Heliyon 10 (2024) e37077

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Frequency-dependent dielectric spectroscopic analysis on phytochemical and antioxidant activities in Radix Glycyrrhizae extract

Ong Hong Liang [a], Cheng Ee Meng [a,b,c], Che Wan Sharifah Robiah Mohamad [a], Nashrul Fazli Mohd Nasir [a,c], Tan Xiao Jian [c,d,e], Beh Chong You [f], Emma Ziezie Mohd Tarmizi [g,*], Lee Kim Yee [h], You Kok Yeow [i], Khor Shing Fhan [j], Leu Kim Fey [k,l], Te Kian Keong [k,l], Mohd Riza Mohd Roslan [f], Siti Aishah Baharuddin [f]

[a] Faculty of Electronic Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Perlis, 02600, Malaysia
[b] Advanced Communication Engineering (ACE) Centre of Excellence, Universiti Malaysia Perlis (UniMAP), Perlis, 02600, Malaysia
[c] Sports Engineering Research Centre (SERC), Universiti Malaysia Perlis (UniMAP), Perlis, 02600, Malaysia
[d] Centre for Multimodal Signal Processing, Tunku Abdul Rahman University of Management and Technology (TAR UMT), Jalan Genting Kelang, Setapak, Kuala Lumpur, 53300, Malaysia
[e] Department of Electrical and Electronics Engineering, Faculty of Engineering and Technology, Tunku Abdul Rahman University of Management and Technology (TAR UMT), Jalan Genting Kelang, Setapak, Kuala Lumpur, 53300, Malaysia
[f] Department of Engineering and Built Environment, Tunku Abdul Rahman University of Management and Technology, Penang Branch, Pulau Pinang, 11200, Malaysia
[g] Centre of Foundation Studies for Agricultural Science, Universiti Putra Malaysia, Serdang, 43400, Malaysia
[h] Lee Kong Chian Faculty of Engineering & Science, Sungai Long Campus, Jalan Sungai Long, Tunku Abdul Rahman University, Kajang, Cheras, Sungai Long City, 43000, Malaysia
[i] Department of Communication Engineering, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, 81310, UTM Johor, Malaysia
[j] Faculty of Electrical Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Kangar, 02600, Malaysia
[k] Department of Chinese Medicine, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman (UTAR), Sungai Long Campus, Jalan Sungai Long, Tunku Abdul Rahman University, Kajang, Cheras, Sungai Long City, 43000, Malaysia
[l] Hospital Universiti Tunku Abdul Rahman, Jalan Hospital UTAR, 31900, Kampar, Perak Darul Ridzuan, Malaysia

ARTICLE INFO

Keywords:
Radix Glycyrrhizae
Phytochemical
Antioxidant
Dielectric

ABSTRACT

Radix Glycyrrhizae, the dried roots of the Glycyrrhiza glabra plant, is a popular Chinese herbal medicine known for its various health benefits. It is particularly effective in relieving respiratory problems like coughs, sore throats, bronchitis, and asthma. However, there is limited research on the electrical properties of Radix Glycyrrhizae, likely due to its complex composition of phytochemical and antioxidant activities. This research aims to investigate the potential of these active biological compounds and understand their electrochemical properties. In this study, High-Performance Liquid Chromatography (HPLC) analysis revealed that Radix Glycyrrhizae decoction contains significant amounts of flavonoids and saponins, compounds known for their health

* Corresponding author.
E-mail addresses: s171151510@studentmail.unimap.edu.my (O. Hong Liang), emcheng@unimap.edu.my (C. Ee Meng), robiah@unimap.edu.my (C.W.S.R. Mohamad), tanxj@tarc.edu.my (T. Xiao Jian), behcy@tarc.edu.my (B. Chong You), emma@upm.edu.my (E. Ziezie Mohd Tarmizi), kylee@utar.edu.my (L. Kim Yee), kyyou@fke.utm.my (Y. Kok Yeow), sfkhor@unimap.edu.my (K. Shing Fhan), leukf@utar.edu.my (L. Kim Fey), tekk@utar.edu.my (T. Kian Keong).

https://doi.org/10.1016/j.heliyon.2024.e37077
Received 19 March 2024; Received in revised form 18 July 2024; Accepted 27 August 2024
Available online 28 August 2024
2405-8440/© 2024 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

benefits and therapeutic effects. Further analysis using Fourier Transform Infrared Spectroscopy (FTIR) identified several functional groups, including phenols, alcohols, alkynes, alkenes, ethers, and glycosides, which contribute to the plant's medicinal potential and affect the impedance and dielectric properties of the extract. The antioxidant activity of Radix Glycyrrhizae decoction was also evaluated using DPPH assays, showing similar radical scavenging activity to gallic acid. Dielectric and impedance measurement of Radix Glycyrrhizae extract were performed using an Agilent vector network analyzer and a Hioki impedance analyzer. The dielectric constant measured was consistent across both analyzers. However, the loss factor showed different trends: the vector network analyzer indicated a decrease in the loss factor with increasing frequency in the range of 5 MHz–20 GHz, while the impedance analyzer showed the opposite trend in the frequency range of 4 Hz–5 MHz.

1. Introduction

Throughout history, humans have recognized and used the medicinal properties of plants in various ways. Scientists have extensively researched these plants and acknowledging their therapeutic potential [1]. These plants are a valuable source for discovering new drugs that are effective, have minimal side effects, and are cost-effective [2]. Over 80 % of the global population relies on medicinal herbs to treat various health conditions [3], showing the long-standing relationship between humans and plants, which dates back thousands of years, as evidenced by fossil records [4]. Numerous studies have highlighted the importance of phytoconstituents found in medicinal plants in various biological activities. These activities include anti-inflammatory, antibacterial, antifungal, antimalarial, anticancer, and antioxidant actions [5]. Reactive Oxygen Species (ROS) are generated by external factors, e.g. drugs, chemicals, smoke, and environmental stress. These factors contribute to atherosclerosis, inflammation, neurodegenerative diseases, cardiovascular diseases, and cancer [1]. The overproduction of free radicals in the human body can lead to cell damage and severe oxidative harm to essential biological molecules.

