# Unveiling the Bioactive Compound Potential of Red Mariposa *Christia vespertilionis* Using Chemometric and ATR-FTIR Spectroscopy

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***Abstract:*** *Red Mariposa* Christia vespertilionis *(MCV) is a medicinal plant containing potential bioactive compounds of interest; however, these have not been fully investigated. This work focuses on the antioxidant properties of red MCV, as evaluated by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy with chemometric methods of analysis such as principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA).The highest total phenolic levels (15.91 ± 0.04 mg GAE/mL) were found in the leaf of aqueous extracts, and total flavonoid content, 15.7 ± 0.04 mg QE/mL, in hexane extracts, compared to other solvents, which is linked to better DPPH radical scavenging activity (74.24 ± 0.00%). The FTIR spectra demonstrated the presence of hydroxyl (O–H) and carbonyl (C=O) functional groups, indicating a significant abundance of polyphenols and flavonoids. The bioactive composition of the red MCV was definitively confirmed through chemometric techniques. The chemometric analysis revealed that methanol and aqueous extracts offer maximum antioxidant and optimum solvent profiles. These results show that red MCV is a feasible source for pharmaceutical and nutraceutical applications dealing with oxidative stress.*

***Keywords:*** *Red Mariposa* Christia vespertilionis*; ATR-FTIR spectroscopy; phenolic compounds; flavonoid compounds*

## ■ INTRODUCTION

For decades, herb plants have been used as a natural resource for treating diseases due to their remarkable chemical diversity and potential biological activity. Much research has been done on the therapeutic advantages and capacity to scavenge free radicals of natural antioxidants, including polyphenols and flavonoids [1]. Antioxidant activity is essential in neutralizing harmful free radicals in the body, which are implicated in various chronic diseases [2]. Known as Butterfly Wing, the non-climbing perennial herb Mariposa *Christia vespertilionis* (MCV) is valued throughout Asia for its aesthetic. It is also used for traditional herbal medicine because of its antioxidant, anti-inflammatory, and antimicrobial effects [3-4].

Despite extensive traditional uses, the specific bioactive compounds that contribute to the antioxidant properties of red MCV have not been thoroughly identified. The previous studies mainly focused on crude extracts, without considering the impact of various solvents or plant parts—elements that significantly influence the efficiency and yield of antioxidant compounds during extraction [5-6]. Therefore, there is a significant knowledge gap because MCV's pharmacological potential is still unknown, and improved compositional profiling is needed to standardize these plants for future medication formulations. Although previous literature was mainly on the bioactivities of crude extracts, the present work demonstrates a functional link between certain functional groups, such as O–H and C=O, and antioxidant capacity [7-9].

This problem can be addressed by using multivariate chemometric approaches. Unlike univariate methods, which examine each variable separately,

multivariate methods, such as principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), analyze all variables simultaneously, and even slight variations between spectra can be captured. They optimize the classification, determine important discriminant variables such as total phenolic content (TPC), total flavonoid content (TFC), and 2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) activity, and give information about the influence of the type of solvent and the part of the plant on the phytochemical composition. PCA and OPLS-DA were chosen over hierarchical clustering methods because they offer enhanced dimensionality reduction, clearer group distinctions, and robust quantitative classification. These features are particularly advantageous for interpreting complex spectroscopic data in natural product research [10-11]. While hierarchical clustering serves well for visualization, chemometric methods like PCA and OPLS-DA deliver superior discrimination and interpretative capabilities, supported by recent progress in metabolomics [12].

The current work aims to evaluate the determination through attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy with multivariate chemometric analysis such as PCA and OPLS-DA. Chemometrics analysis is required to analyze data constructed from the FTIR findings since the findings are extremely complicated and have overlapping spectra [5-6]. This method is valuable for classification, increasing the detection of hidden structures and extraction methods. This comprehensive approach will not only provide better differentiation of parts of plants but also promote the solvent effectiveness and the spectral technique interpretability in the field of natural product investigation [11,13]. This integrated methodology influences the phytochemical composition and antioxidant activity of red MCV, uncovering hidden patterns and optimizing extraction methods for pharmaceutical applications. This research is innovative in its combination of chemometric and spectroscopic analysis to identify and differentiate antioxidant compounds across various parts of red MCV. By clarifying these relationships, our study lays a scientific groundwork for refining extraction techniques and guiding future pharmaceutical and nutraceutical developments with a liquid chromatography tandem mass spectrometry (LC-MS/MS) metabolomic study still recommended for pharmacokinetics, safety, and bioavailability exploration when used in bioactive drug development.

## ■ EXPERIMENTAL SECTION

### Materials

The chemicals used in this study, such as potassium chloride, sodium nitrate, DPPH, methanol, ethyl acetate, chloroform, *n*-hexane, aluminium chloride, sodium carbonate, sodium hydroxide, and the Folin-Ciocalteu reagent standards (gallic acid and quercetin), were purchased from E-Merck.

### Instrumentation

The experimental work involved laboratory glassware, a rotary evaporator, a hot plate, a 96-well plate, and 1 mL and 100 µL micropipettes. Analytical instruments included an FTIR spectrometer (Shimadzu Corp IR Tracer-100), an Infinite F200 Pro microplate reader (Tecan), and a UV-vis spectrometer (Cary 100 Bio UV-vis Spectrophotometer, Varian Inc. Agilent Technologies, Canada).

