Asian Journal of Fundamental and Applied Sciences
e-ISSN: 2716-5957 | Vol. 4, No. 4, 1-9, 2023
http://myjms.mohe.gov.my/index.php/ajfas

Functional Group and Phytochemical Properties Detection of Peperomia Pellucida Leaves Extract

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Received: 27 October 2023 | Accepted: 13 December 2023 | Published: 31 December 2023

DOI: https://doi.org/10.55057/ajfas.2023.4.4.1

Abstract: *Since prehistoric times, medicinal plants, often known as medicinal herbs, have been identified and employed in traditional healing practises. Because of the existence of phytochemical ingredients, medicinal plants synthesise hundreds of chemical compounds and are a key source of molecules with therapeutic qualities. Wild herb leaves were taken from the green region of Universiti Sains Islam Malaysia, specifically Peperomia pellucida, also known as 'sireh cina' in Malaysia. The dried wild herb leaves were crushed. For 48 hours, the powdered materials were treated to a solid-liquid extraction method utilising hot water and ethyl acetate as a solvent. For all examined samples, a phytochemical screening test of hot water and ethanolic leaves extract confirmed the presence of antioxidant chemicals such as flavonoid and phenolics. Meanwhile, resins were only found in ethanolic leaves extract, and steroid was not found in any of the studied samples. Fourier-Transform Infrared Spectroscopy (FTIR) was used to study the organic component in greater depth by evaluating the chemical ingredients in the crude extracted samples of ethyl acetate. The study of FTIR spectra revealed that the hydroxyl group, alkanes, alkyl groups, benzoic compounds, and phenols were the primary functional groups. The identification of functional groups indicates a possible source of antioxidants and highlights a potential prospect for the development of natural products from these wild plants in the drug discovery business. Disc diffusion analysis of antibacterial properties exhibited significant clear zone formation against Gram-positive and Gram-negative tested bacteria.*

Keywords: Peperomia pellucida, sireh cina, phytochemical constituents, solid-liquid extraction

1. Introduction

In recent years, leading a healthy lifestyle has become more important and concerning. Medicinal plants have become the primary source of healthcare due to the minimal side effect but beneficial to human body (Parekh et al., 2005). The most significant chemicals found in plants are alkaloids, flavonoids, steroids, terpenoids, carotenoids, tannins, and glycosides. These compounds are also in charge of giving the plants their bioactivities, which include antiviral, antifungal, antimicrobial, analgesic, antioxidant, and antimalarial properties (Raina et al., 2014).

Pepperomia pellucida (L.) is one of the well-known medicinal plants worldwide, belongs to the family of Piperaceae (Rojas-Martinez et al., 2013). *Pepperomia pellucida* is a slender herb (reaching 30-50 cm in length) with straight and succulent stem and is cosmopolitan in distribution. Leaves are opposite and alternate, up to 2.5 x 2 cm, ovate-deltoid, obtuse to acute at apex. Leaves are thin, fleshy, smooth, membranous when dry, 5-7 nerved from the base. Petiole is up to 1.5 cm long. Spikes are terminal and leaf-opposed, up to 5 cm long. Flowering occurs more or less throughout year. Fruits are ribbed and reticulate, minute in size and almost dry [Bhat, 2014; Melo et al., 2016]. The *Pepperomia pellucida* leaves have been used as food and traditional medicine (Tablang et al., 2020) and this herb easily found in Malaysia where the surrounding environment are humid places. This plant normally grown as a wild plant.

Generally, the origin of the plant sample will affect the plant growth as well as the plant's chemical compounds (Pandey & Tripathi, 2014). Thus, this study was conducted to investigate the phytochemical properties, elucidation functional groups using Attenuated Total Reflectance-Fourier Transform Spectroscopy (ATR-FTIR) and antibacterial properties of *Pepperomia pellucida* leaves extract through disc diffusion method. The findings of this study will add more scientific information about *Pepperomia pellucida* that grows around our campus area.

2. Materials and Method

2.1 Sample Preparation

This research was conducted around Universiti Sains Islam Malaysia, (USIM) with coordinate of 2.48° N, 101.78 ° E. Wild herbs were collected around this university green area and the leaves of the collected herbs were washed and measured the weight. Then, the leaves were dried in oven at temperature 60℃ for 24 hours. The dried sample were grounded to obtain the powdered by using a grinder. The grounded samples were weighted, sorted and kept in a Schott Duran bottle for further use.

2.2 Solid-Liquid Extraction

This method was conducted by using two different solid-liquid extraction solvent such as water and ethyl acetate.

Hot water extraction

Approximately 3 g of each sample powder were placed in 500 ml beaker and 300 ml of distilled water was added into that beaker. Extraction process started by putting all beakers onto the stirring hotplate machine. The samples were heated at temperature 80℃ for 2 hours. The samples were filtered by using filter paper to separate the solute and the solution of the samples (Ahmad et al., 2018). The extract solution (HW) of the samples were used for phytochemical screening.