Natural antioxidant, i.e. phenolic compounds offer protection against diseases caused by free radicals. These compounds are found in a various natural source, including curcuminoids, phenolics, lignans, tannins, stilbenes, quinones, coumarins, and flavonoids [6]. The health and food industries have shown significant interest in antioxidants of natural origin, particularly in identifying secondary metabolites. These antioxidants protect the body from radical damage by neutralizing ROS [7].

Antioxidants are molecular compounds that protect cells and biological structures from oxidative stress, which results from an imbalance in the body's defenses against free radicals. Free radicals are molecules with an unpaired electron. It triggers chemical chain reactions that damage DNA, lipids, proteins, cells, and other body parts, leading to disease development and accelerating the aging process. Antioxidants prevent this damage by binding to free radicals, reducing their harmful effects, and supporting the integrity and overall health of biological systems.

Radix Glycyrrhizae, a plant from the Fabaceae family, is widely used in traditional medicine practices [8]. It is primarily found in central and eastern Asia, with around 30 known species [9]. This plant is the source of various secondary metabolites or phytoconstituents, including triterpenoids, flavonoids, isoflavonoids, and chalcones, which are responsible for many biological activities [10]. Understanding the phytochemical profile of Radix Glycyrrhizae extract is crucial for revealing the potential health benefits of these constituents. This research uses High-Performance Liquid Chromatography (HPLC) analyses to identify the phytochemical constituents in a water extract of Radix Glycyrrhizae. The study aims to uncover potent phytochemicals that could redefine the understanding and use of electrochemical properties in various applications.

Dielectric analysis offers a unique perspective on the electrical characteristics of Radix Glycyrrhizae extract. The extract's response to an electric field is linked to molecular interactions and structural dynamics. This analysis plays a significant role in exploring the electrical properties of the extract and their potential applications. Frequency domain dielectric spectroscopy enhances traditional chemical analyses, uncovering new dimensions of the extract's bioactivity and therapeutic potential. This interdisciplinary approach bridges herbal medicine with modern scientific inquiry, fostering advancements in pharmacology, biotechnology, and biomedical devices. Exploring Radix Glycyrrhizae extract through dielectric analysis promises to optimize its biomedical applications, underscoring the importance of diverse analytical approaches in botanical research.

2. Materials and methods

2.1. Reagents

The reference standards of glycyrrhizin (HPLC grade) is product of Sigma Aldrich (MERCK-Sigma Aldrich, US). Folin-Ciocalteu reagent, aluminium chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, and gallic acid are also products of Sigma Aldrich (MERCK-Sigma Aldrich, US). All other reagents used in these experiments are of analytical grade.

2.2. Plant collection and preparation

Radix Glycyrrhizae was obtained from a reputable vendor specializing in traditional Chinese medicinal and identified by Dr. Te Kian Keong, a traditional Chinese medicine pharmacist from the Department of Chinese Medicine, Universiti Tunku Abdul Rahman,

Malaysia. The plant was air-dried at room temperature for three days. Subsequently, the plant was ground into a fine powder and stored in sealed plastic containers until extraction [11].

2.3. Extraction method

A 50 g portion of the dried Radix Glycyrrhizae powder was subjected to a water decoction process. The plant material was boiled in 1000 mL of water for 2 h to extract the active compounds. After cooling, the decoction was filtered to obtain the aqueous extract. This extract was then evaporated using rotary evaporators, resulting in solid, amorphous masses. The crude extract (50 mg) was then dissolved in 50 mL of water.

2.4. Quantitative analysis

The quantitative analysis of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) was determined using a UV–Vis spectrophotometer (Shimadzu UV-1280, Shimadzu Corporation, Kyoto, Japan).

2.4.1. Total phenolic content (TPC)

The Folin–Ciocalteu reagent method is a widely used chemical assay for determining the Total Phenolic Content (TPC) in various samples, particularly plant extracts. It quantifies phenolics by their ability to reduce the reagent, forming a blue complex, and is crucial in assessing antioxidant capacity across disciplines like natural product research and pharmaceutical analysis. In a previous study [12], 0.5 mL of Radix Glycyrrhizae decoctions was combined with 2.5 mL of Folin–Ciocalteu reagent. After incubation for 15 min at 37 °C, 2 mL of a 7.5 % w/v Na_2CO_3 solution was added, followed by dilution to a 10 mL volume with distilled water. The mixtures were incubated for additional 30 min. The absorbance was then measured at 765 nm using an ultraviolet–visible spectrophotometer. A reference curve was established using Gallic acid as a standard for TPC determination, with concentrations ranging from 50 to 250 g/mL. The total phenol values were expressed as Gallic acid equivalents (GAE) mg/g of dry weight, using the equation $y = 0.0037x\text{-}0.094$, $R^2 = 0.999$ [12] where y is concentration of TPC in mg GAE/g and x is absorbance value obtained from ultraviolet–visible spectrophotometer. The formula

$$T_p = C_g \text{ x } V/ M \quad (1)$$

was used, where T_p represents the total phenolic content in mg GAE/g, C_g is the concentration of decoctions derived from the Gallic acid calibration curve, V signifies the volume of extract in milliliters (mL), and M denotes the weight of the dry plant extract in grams (g).

2.4.2. Total Flavonoid Content (TFC)

The protocol outlined by Zengin et al. [13] was employed to determine the Total Flavonoid Content (TFC) using the aluminium chloride ($AlCl_3$) colorimetric method. Quercetin, dissolved in methanol, served as the reference for the standard curve, with concentrations ranging from 50 to 250 g/mL. A concoction was prepared by combining 0.5 mL of plant decoction solution, 0.1 mL of 10 % w/v $AlCl_3$ solution, and 0.1 mL of a 0.1 mM potassium acetates solution in a flask. The volume was then adjusted to 5 mL with distilled water. The mixture was maintained at 37 °C for 30 min. The absorbance was measured using a UV–Vis spectrophotometer at 415 nm. The results were expressed in grams of Quercetin Equivalent (QE) per mg/g of the extract, using the equation $y = 0.0042x\text{-}0.1149$, $R^2 = 0.998$ [13]. The flavonoid content was calculated using the following formula:

$$T_f = C_q \text{ x } V/M \quad (2)$$

where T_f represents the TFC mg/g extract in quercetin equivalents and C_q is the concentration of the extract derived from the quercetin calibration curve.