### Procedure

#### *Extraction of red MCV*

The dried red MCV samples were obtained from the Botanical Bukit Jalil, Kuala Lumpur. The leaves and stems of the red MCV were ground into a fine powder and stored in an airtight container for later use. The dried powdered samples (2 g) were extracted with 50 mL of different solvents (hexane, ethyl acetate, methanol, chloroform, and water) using the maceration method. The maceration was carried out for 48 h with occasional shaking at a room temperature (25 ± 2 °C) to achieve exhaustive extraction without loss of thermolabile components [14]. Methanol and water solvents were selected because they effectively extract polar polyphenolic and hydrophilic antioxidant compounds, respectively, while non-polar bioactive constituents such as lipid-soluble phytochemicals were specifically

extracted by hexane solvent [15]. The sample extract was filtered and concentrated using a rotary evaporator. Then, it was further filtered to remove residual particles. The samples were kept in microcentrifuge tubes at 4 °C for future use.

### *Quantitative analysis*

**TPC.** This method allows for accurate measurement of phenolic content by addressing potential interferences and optimizing reaction conditions to ensure reliable results. As described in related studies, the modified method makes it suitable for various plant extracts [16]. The Folin-Ciocalteu reagent method was used to determine the TPC. The extracts were prepared by dissolving them in methanol at a 5 mg/mL concentration. A volume of 20 µL of the extract was combined with 100 µL of Folin-Ciocalteu reagent (which was incubated for 5 min) and 80 µL of 7.5% (w/v) $Na_2CO_3$. Following this, the mixture was allowed to incubate for 2 h at room temperature, after which the absorbance was measured at 760 nm using a spectrophotometer. Calibration curves were generated with eight different concentrations of gallic acid [17].

**TFC.** A modified approach was employed to quantify the TFC test. A mixture of 120 µL of distilled water, 10 µL of 5% sodium nitrate, and 20 µL of the sample extract (at a concentration of 5 mg/mL in methanol) was prepared. After 6 min, 10 µL of a 10% aluminium chloride solution was incorporated. Following another 6-min incubation, 40 µL of sodium hydroxide (1 mol/L) was added to the well. The plate was then left at room temperature in the dark for 15 min. Finally, the absorbance was recorded at 430 nm using a microplate reader [17-18].

**DPPH radical scavenging assay.** The free radical scavenging activity of MCV leaf and stem extracts was tested using the DPPH assay [19]. As much as 5 mg of crude extract was dissolved in methanol. The samples underwent sonication and vortexing to create homogenized aliquots. A 100 µL of methanol was added to a 96-well plate, followed by 100 µL of the sample solution. Subsequently, 100 µL of DPPH was introduced to all wells (2.4 mg in 50 mL of methanol). Quercetin served as the positive control. The absorbance value was recorded at 517 nm with a microplate reader after 30-min incubation in the dark [20]. The findings are expressed as the mean ± standard deviation of the percentage inhibition towards DPPH at a concentration of 500 µg/mL. Gallic acid served as the positive control [21]. The percentage of inhibition was calculated by using Eq. (1);

$$\%\text{Inhibition} = \frac{AB - AS}{AB} \times 100\% \quad (1)$$

where AB and AS are the absorbance of the reagent blank and the tested samples, respectively.

### *ATR–FTIR spectroscopy*

The crude extracts were dissolved in 1 mL of analytical acetone for analysis using an FTIR spectrometer with a scan range of 400 to 4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. Approximately 0.1 mL of the sample (1 mg/mL) was applied to the ATR crystal center. The samples were dried at room temperature for about 40 s. A spectrum of the ATR crystal was captured before each sample scan, maintaining the same instrumental conditions as the background measurement. The ATR base was thoroughly cleaned with acetone and allowed to dry before measuring the following sample [22]. Six biological replicates and three technical replicates were utilized for the FTIR analysis of the samples.

### *Statistical analysis*

The FTIR spectra data were transformed from point scan to single-beam format and baseline corrected by spectrum software (Perkin Elmer, Waltham, MA, USA). The processed spectral data were saved in an ASCII format, and the quantitative phytochemical and antioxidant results were obtained (TPC, TFC, DPPH inhibition, and $IC_{50}$ values). Correlation coefficients between the four variables were estimated for leaves and stems. These coefficients were plotted as a correlation heatmap based on Origin software (OriginLab Corporation, Northampton, MA, USA). In Fig. 1, we use a color scale on that heatmap where dark blue is strong positive correlation ($r > +0.90$), light orange is medium positive correlation, light blue is neutral relationships, red is medium negative correlation, and purple is strong negative correlation ($r < -0.90$). This method offers a simple visualization and interpretation of statistical correlation between TPC, TFC, DPPH inhibition, and

$IC_{50}$ values [22-23]. The SIMCA-P software (v.14.1 Umetrics, Umeå, Sweden) was employed for multivariate data analysis (MVDA), which included PCA and OPLS-DA methods. PCA and OPLS-DA were utilized to reduce dimensionality and classification, highlighting variations in phytochemical composition influenced by solvent polarity and different plant parts. This approach is commonly used for metabolite profiling and classification [3].

