-Pages/plant-detail.aspx?id=1811

[3] Abdullah, M. K. (2015, March 13). UPM Runs Stage Two Of Anti-Cancerous Red Butterfly Wing Research. (A. Zakaria, Interviewer)

[4] Mohammad A. Hossain, Wafa A.S. Al-Toubi, Afaf M. Weli, Qasim A. Al-Riyami, Jamal N. Al-Sabahi. (2013, May 31). Identification and chracaterization of chemical compounds in different crude extracts from leaves of Omani neem. *Juornal of Taibah University for Science 7*, 181-188.

[5] Lethika. D. Nair, Santosh K. Sar, Arun Arora. Review Paper on Chemical Characterization of Millettia Pinnata of Bhilai – Durg Region of Chhattisgarh. Science Innovation. Vol. 4, No. 5, 2016, pp. 242-246.doi:10.11648/j.si.20160405.14

[6] Kim, M.-S., Kim, J.-E., Lim, D. Y., Huang, Z., Chen, H., Langfald, A., Bode, A. M. (2014). Naproxen induces cell cycle arrest and apoptosis in human urinary bladder cancer cell lines and chemically induced cancers by targeting PI3-K. *Cancer Prevention Research (Philadelphia, Pa.)*, *7*(2), 236–245. http://doi.org/10.1158/1940-6207.CAPR-13-0288

[7] Jiang X, Sun L, Qiu JJ, Sun X, Li S, Wang X, et al. (2013) A Novel Application of Furazolidone: Anti-Leukemic Activity in Acute Myeloid Leukemia. PLoS ONE 8(8): e72335. https://doi.org/10.1371/journal.pone.0072335

[8] Shi X-N, Li H, Yao H, Liu X, Li L, Leung K-S, et al. (2015) *In Silico* Identification and *In Vitro* and *In Vivo* Validation of Anti-Psychotic Drug Fluspirilene as a Potential CDK2 Inhibitor and a Candidate Anti-Cancer Drug. PLoS ONE 10(7): e0132072. https://doi.org/10.1371/journal.pone.0132072

[9] Dr.Subin Mary Zachariah, Mridula Ramkumar, Namy George, Mohammad Salam Ashif. (2015, January-February). Azetidinones : An Overview. 211-218.

[10] Carla de M. Martins, Evandro A. do Nascimento, Sérgio A. L. de Morais, Alberto de Oliveira, Roberto Chang, Luís C. S. Cunha, Mário M. Martins, Carlos Henrique G. Martins, Thaís da S. Moraes, Paulla V. Rodrigues, Cláudio V. da Silva, and Francisco J. T. d. (2015, March 27). Chemical Constituents and Evaluation of Antimicrobial and Cytotoxic Activities of Kielmeyera coriacea Mart. & Zucc. Essential Oils. 1-9.

[11] Sheeba Gnanadeebam D and Viswanathan P. (2014). GC-MS Analysis of Phytocomponents in Spermacoce. *Research in Pharmacy, 4*, 1-7.

[12] Yasui M, Kato A, Kanemasa T, Murata S, Nishitomi K, Koike K, Tai N, Shinohara S, Tokomura M, Horiuchi M, Abe K (June 2005). "[Pharmacological profiles of benzodiazepinergic hypnotics and correlations with receptor subtypes]". Nihon Shinkei Seishin Yakurigaku Zasshi. **25** (3): 143–51. *PMID 16045197*v

[13] G. Belakhdar, A. B. (2015, September 15). Determination of some bioactive chemical constituents from Thesium humile Vahl. *J. Mater. Environ. Sci*, 2778-2783.

[14] Vijisaral Elezabeth D. and Arumugam S. (2014). GC-MS analysis of bioactive constituents of Indigofera suffruticosa leaves. *Journal of Chemical and Pharmaceutical Research*, 294-300.

[15] Silverstein, R.M.; Bassler, G.C.; and Morrill, T.C. . (n.d.). *Characteristic IR Absorption Frequencies of Organic Functional Groups*. Retrieved from http://www.ifsc.usp.br/~lavfis2/BancoApostilasImagens/ApLuminescencia/IR-Spectras-http.pdf

[16] Professor Craig A. Merlic, Dr. Jane Strouse. (n.d.). *Table of IR Absorptions*. Retrieved from Web Spectra: https://webspectra.chem.ucla.edu/irtable.html