2.5. Antioxidant activity (DPPH)

The procedure for evaluating the radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was adapted from the method proposed by Greco et al. [14], with some alterations. In a dark environment, 1 mL of a 90 M DPPH solution was mixed with 1 mL of various concentrations of the decoction and gallic acid (used as the control) at concentrations of 12, 6, 3, 1.5, 0.75, and 0.375 mg/mL. The concoction was then incubated for 30 min at 37 °C. The absorbance was measured at 517 nm, and the activity was expressed as percentage inhibition [15]. The formula for calculating the radical scavenging activity is as follows:

$$\% \text{ radical scavenging activity} = (\text{Absorbance of control - Absorbance of sample}) / \text{Absorbance of control x } 100\ \% \quad (3)$$

2.6. Fourier-Transform Infrared Spectroscopy (FTIR)

Decoction extracts of Radix Glycyrrhizae were combined with KBr salt, ground using a mortar and pestle, and then pressed into a thin pellet [16]. Spectroscopic measurements of the samples were performed using a FTIR spectrometer (PerkinElmer Spectrum Two,

PerkinElmer Inc., Waltham, Massachusetts, USA), scanning within the range of 4000 and 500 cm^{-1}.

2.7. High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) was used on the plant extract decoctions for the quantitative analysis of phenolics and flavonoids. The HPLC analysis was performed using an Agilent 1260 Infinity II system (Agilent Technologies, Santa Clara, California, USA). The column used was a shim-packed CLC ODS C-18 (2.5 cm, 4.6 mm, 5 μm in diameter), selected for its high efficiency in separating phenolic compounds and flavonoids, which are key components in Radix Glycyrrhizae. Plant extract decoctions were prepared at a concentration of 10 mg/mL in their respective solvents, chosen based on preliminary tests to ensure clear and distinct peaks. A volume of 20 μL of the decoctions was injected into the HPLC system after evaluating different volumes (10 μL, 20 μL, and 30 μL) to provide the best sensitivity without overloading the column. The mobile phases were A (H_2O: acetoacetate 94:6, pH 2.27) and B (100 % acetonitrile), with a gradient elution program developed through several trials to ensure sharp and well-resolved peaks. The specific gradient conditions were set at 15 % B for 0 min, transitioning to 45 % B for 15–30 min and reaching 100 % B at 45 % for 35–40 min, at a flow rate of 1 mL/min. This flow rate was optimized after testing different rates (0.8 mL/min, 1.0 mL/min, and 1.2 mL/min) to find the optimal balance between run time and resolution. The UV–visible detector spectra for all samples were recorded at 254 nm [17], chosen after scanning the UV spectra of the reference standards and samples as it provided the highest absorbance for saponins, particularly glycyrrhizin. The column temperature was maintained at 30 °C, with different temperatures (25 °C, 30 °C, and 35 °C) tested to achieve optimal peak shapes and retention times. The optimized method was validated for parameters such as linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The method showed good linearity ($R^2 > 0.999$) for all compounds, with acceptable precision (RSD <2 %) and accuracy (recovery rate within 98–102 %). The LOD was determined to be 0.05 μg/mL, and the LOQ was determined to be 0.2 μg/mL [18], ensuring the sensitivity of the method for detecting and quantifying low concentrations of phenolics and flavonoids.

2.8. Dielectric spectroscopy - 4 Hz to 5 MHz (impedance analyzer)

The method for measuring the decoction extract of Radix Glycyrrhizae from 4 Hz to 5 MHz involves the use of customized capacitive electrodes and an impedance analyzer (Hioki IM3570, Hioki E.E. Corporation, Nagano, Japan). The Radix Glycyrrhizae sample is prepared as a decoction and placed between the capacitive electrodes. The HIOKI impedance analyzer is then used to measure the parallel capacitance (Cp) and conductance (G) of the sample.

The obtained Cp and G values are used to calculate the dielectric constant and the dielectric loss factor. The dielectric constant (ε') is calculated using the formula

$$\varepsilon' = (Cp*d)/(A*\varepsilon_o) \quad (4)$$

where d is the sample thickness, A is the sample area, and ε_o is the permittivity of free space. The dielectric loss factor (ε'') is calculated using the formula

$$\varepsilon'' = (G*d)/(\omega*A*\varepsilon_o) \quad (5)$$

where ω is the angular frequency.

2.9. Dielectric spectroscopy - 5 MHz to 20 GHz (vector network analyzer)

To measure the decoction extract of Radix Glycyrrhizae from 5 MHz to 20 GHz, an open-ended coaxial probe is used with a vector network analyzer (Agilent E5071C ENA Series, Agilent Technologies, Santa Clara, California, USA). The Radix Glycyrrhizae extract is prepared as a decoction, and the open-ended coaxial probe is placed in direct contact with the sample. The probe is connected to the vector network analyzer, which transmits a range of frequencies into the sample. The reflected signals are then captured and measured by the vector network analyzer. The resulting data represents the complex permittivity of the sample, including both the dielectric constant and the dielectric loss factor.

3. Results

3.1. Total phenolic content and Total Flavonoid Content

Decoctions extracted from Radix Glycyrrhizae were found to contain a significant amount of Total Phenolic Content (TPC), measured at 137.5 ± 1.46 mg/g of dry weight, equivalent to Gallic acid equivalent (GAE).