## ■ RESULTS AND DISCUSSION

### Extraction of Red MCV

The yield of bioactive compound extraction is primarily influenced by the polarity of the solvent, which is crucial for determining extraction efficiency. Table 1 shows the percentage yield of the different plant parts (leaf and stem) of red MCV extract solvents. The methanol solvent, owing to its significant polarity, achieved the highest extraction yields (26.45% for the leaf and 25.61% for the stem), and aqueous (water) (24.11% for leaves and 22.91% for stems) solvents with higher polarity gave the best extraction rates. This result showed the effectiveness of the methanol extraction for both polar and semi-polar compounds, including polyphenols and flavonoids [24]. Aqueous extracts also gave good results (24.11% for leaves and 22.91% for stems) because water can dissolve hydrophilic bioactive compounds such as phenolic acids, glycosylated flavonoids, and other water-soluble phytochemicals. Hexane, as a non-polar solvent, showed lower yields (11.96% for leaves and 10.54% for stems) since it preferentially extracts non-polar compounds, i.e., terpenoids and other lipid-soluble metabolites. These results correspond with previous studies, highlighting solvent polarity's importance in the extraction yield and selectivity. The higher yields from leaves agree with their higher metabolic activity and secondary metabolite biosynthesis [25].

Ethyl acetate was chosen due to its moderate polarity, which allows the extraction of semi-polar compounds that are not completely extracted by highly polar solvents (methanol or water) or non-polar solvents (hexane). Its polar carbonyl group with slightly higher hydrogen bond acceptor contributes to ethyl acetate being an efficient solvent for flavonoid aglycones, aliphatic and aromatic phenolic acids, some alkaloids, and relatively polar glycosides—phytochemicals. These compounds are well known for their potent antioxidant, antimicrobial, and anti-inflammatory activities [26].

**Table 1.** Percentage yield of plant extracts

| Solvents | Parts of the plant extracts (%) | |
|---|---|---|
| | Leaf | Stem |
| Methanol | 26.45 | 25.61 |
| Water | 24.11 | 22.91 |
| Ethyl acetate | 17.20 | 15.16 |
| Chloroform | 13.54 | 8.95 |
| Hexane | 11.96 | 10.54 |

Both ethyl acetate and chloroform have similar polarity indices of ca. 4.1 but show very different solvation tendencies. Chloroform is less hydrogen-bonding than water and has a lower dipole moment (but greater solvating power for relatively hydrophobic compounds, such as organochlorides and steroids); accordingly, it is classified as a medium polarity solvent [24-25]. With ethyl acetate and chloroform as two specific solvents in extraction, the recovery of bioactive compounds can be enriched—this is key in untargeted screening and bioactivity-guided fractionation approaches.

### TPC and TFC

Phenolic and flavonoid compounds are crucial in the antioxidant functions because they can scavenge free radicals. Table 2 shows the results of the quantitative analyses, which indicate significant differences for TPC and TFC with the solvent type and plant parts. The aqueous extract of red MCV leaf exhibited a greater TPC at 15.91 ± 0.04 mg GAE/mL, and the methanol extracts showed lower levels at 6.45 ± 0.04 mg GAE/mL, which means water was more efficient than methanol solvent to extract hydrophilic phenolic compounds from the leaf tissue. Similarly, the TPC was higher in the aqueous extract (4.28 ± 0.24 mg GAE/mL) for the stem extracts than in the methanol extract (3.35 ± 0.05 mg GAE/mL). The aqueous extract consistently yielded higher TPC than the methanol extract, although the levels varied between leaves and stems.

**Table 2.** Total phenolic contents and total flavonoid contents of the solvent extracts

| Solvents | Total phenolic content (mg GAE/mL) | | Total flavonoid content (mg QE/mL) | |
|---|---|---|---|---|
| | Leaf | Stem | Leaf | Stem |
| Hexane | 3.64 ± 0.17 | 2.41 ± 0.05 | 15.70 ± 0.04 | 13.97 ± 0.03 |
| Chloroform | 3.79 ± 0.04 | 2.60 ± 0.06 | 13.42 ± 0.01 | 6.21 ± 0.04 |
| Ethyl acetate | 3.83 ± 0.04 | 3.22 ± 0.18 | 10.20 ± 0.05 | 3.40 ± 0.03 |
| Methanol | 6.45 ± 0.04 | 3.35 ± 0.05 | 12.75 ± 0.03 | 12.86 ± 0.02 |
| Aqueous | 15.91 ± 0.04 | 4.28 ± 0.24 | 9.36 ± 0.04 | 3.64 ± 0.04 |

Conversely, the greatest values of TFC were detected in hexane extracts (15.7 ± 0.04 mg QE/mL, leaf). This result showed that flavonoid compounds accumulated in non-polar extracts, as found in semi-polar and polar extracts. This result indicates that the non-polar solvents are more efficient in extracting lipophilic flavonoid compounds. These findings align with earlier research, which suggested that the polarity of solvents significantly influences the yield of flavonoid and phenolic compounds for diverse uses [22].

## Statistical Analysis of TPC, TFC, and DPPH

Statistical analysis of differences between TPC, TFC, DPPH radical scavenging activity, and $IC_{50}$ values was performed on leaf and stem using one-way ANOVA and Tukey's post hoc test. DPPH activity was significantly influenced by the type of solvent used for leaf extracts ($F = 137.34$, $p < 0.001$), and Tukey's HSD revealed that differences in DPPH inhibition were highly significant between most solvents, especially between aqueous and all other solvents, as well as between ethyl acetate and the non-polar solvents. In contrast, the TPC and TFC did not significantly vary in leaf surface extracts from different solvents ($p > 0.05$). For stem extractions, the effect of solvent on TFC ($F = 4.07$, $p = 0.007$) and DPPH activity ($F = 119.91$, $p < 0.001$) was significant, while that on TPC ($p = 0.98$) was found not to be substantial based on ANOVA. Tukey's test identified important differences in TFC and DPPH values (across both stems and leaves), particularly between aqueous and non-polar extracts and methanol and hexane for stems. $IC_{50}$ could not be statistically analyzed since there were no replicates per group. These results suggest that solvent polarity significantly affects antioxidant capacity and, to a smaller degree, flavonoid yield in red MCV. They also indicate that the group means and low standard deviation of parameters confirm the reliability of these findings. Similar trends were observed in an earlier study where the extraction solvent largely influenced smoking statistics, and polar solvents usually resulted in maximal radical scavenging and phenolic yields [27].