Total Flavonoid Content (TFC) specifically measures flavonoids, a subclass of phenolic compounds characterized by a common structure of 15 carbon atoms arranged in three rings (C_6-C_3-C_6). Flavonoids include various subclasses such as flavones, flavonols, flavanones, flavan-3-ols (catechins), anthocyanins, and isoflavones, distinguished by different levels of oxidation and substitutions on these rings. TFC focuses specifically on flavonoids with their characteristic C_6-C_3-C_6 structure.

The flavonoid content in the Radix Glycyrrhizae decoction was found to be 91.5 ± 0.87 mg/g of dry weight, equivalent to quercetin.

3.2. High-Performance Liquid Chromatography (HPLC)

Table 1 presents the quantitative analysis of the Radix Glycyrrhizae decoction. The components identified in the decoction, based on retention time, include glycyrrhizin (a triterpenoide glycoside (saponin) with glycyrrhetinic acid). The HPLC analysis results revealed that glycyrrhizin exhibits a concentration 0.732 mg/g. The HPLC chromatogram, shown in Fig. 1(a), demonstrates that glycyrrhizin is the most abundant saponin in the Radix Glycyrrhizae. The retention time of the identified peak for glycyrrhizin is 1.824 min. This confirms the quantitative results, with glycyrrhizin showing the highest peak, indicating its predominance in the decoction.

3.3. Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-Transform Infrared Spectroscopy (FTIR) analysis confirmed the presence of multiple bioactive constituents in the decoction extract of Radix Glycyrrhizae. These constituents include alcohols, phenols, alkynes, alkenes, ethers, and glycosides. Six functional groups were identified from the extract, detailed in Table 2 and illustrated in Fig. 2. The most prominent peak is observed at peak number 3265, corresponds to the O-H stretch, likely originating from the benzene ring of TPC and TFC. These functional groups present in extract significantly influence dielectric parameters.

The FTIR spectrum in Fig. 2 reveals key functional groups in Radix Glycyrrhizae extract. A broad O-H stretch at 3265 cm^{-1} suggests strong hydrogen bonding in alcohols and phenols, enhancing antioxidant properties. A sharp C ≡ C stretch at 2142 cm^{-1} indicates alkynes, contributing to pharmacological activity. The C=C stretch at 1635 cm^{-1} represents alkenes, linked to anti-inflammatory effects. Multiple C-O stretches (1055, 1033, 1016 cm^{-1}) suggest ethers and glycosides, indicating antimicrobial properties. The fingerprint region below 1000 cm^{-1} shows complex structures, highlighting the extract's rich bioactive compounds and therapeutic potential.

3.4. Antioxidant activity (DPPH)

The antioxidant potential of Radix Glycyrrhizae decoctions was evaluated using the DPPH method. Results indicated that antioxidant activity increased proportionally with concentration. Both decoction extract and Gallic acid showed higher antioxidant efficacy at increased concentrations. The DPPH method measures antioxidant capacity through an electron-transfer process, turning a violet solution colorless in the presence of antioxidants. This color change, quantified using a UV–visible spectrophotometer, correlates with antioxidant concentration.

In the DPPH assay, the decoction exhibited a radial scavenging activity of 82.3 % ± 0.8 at 12 mg/mL, comparable to the standard Gallic acid, as shown in Fig. 3. The figure demonstrates that as the concentration of both the decoction extract and Gallic acid increases, the radical scavenging activity also rises, confirming the concentration-dependent antioxidant efficacy. Radix Glycyrrhizae demonstrated significant variability ($p < 0.05$) in its ability to neutralize free radicals, further emphasizing its potential as a strong antioxidant.

3.5. Dielectric spectroscopy- 4 Hz to 5 MHz (HIOKI IM 3570 impedance analyzer)

Fig. 4 illustrates the variation in measured dielectric constant and loss factor of Radix Glycyrrhizae decoction extract from 4 Hz to 5 MHz using a HIOKI impedance analyzer. The data reveals that both the dielectric constant and loss factor exhibit frequency-dependent behavior.

In Fig. 4(a), the dielectric constant is high at the lower frequency of around 4 Hz, indicating a strong polarization response. As frequency increases, the dielectric constant decreases, suggesting reduced polarization. It continues to decrease until it stabilizes beyond 1 MHz, showing minor fluctuations up to 5 MHz.

Distilled water serves as a control for measuring dielectric constant and loss factor. Its frequency-dependent variation is negligible compared to the decoction extract, indicating that the presence of distilled water, a major component of the extract, does not dominate its dielectric behavior.

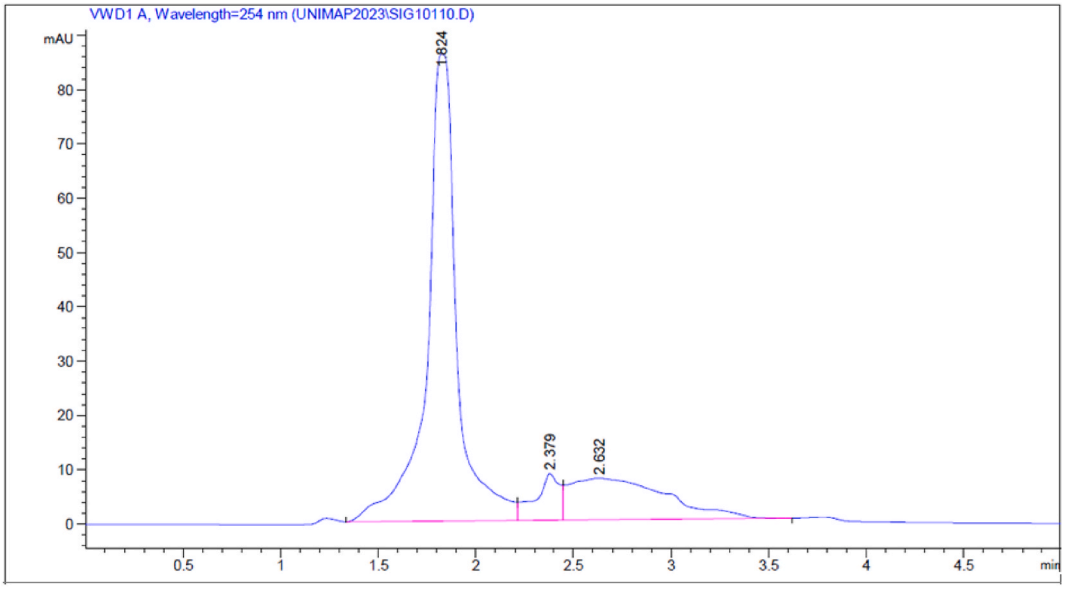
Similarly, Fig. 4(b) depicts the loss factor of extract, which is highest at the lower frequencies and diminishes as frequency increases. It reaches a minimum around 5 MHz, showing a frequency-dependent behavior in the Radix Glycyrrhizae extract.

3.6. Dielectric spectroscopy - 5 MHz to 20 GHz (vector network analyzer)

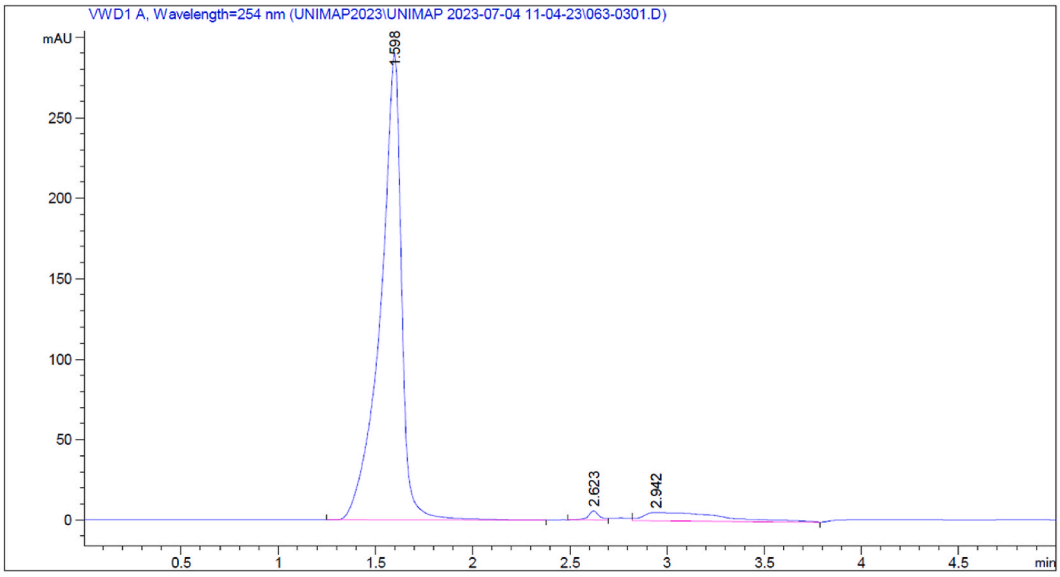
In the frequency range of 5 MHz–20 GHz (RF and microwave spectrum), the dielectric constant and loss factor of the Radix Glycyrrhizae extract were measured using the Agilent Technologies E5071C vector network analyzer. The measured dielectric constant decreases as frequency increases. This occurs because at higher frequencies, the material's polarization mechanisms cannot

Table 1
Analysis of saponin and flavonoids in Radix Glycyrrhizae decoctions using HPLC.

Phytochemicals	Concentration (mg/g)	Retention Time (min)
Saponin		
Glycyrrhizin	0.732	1.824



(**a**)



(**b**)

Fig. 1. Chromatographic representation of (a) saponin and flavonoids in Radix Glycyrrhizae decoctions and (b) standard compound (glycyrrhizin).

Table 2
Interpretation of FTIR for compounds found in the decoction extract of Radix Glycyrrhizae.

No	Wave Number cm^{-1}	Function Groups	Identified Phytocompounds
1	3265	O-H stretch	Alcohols, phenols
2	2142	C ≡ C stretch	Alkynes
3	1635	C=C stretch	Alkenes
4	1055	C-O stretch	Ethers
5	1033	C-O stretch	Ethers
6	1016	C-O stretch	Glycosides

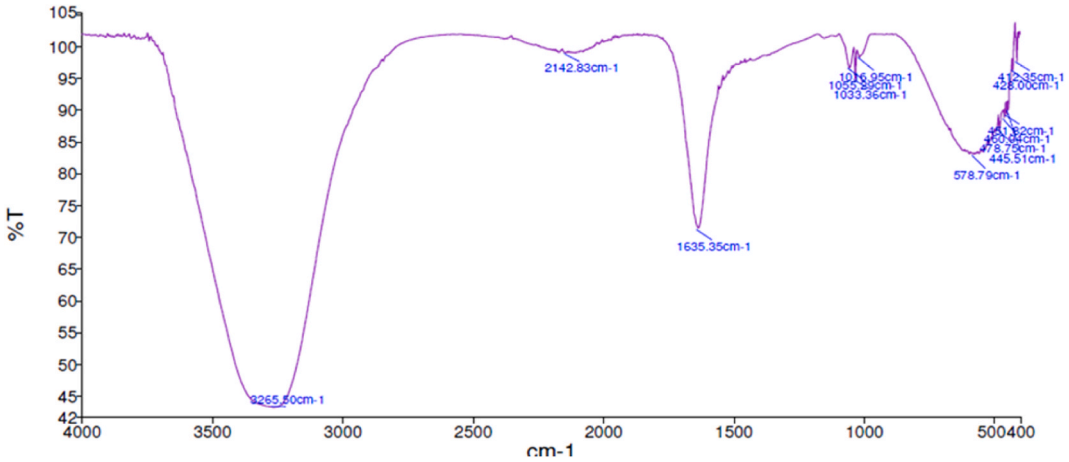


Fig. 2. Analysis of the FTIR spectrum for the decoction extract derived from the Radix Glycyrrhizae plant.