## DPPH Radical Scavenging Assay

A DPPH is a stable free radical that transitions into a stable diamagnetic compound by accepting either an electron or a hydrogen radical. A higher percentage of inhibition indicates greater antioxidant activity, which demonstrates the compound's capacity to neutralize free radicals and mitigate oxidative stress. A lower $IC_{50}$ indicates more potent antioxidant activity **in** a compound [28]. The findings from the DPPH assay presented in Table 3 suggest that the aqueous leaf extract possesses the highest antioxidant activity (74.24% inhibition, $IC_{50} = 4.574$ mg/mL) compared to the stem extracts and other solvents. Aqueous solvents serve as efficient polar solvents for the extraction of phytochemicals, especially phenolics, which greatly improve the antioxidant potential of the extracts. This is closely linked to the increased phenolic concentration observed in the aqueous extracts, as phenolics effectively scavenge radicals due to their capacity to transfer hydrogen atoms, thereby neutralizing free radicals [29-30].

A positive relationship was found when TPC was compared with DPPH for all the different species (Table 3). For instance, the TPC of the methanol leaves extract was the second highest of all leaf extracts, but the DPPH %inhibition was the third highest. Various theories might explain this discrepancy. First, TPC is a measure of total phenolics, but not all phenolics are equally antioxidant; methanol could extract more phenolics but

**Table 3.** Inhibition percentages and $IC_{50}$ values of Red MCV extracts

| Solvents | Leaf inhibition (% ± SD) | Leaf $IC_{50}$ (mg/mL) | Stem inhibition (% ± SD) | Stem $IC_{50}$ (mg/mL) |
|---|---|---|---|---|
| Hexane | 19.24 ± 0.02 | 1.614 | 4.43 ± 0.02 | 2.645 |
| Chloroform | 39.83 ± 0.03 | 3.794 | 9.39 ± 0.03 | 2.608 |
| Ethyl acetate | 60.57 ± 0.09 | 3.586 | 19.29 ± 0.02 | 3.016 |
| Methanol | 43.51 ± 0.08 | 3.647 | 46.67 ± 0.07 | 3.693 |
| Aqueous | 74.24 ± 0.00 | 4.574 | 20.44 ± 0.01 | 3.114 |

has a lower ability to scavenge radicals compared with more active phenolics in the ethyl acetate extract. Second, DPPH antioxidant activity is not only phenolic compounds–other bioactive compounds such as flavonoids, vitamins, or synergistic phytochemicals are also involved in the overall free radical scavenging, which may be more efficiently extracted with ethyl acetate or aqueous (water) solvents. The differences in extract selectivity and the eventual synergism may result in a higher antioxidant activity of an extract with less amount of total phenolics [27]. The DPPH assay is selective for hydrogen-donating antioxidants. If the phenolic compound(s) in the methanol extract were less effective, the %inhibition would also be less. Therefore, these results indicate that the polyphenolic profile and solvent-mediated synergistic bioactive co-extraction are crucial in determining the variety of antioxidant potential rather than the TPC alone.

The heatmap in Fig. 1 indicates a strongly positive correlation between TPC and DPPH inhibition, with correlation coefficients r = +0.93 for leaf and +0.91 for stems, indicating the phenolic compounds are significant antioxidants in red MCV. Interestingly, a negative correlation was identified for the stems' TFC value and antioxidant activity, suggesting that the extraction of non-polar flavonoids with hexane may have less capacity in free radical scavenging. $IC_{50}$ values demonstrate an excellent negative correlation with inhibition percentages as anticipated ($r \approx -0.85$), validating that low $IC_{50}$ corresponds to high bioactivity. Yield is moderately correlated with TPC but less with TFC, since it seems that the amount of extract does not necessarily equate to bioactivity.

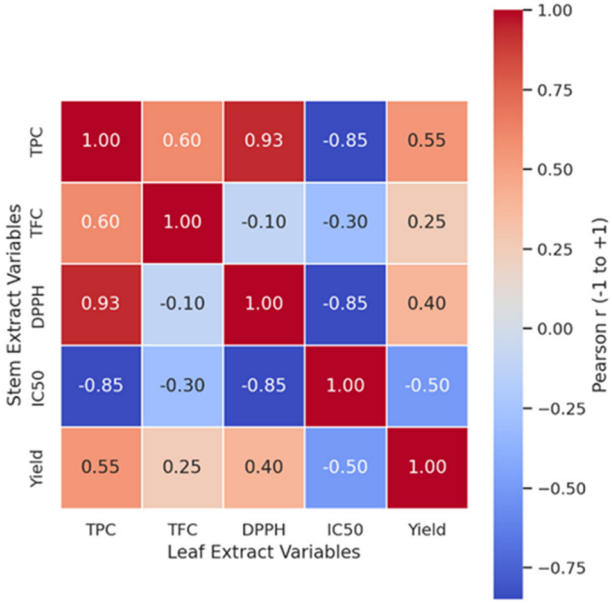


**Fig 1.** Correlation heatmap of phytochemicals metrics and antioxidant activity in red MCV extracts. Red color indicates the strong positive correlation (r = +1), dark blue indicates strong negative correlation (r = −1), and the cell labels show the exact correlation coefficients

## ATR-FTIR Spectra Overlay of Red MCV

ATR-FTIR analyses were performed on the extracted compounds by dissolving them in acetone, which is very soluble and evaporates quickly. However, the IR spectrum of acetone features several relatively strong and diagnostic absorption bands such as the strong C=O stretching vibration (1715 $cm^{-1}$) and the C–H bending near 1365 $cm^{-1}$. To reduce the contribution of solvent and any background spectral obscuration, all samples were dried under high vacuum before analysis to eliminate residual acetone, and all spectra were baseline-corrected to reduce any solvent effects further [31].