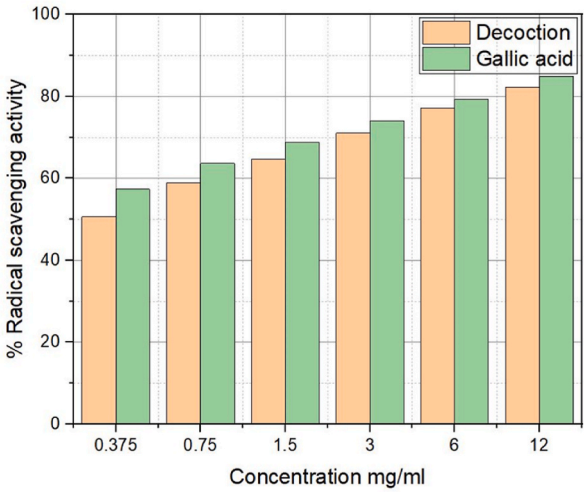


Fig. 3. Assessment of the antioxidant activity of Radix Glycyrrhizae decoctions using the DPPH assay.

respond fast enough to the changing electric field, resulting in reduced ability to store electrical energy (represented by the dielectric constant).

Simultaneously, the loss factor increases with frequency. The loss factor denotes the ability of bioactive compound in extract to dissipate electrical energy when subjected to electric field. The increase in loss factor corresponds to the decrease in dielectric constant, where incomplete dielectric polarization reduces friction among the functional groups of bioactive compounds.

4. Discussion

The therapeutic potential of Radix Glycyrrhizae decoctions can be attributed to the presence of phytoconstituents, which were identified through HPLC analysis. Previous studies have documented various phytoconstituents in Radix Glycyrrhizae extract, including flavonoids and saponins [19]. The decoctions exhibited TPC and TFC of 137.5 ± 1.46 and 91.5 ± 0.87, respectively, consistent with previous research findings reporting TPC content at 45.13 ± 8.74 mg GAE/g and TFC at 24.99 ± 2.65 mg QE/g [20]. HPLC analysis revealed high concentrations of saponins such as glycyrrhizin in Radix Glycyrrhizae decoction. FTIR analysis further validated the presence of alcohols, phenols, alkynes, alkenes, ethers, and glycosides, supporting the identification of biologically active compounds as revealed by HPLC. Glycyrrhizin features hydroxyl groups within its triterpene structure, alongside carboxylic acid groups and glycosidic linkages. According to Qiao et al. [21], glycyrrhizin emerged as the predominant saponin compound in HPLC in the decoction extract.

Phytoconstituents such as phenolics and flavonoids significantly contribute to the antioxidant properties due to their hydrogen-donating ability and structural requirements essential for potent radical scavenging activity [22]. The broad spectrum of biological activities attributed to flavonoids includes, antimicrobial, antioxidant, anti-inflammatory, anticancer, and anti-allergic properties

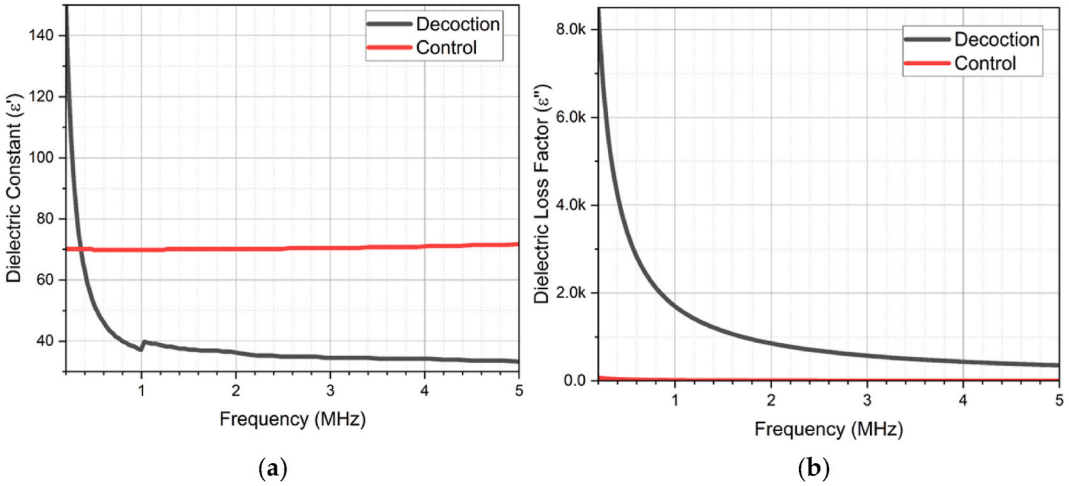


Fig. 4. (a) Dielectric constant and (b) loss factor of Radix Glycyrrhizae decoction extract and control (distilled water) using a Hioki impedance analyzer in the frequency range of 4 Hz–5 MHz.

[22]. Phenolic compounds like tannins are responsible for antioxidant activity, while saponins exhibit antimicrobial, allelopathic, and hemolytic properties, and alkaloids contribute to antimicrobial and anticancer effects [23]. Radix Glycyrrhizae demonstrated significant antioxidant potential in the DPPH assay, with an observed activity of 82.3 ± 0.8. Both quantitative and qualitative analyses revealed a high concentration of polyphenols, suggesting a direct correlation between scavenging activity and polyphenolic content. The findings align with Grigore et al. [24], who demonstrated significant radical scavenging activity in extracts with high concentrations of polyphenols and quercetin at doses over 3 mg/mL, resulting in over 50 % inhibition.

Data processing and analysis were performed using OriginPro 2023 for dielectric spectroscopy measurements. In Fig. 4(a), the decrease in dielectric constant of Radix Glycyrrhizae decoction extract from 4 Hz to 50 MHz is attributed to molecular dipole relaxation. Polar covalent bonds, such as those involving oxygen (e.g., in hydroxyl groups of glycyrrhizins or other polar groups in the glycosides and glycyrrhetinic acid), contribute to dipole polarization. As the frequency increases, molecules may not have sufficient time to reorient in response to changing electric field, reducing polarization effects. Ionic metabolites, electrolytes, or mineral in the extract experience limited mobility with increasing frequency due to asynchrony between oscillations and electric flied frequency, further reducing polarization effect. Conversely, the decrease in the loss factor from 4 Hz to 50 MHz as shown in Fig. 4(b) is attributed to reduced energy dissipation during polarization due to friction among phytochemical polar groups (e.g., hydroxyl, carboxylic acid, carbonyl, ester groups).

The dielectric spectrum differs between 4 Hz and 50 MHz and 50 MHz to 20 GHz, where the dielectric loss factor increases in the latter range. The mobility of ionic metabolites, electrolytes, and minerals within the extract decreases, leading to reduced ionic conductivity and a decline in dielectric constant (Fig. 5(a)). Meanwhile, the loss factor increases due to intensified frictional activities during ion oscillations at higher frequencies compared to lower frequencies. Additionally, hindered response of water molecules to electric fields contributes to decreased dielectric constant at higher frequencies, while increased loss factor results from damping of molecular motions. Both dielectric constant and loss factor show minimal differences between the decoction extract and distilled water, largely influenced by water molecules as the major component of the decoction.

The dielectric properties of Radix Glycyrrhizae decoctions, including dielectric constant and loss factor, significantly influence their therapeutic potential. The dielectric constant, which measures energy storage in an electric field, is affected by concentrations and types of phytoconstituents such as glycyrrhizin, altering interactions with tissues [11]. The dielectric loss factor, quantifying electromagnetic energy dissipation, varies with phytoconstituent interactions, potentially enhancing therapeutic effects [12]. Moisture content, crucial in aqueous decoctions, also influences dielectric properties [13]. Concurrently, dielectric spectroscopy analyzes these properties from 4 Hz to 5 MHz with the HIOKI IM 3570 Impedance Analyzer, highlighting robust polarization mechanisms influenced by phenols and flavonoids. Higher TPC and TFC correspond to enhanced dielectric responses. In the range of 5 MHz–20 GHz, analyzed with a vector network analyzer, polarization decreases with frequency, while the loss factor increases, reflecting effective energy dissipation. These findings are supported by FTIR, identifying molecular vibrations affecting dielectric behavior. Antioxidant activity, assessed via the DPPH assay, further impacts dielectric properties, suggesting stable electrical performance alongside antioxidant benefits. This integrated approach informs potential applications in pharmaceuticals, biomedicine, and food science, highlighting the critical role of both electrical and bioactive properties in Radix Glycyrrhizae decoctions.

5. Conclusions

The determination of total phenolic and flavonoid content revealed significant amounts of TPC and TFC in the decoction. HPLC analysis further confirmed high quantities of phenolics and flavonoids, recognized for their potent antioxidant and anti-inflammatory

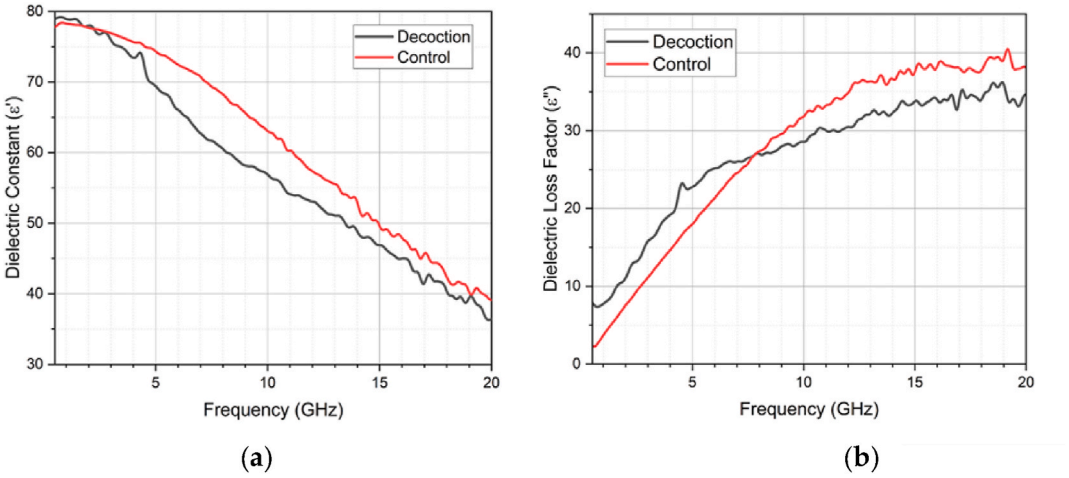


Fig. 5. Comparative study of the dielectric properties of Radix Glycyrrhizae decoction and a control using vector network analyzer within the frequency range of 5 MHz–20 GHz. (a) Fluctuation of the dielectric constant with frequency. (b) Fluctuation of the dielectric loss factor with frequency.

properties. FTIR spectroscopy analysis indicated the presence of functional groups, corroborating the phytoconstituents identified via HPLC. The decoction demonstrated robust antioxidant properties, largely attributed to the phenolic and flavonoid compounds due to their strong hydrogen-donating ability. The pharmacological and medicinal potential of Radix Glycyrrhizae suggests it holds great promise as a versatile therapeutic plant, warranting further exploration. Additionally, dielectric spectroscopy shows promising results in investigating phytochemical contents in Radix Glycyrrhizae decoction extract.

CRediT authorship contribution statement

Ong Hong Liang: Writing – review & editing, Methodology. **Cheng Ee Meng:** Writing – review & editing, Supervision, Conceptualization. **Che Wan Sharifah Robiah Mohamad:** Supervision, Funding acquisition, Conceptualization. **Nashrul Fazli Mohd Nasir:** Investigation. **Tan Xiao Jian:** Visualization, Software, Data curation. **Beh Chong You:** Visualization. **Emma Ziezie Mohd Tarmizi:** Validation. **Lee Kim Yee:** Formal analysis. **You Kok Yeow:** Formal analysis. **Khor Shing Fhan:** Validation. **Leu Kim Fey:** Methodology. **Te Kian Keong:** Resources. **Mohd Riza Mohd Roslan:** Software. **Siti Aishah Baharuddin:** Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge Hospital Universiti Tunku Abdul Rahman for offering support in this project. This research was financed by Universiti Malaysia Perlis through a postgraduate grant. The article processing charge was sponsored by Universiti Putra Malaysia.