The bioactive compounds in the red MCV extracts were examined by ATR-FTIR spectroscopy, and the representative spectra are shown in Fig. 2 together with the assigned peaks summarized in Table 4. The wavenumber at 3379 $cm^{-1}$ showed O–H stretching and indicates the presence of hydroxyl groups, as with the

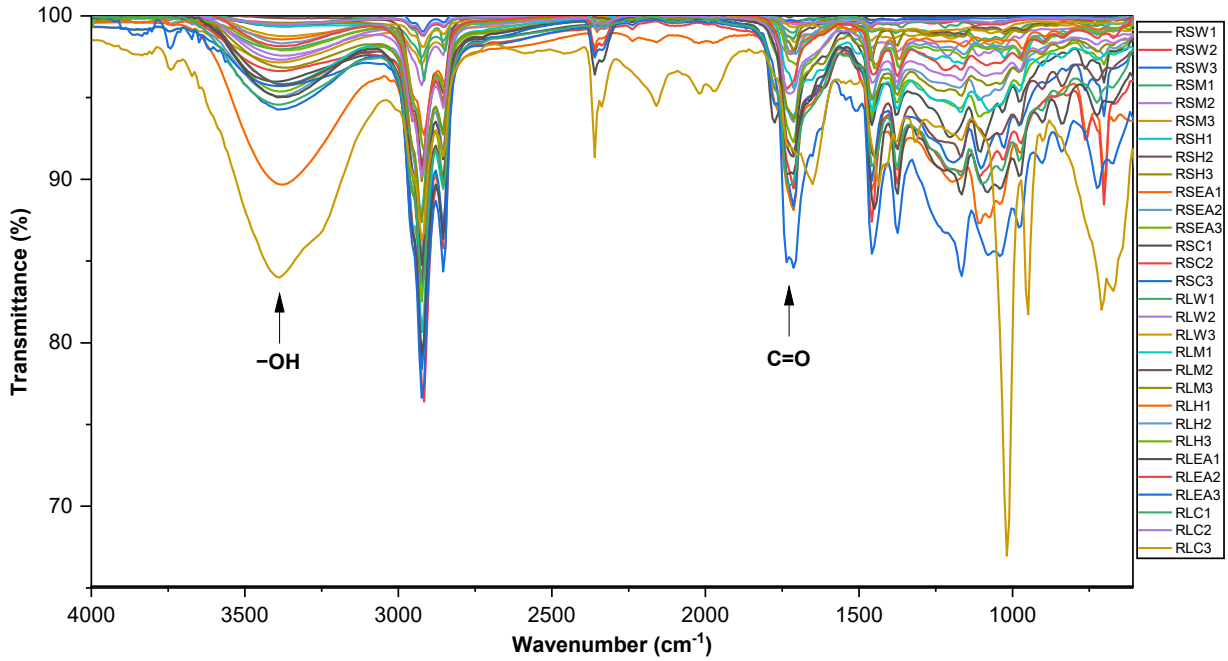


**Fig 2.** ATR-FTIR spectra show the overlay of leaf (L) and stem (S) red MCV extracts in a variety of solvents. R = red, SW = stem water, SM = stem methanol, SH = stem hexane, SEA = stem ethyl acetate, SC = stem chloroform, LW = leaf water, LM = leaf methanol, LH = leaf hexane, LEA = leaf ethyl acetate, LC =leaf chloroform

**Table 4.** Assignment of FTIR spectrum in red MCV

| Wavenumber ($cm^{-1}$) | Assignments | Main attribution |
|---|---|---|
| 3402 | O–H stretch | Alcohol, phenols, carboxylic acid |
| 2854–2924 | C–H stretch | Alkanes |
| 1720 | C=O | Aldehydes, ester |
| 1604 | C=C, N–H bend | Alkene, primary and secondary amines, and amide |
| 1381–1411 | C–H | Alkanes |
| 103–1203 | C–O | Alcohol, ethers, esters, carboxylic acid, and anhydrides |
| 663 | C–H out-of-plane bend | Aromatic |

phenolic and flavonoid compounds. This fingerprint is highly correlated with TPC and DPPH scavenging, which further points to phenols as the major bioactive compounds. Saturated ($sp^3$) C–H stretching (2885–2947 $cm^{-1}$) is assignable to alkyl groups. The C=O stretch at 1712 $cm^{-1}$ is an indicator of flavonoids, phenolic acids, tannins, and various polyphenol compounds. The OPLS-DA loading plot shows that this region is related to the $IC_{50}$ potency of the polar extracts. Alkane C–H bending vibrations (1342–1465 $cm^{-1}$), while the C–O stretching ranged between 1033 and 1280 $cm^{-1}$ were observed [32]. All are typical of phenolic and flavonoid structures believed to play a role in free radical scavenging.

The intensities of the FTIR bands, especially for the functional group of O–H and C=O, were in good correlation with TPC, showing that methanol and water extracts have higher absorbances, due to the high polyphenolic content. On the other hand, the typical specific bands for the hexane extracts were noticeably weaker, so the phenolic content and antioxidant activity (obtained by the DPPH method) were also lower. These spectral outcomes validate the critical role played by the phenolic and flavonoid groups as primary active constituents in the antioxidant actions of red MCV extracts.

### PCA Score Plot Data

The quantified FTIR absorbance of different parts of red MCV extract samples for each wavenumber was used as input for PCA, which was then transformed into a set of values of linearly uncorrelated variables called principal components (PCs) [33]. Chemometric modelling afforded solid validation, pointing toward methanol extracts and aqueous extracts in PCA and OPLS-DA loading plot, as rich sources of active bio-compounds. Fig. 3 demonstrates that the various components (leaf and stem) of red MCV treated with different solvents can be categorized into separate groups. The distribution plot serves as a tool for analyzing the segregation of samples (leaf and stem) based on their solvent, which can be inferred by examining the proximity of the centroids for each sample group. The PCA score plots indicate that the extract solvent separated samples based on polarity and phytochemical profiles, with the aqueous and methanol extracts appearing in the same cluster, owing to the efficiency of these solvents in extracting the phenolic compounds. In contrast, hexane and chloroform extracts were clustered separately, aligning with their efficiency in

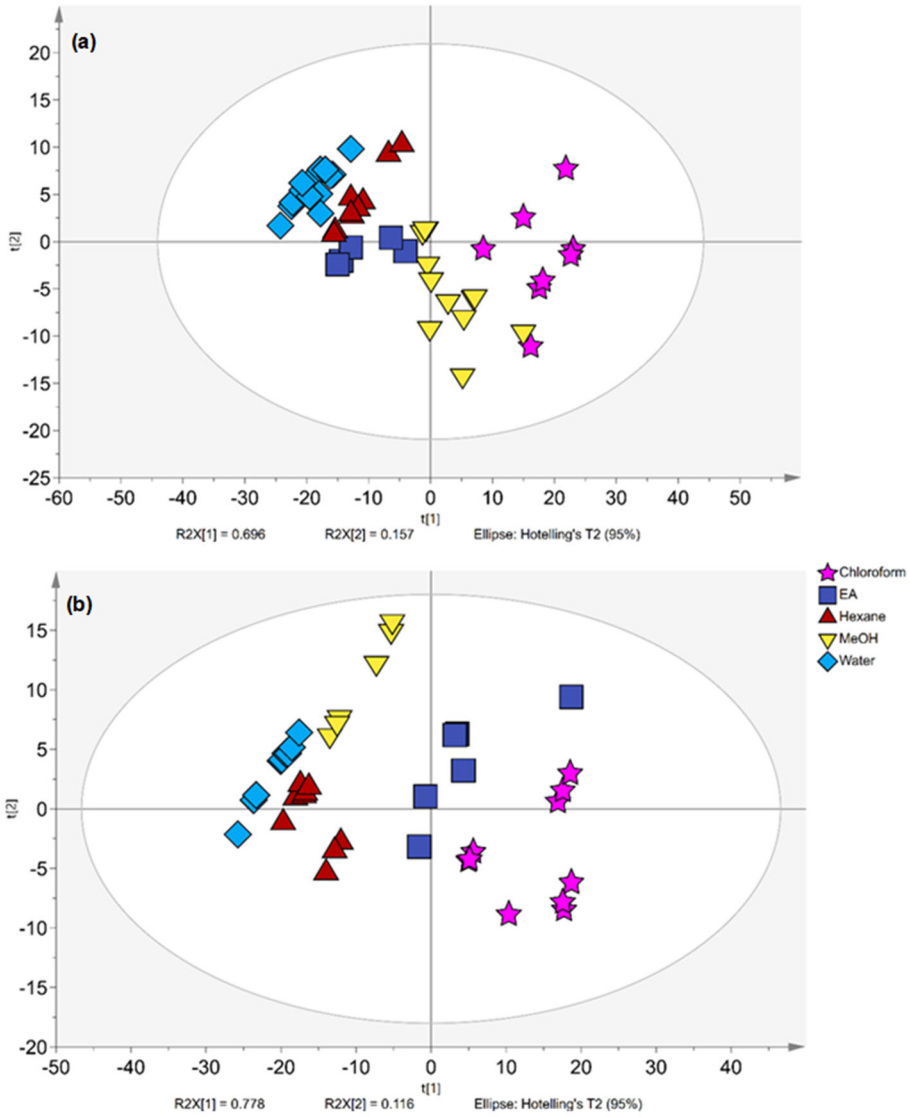


**Fig 3.** PCA score plot data for different parts of the red MCV from the (a) leaf extracts and (b) stem extracts following their extraction solvent. Dark Blue = ethyl acetate extract; Red = hexane extract; Yellow = methanol extract; Light Blue = water extract, and Pink = chloroform extract

extracting non-polar flavonoid compounds [34].

### OPLS-DA Loading Plots for Leaf and Stem Extracts of Red MCV

The OPLS-DA is a statistical method used in chemometric analysis. The analysis of MCV extracts provided a comprehensive understanding of the chemical diversity between the leaf and stem of red MCV (Fig. 4) and the effectiveness of various types of solvents in extracting bioactive antioxidant compounds. The OPLS-DA loading plot classified functional groups such as O–H and C=O stretches as active regions associated with antioxidant activity, while the weaker contributors were categorized as non-active regions. The study revealed that the methanol and aqueous red MCV extracts have a superior antioxidant profile. The elevated levels of O–H and C=O functional groups in red MCV suggest a higher concentration of phenolic and flavonoid compounds directly linked to the antioxidant effectiveness.