References

[1] K. Meitha, Y. Pramesti, S. Suhandono, Reactive oxygen species and antioxidants in postharvest vegetables and fruits, International Journal of Food Science 2020 (2020) 1–11.
[2] M. Ashraf, Phytochemicals as potential anticancer drugs: time to ponder nature's bounty, BioMed Res. Int. 2020 (2020) 1–7.
[3] S. Fialová, K. Rendeková, P. Mučaji, M. Nagy, L. Slobodníková, Antibacterial activity of medicinal plants and their constituents in the context of skin and wound infections, considering european legislation and folk medicine—a review, Int. J. Mol. Sci. 22 (19) (2021) 10746.
[4] M. Mohanty, S. Mohanty, S. Bhuyan, R. Bhuyan, Phytoperspective of moringa oleifera for oral health care: an innovative ethnomedicinal approach, Phytother Res. 35 (3) (2020) 1345–1357.
[5] J. Jeeva, J. Sunitha, R. Ananthalakshmi, S. Rajkumari, M. Ramesh, K. Ravindran, Enzymatic antioxidants and its role in oral diseases, J. Pharm. BioAllied Sci. 7 (6) (2015) 331.
[6] K. Ecevit, A.A. Barros, J.M. Silva, R.L. Reis, Preventing microbial infections with natural phenolic compounds, Future Pharmacology 2 (4) (2022) 460–498.
[7] A. Saleem, et al., HPLC, FTIR and GC-MS analyses of Thymus vulgaris phytochemicals executing in vitro and in vivo biological activities and effects on COX-1, COX-2 and gastric cancer genes computationally, Molecules 27 (23) (2022) 8512.
[8] M. Ramalingam, H. Kim, Y. Lee, Y.-I. Lee, Phytochemical and pharmacological role of liquiritigenin and isoliquiritigenin from Radix Glycyrrhizae in human health and disease models, Front. Aging Neurosci. 10 (2018).
[9] Y. Ding, E. Brand, W. Wang, Z. Zhao, Licorice: resources, applications in ancient and modern times, J. Ethnopharmacol. 298 (2022) 115594.

[10] S. Wahab, et al., Glycyrrhiza glabra (Licorice): a comprehensive review on its phytochemistry, biological activities, clinical evidence and toxicology, Plants 10 (12) (2021) 2751.
[11] A. Kraszewski, S. Trabelsi, S. Nelson, Microwave dielectric properties of plant materials, IEEE Trans. Microw. Theor. Tech. 55 (6) (2007) 1298–1304.
[12] M.H. Alu'datt, et al., Herbal yield, nutritive composition, phenolic contents and antioxidant activity of purslane (Portulaca oleracea L.) grown in different soilless media in a closed system, Ind. Crop. Prod. 141 (2019) 111746.
[13] G. Zengin, et al., Screening of in vitro antioxidant and enzyme inhibitory activities of different extracts from two uninvestigated wild plants: centranthus longiflorus subsp. longiflorus and Cerinthe minor subsp. Auriculata, European Journal of Integrative Medicine (8) (2015) 286–292.
[14] I. Greco, et al., Correlation between hemolytic activity, cytotoxicity and systemic in vivo toxicity of synthetic antimicrobial peptides, Sci. Rep. (10) (2020) 13206.
[15] A. Navarrete, et al., Measurement and estimation of aromatic plant dielectric properties. Application to low moisture rosemary, Ind. Crop. Prod. 33 (3) (2011) 697–703.
[16] V.A. Kostikova, T.N. Veklich, HPLC analysis of phenolic compounds in leaves and inflorescences of Sorbaria pallasii, BIO Web of Conferences 24 (2020) 00040.
[17] M. de A Cavalcante, et al., An HPLC method to determine phenolic compounds of plant extracts: application to Byrsonima crassifolia and Senna alata leaves, Pharmacogn. Res. 14 (4) (2022) 395–404.
[18] G. Sharma, T. Mishra, Phytochemical analysis, high-performance liquid chromatography, and ex vivo antioxidant potential of a herbal fruit extract, Asian J. Pharmaceut. Clin. Res. (2019) 256–260.
[19] Y. Seong, Inhibitory effect of an ethanol extract mixture of aralia elata leaf, chaenomeles sinensis fruit and glycyrrhizae radix on aβ (25–35)-induced memory impairment, Journal of Biomedical Translational Research 22 (2) (2021) 78–84.
[20] H.-J. Chang, Y.-H. Kim, Y.-H. Kang, M.-H. Choi, J.-H. Lee, Antioxidant and antibacterial effects of medicinal plants and their stick-type medicinal concentrated beverages, Food Sci. Biotechnol. 29 (10) (2020) 1413–1423.
[21] X. Qiao, W. Song, S. Ji, Q. Wang, D. Guo, M. Ye, Separation and characterization of phenolic compounds and triterpenoid saponins in licorice (Glycyrrhiza uralensis) using mobile phase-dependent reversed-phase × reversed-phase comprehensive two-dimensional liquid chromatography coupled with mass spectrometry, J. Chromatogr. A 1402 (2015) 36–45.
[22] A. Ullah, et al., Important flavonoids and their role as a therapeutic agent, Molecules 25 (22) (2020) 5243.
[23] M. Abid, et al., Antioxidant properties and phenolic profile characterization by LC–MS/MS of selected Tunisian pomegranate peels, J. Food Sci. Technol. 54 (9) (2017) 2890–2901.
[24] A. Grigore, I. Paraschiv, S. Colceru-Mihul, C. Bubueanu, E. Draghici, M. Ichim, Chemical composition and antioxidant activity of Thymus vulgaris L. volatile oil obtained by two different methods, Romanian Biotechnological Letters 15 (4) (2010) 5436–5443.