The red MCV demonstrated not only an increased presence of phenolic compounds in the leaf extracts but also indicated that the choice of solvent for extraction affects the profile of bioactive compounds. Methanol and water were found to be the most effective solvents for extracting phenolics, as evidenced by the OPLS-DA

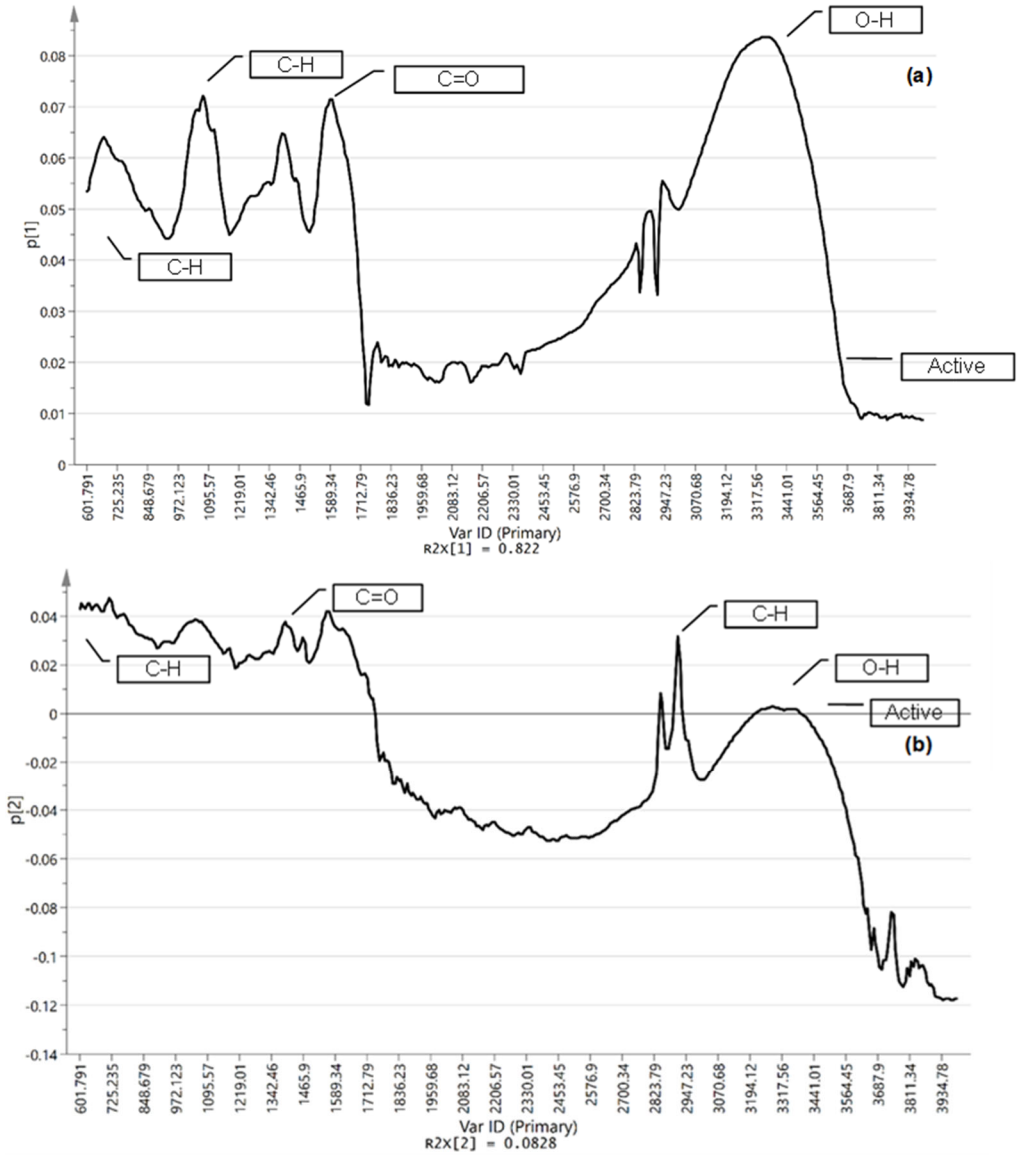


**Fig 4.** Loading plot for OPLS-DA of methanol extraction: (a) leaf extract and (b) stem extract from Red MCV

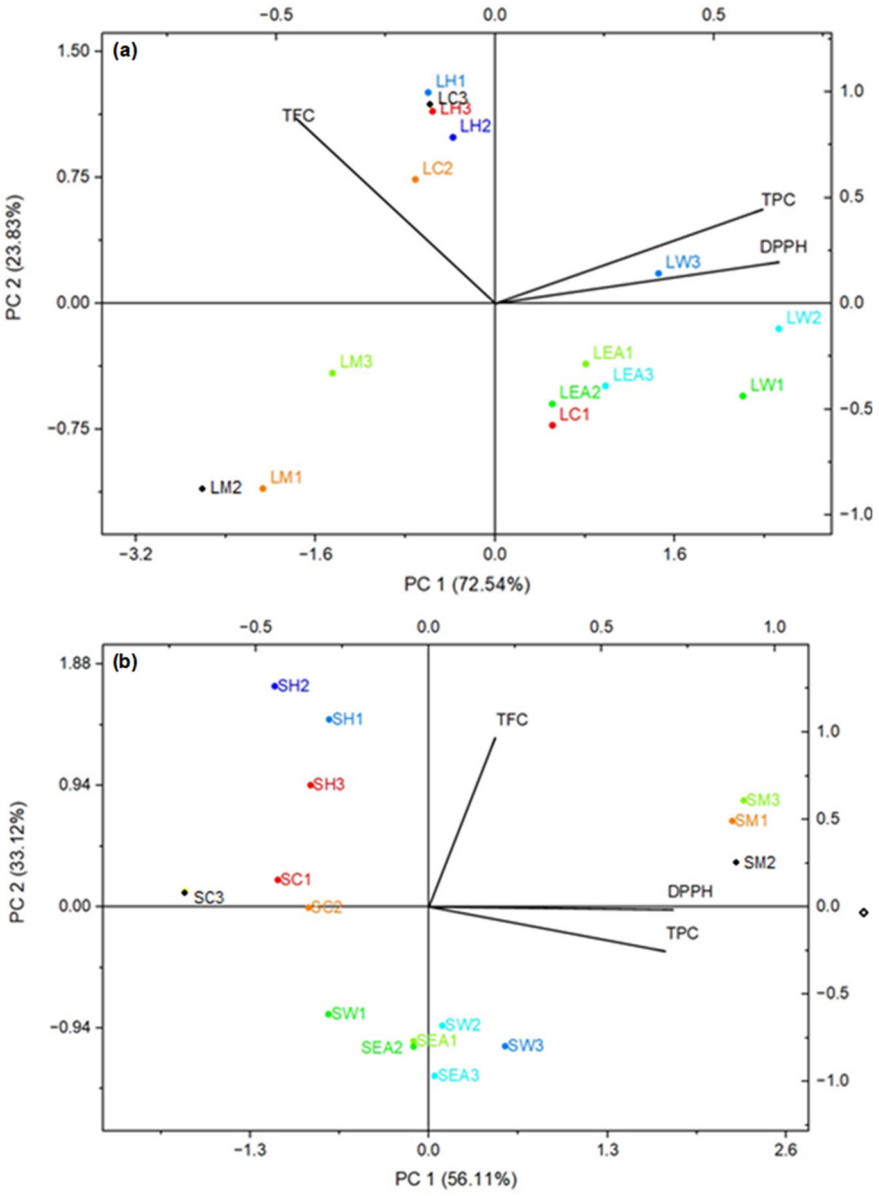


**Fig 5.** (a) Biplot of antioxidant activity of the leaf and stem solvent extracts and (b) type solvent extracts in Mariposa plant extracts (triplicate). DPPH: 2,2-Diphenyl-1-picryl-hydrazyl radical scavenging activity; TFC: total flavonoid content; TPC: total phenolic content; W: water extract; M: methanol extract; EA: ethyl acetate; C: chloroform extract; H: hexane extract; L: leaf part; S: stem part

data. The biplot was derived using PCA to identify the variables that cause the most influence on the antioxidant activity for both MCV extracts [35]. Fig. 5 shows a PCA biplot evaluating three key experimental variables (extraction solvent, antioxidant activity, and plant variety) in different plant solvent extracts. The first two principal components, PC1 (45.03%) and PC2 (31.53%), account for 76.56% of the variation in the data set. The biplot showed that polar solvents such as water and methanol are positioned in the positive region of PC1 (45.03%). This indicates that these solvents extract phenolic compounds linked to the antioxidant activity more efficiently. The negative values on the PC1 (lower region) are associated with less polar solvents (chloroform and hexane), indicating that these solvents are extracting the flavonoid compounds, as suggested by

their proximity to TFC in the biplot.

Both red MCV extracts are predominantly located in the positive region of PC2. These extracts have higher phenolic content and antioxidant activity. In contrast, the green MCV extracts are found in the negative region of PC2, indicating lower phenolic content and antioxidant efficacy than the red MCV. This separation highlights the distinct chemical profiles and bioactive compound concentrations between the red MCV extracts. The association of variables (TPC, TFC, and DPPH) provides deeper insights into the antioxidant mechanism of MCV extracts. TPC and DPPH inhibition are located in the upper-right quadrant of the biplot, indicating a positive correlation between phenolic content and antioxidant activity. On the other hand, TFC is positioned closer to non-polar solvents (hexane), suggesting that flavonoids are present in higher concentrations in non-polar solvent extracts.

## ■ CONCLUSION

After the aqueous and methanolic extracts, the red MCV leaf extract has the highest concentration of antioxidant components. The findings of the DPPH experiment showed greater antioxidant activity from these solvent extracts. Correlation between TPC and TFC indicates more antioxidant activity in polar solvents (methanol and water). These results were confirmed by data analysis from ATR-FTIR spectroscopy and chemometric analysis. The functional groups that evidenced the presence of flavonoids and phenolic antioxidant molecules included the carbonyl (C=O) and hydroxyl (O–H) groups. Different parts of red MCV consistently showed higher concentrations of beneficial chemicals compared to other plants, as revealed by chemometric analysis, highlighting the unique chemical profiles. This study did not study the bioavailability, toxicity, and pharmacodynamics of the identified compounds in biological systems. The therapeutic relevance, safety, and effectiveness of red MCV–derived compounds should be assessed in future studies using human cell lines and animal model assays, LC-MS /MS-based metabolite profiling, and *in vivo* antioxidant testing.

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## ■ CONFLICT OF INTEREST

We have no conflicts of interest to disclose.

## ■ AUTHOR CONTRIBUTIONS

Nur Firzana Liyana Abdul Jaafar did the experiment. Soraya Shafawati Binti Mohamad Tahier and Nur Firzana Liyana Abdul Jaafar wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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