Ethyl acetate extraction

Approximately, 3 g of seed powder sample were placed in 500 mL Schott Duran bottle and 3000 mL of ethyl acetate solvent was added and left for extraction at room temperature for 48 hours. After 48 hours, the solvent (ethyl acetate) was pipetted out carefully from the bottle until the 50% of the total volume reduced. Then the ethyl acetate was removed using rotary evaporator at 50°C, producing concentrated ethyl acetate oil extract which will be further used in ATR-FTIR analysis.

2.3 Phytochemical screening

Two different extracts will be used for the screening of phytochemicals, namely hot water extract (HW) and ethanol extract. Ethanol extract (EE) was prepared by adding few drops of ethanol solution to the oil obtained from the ethyl acetate extraction.

Detection of flavonoid

1 mL of the extract was added into the test tube (triplicate) and 1 ml of 10% NaOH was added to each test tube. The presence of flavonoid was indicated by a yellow colour (Edewaor & Usman, 2011).

Detection of phenolic

1 ml of each extract was dropped on a blue litmus paper. The presence of phenolic compounds was identified when the blue litmus paper turned red (Ghasemzadeh & Ghasemzadeh, 2011).

Detection of resins

1 ml of hydrochloric acid solution, HCl was added to 1 ml of the extract in the test tube. The presence of Resins was indicated by the appearance of turbidity (Edewaor & Usman, 2011). Experiment was conducted in triplicate.

Detection of steroid

Three test tubes were taken and 1 ml of the extract was added into each test tubes. 1 ml of sulphuric acid, H_2SO_4 was added to each test tubes. The presence of steroid was indicated by a red precipitate (Edewaor & Usman, 2011).

Determination of Total Phenolic Content

Total soluble phenolic content were determined with Folin-Ciocalteau's phenol reagent (FCR) according to the method of Ahmad et al., 2014 and Heleno et al., 2010. Briefly, 1 mL of extract solution (2 g/L) was added with 46 mL of distilled water and 1 mL of Folin-Ciocalteau's phenol reagent and the content of the flask were mixed thoroughly. After 3 min, 3 mL of Na_2CO_3 (2%) was added and the mixture was allowed to stand for reading absorbance at 760 nm. Gallic acid (0.05 - 0.8 mM) was used as a standard, and the results were expressed as mg of Gallic acid equivalents per gram of extract.

Determination of Flavanoid Content

Total flavonoid compound in the various crude extracts were determined according to the method of (Park et al., 1997). Briefly, 1 mL sample from various extracts solution (2 mg/mL) were diluted with 4.3 mL of 80% aqueous ethanol. The mixtures were then added with 0.1 mL of 10% aluminium nitrate and 0.1 mL aqueous potassium acetate (1M). The mixtures were incubated at room temperature for 40 min before read the absorbance using spectrophotometer at 415 nm. Total flavonoid compound was calculated using Quercetin as a standard and the data was depicted as mg of Quercetin equivalents per gram of extracts.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The functional group containing in *Peperomia pellucida* extract was investigated using ATR-FTIR. The powder sample of Peperomia pellucida was characterized within the frequency range of 4000-600 cm^{-1}. Briefly, the samples were placed on the clean window of Agilent Cary 630

equipped with diamond ATR (Attenuated Total reflectance). Then, the pressure clamp was closed until a click sound was heard and the absorbance peak were analysed using a real-time Micro-Lab Software.

Antibacterial test

Antibacterial analysis of HW extract was further tested tested on different Gram-positive and Gram-negative bacterial using disc diffusion method. The tested five common bacterial species were used: *Escherichia coli* (*E. coli*), *Ralstonia* sp., *Salmonella* sp., *Staphylococcus aureus*, and *Streptococcus* sp. Initially, the each of the bacterial species were cultured in 10 mL Nutrient broth (NB) (Sigma-Aldrich, Dorset, UK) at room temperature for 24 hours before being adjusted to 1.0 OD concentration. Approximately, 100 mL of the bacteria liquid culture, grown in NB (13 g/L) (Sigma-Aldrich, Dorset, UK), for 24 h. The bacterial culture was then transferred onto the middle of nutrient agar (NA) (Sigma-Aldrich, Dorset, UK) plate. The bacteria were spread using 'L' shaped hockey stick (spread plate technique). Sterile paper discs were immersed in the HW extract solution and placed on the NA plate and labelled. Antibiotic (Penicillin-streptomycin solution ATCC® 30–2300TM) was used as positive control while sterile distilled water was used as negative control. The bacteria plates were incubated at 37°C for 24 h and the zone of inhibition was measured in millimetre (mm) (Ramesh & Manihar, 2010).

3. Results and Discussion

Phytochemical properties

Qualitative phytochemical properties are the characteristics of phytochemicals that can be observed or measured without the use of sophisticated analytical instruments. These properties can be used to identify the presence of different types of phytochemicals in a plant extract or sample. Some common qualitative phytochemical properties include odor where some phytochemicals have a characteristic odor. For example, terpenoids often have a strong, aromatic odor; taste where some phytochemicals have a characteristic taste. For example, tannins often have a bitter taste; solubility where phytochemicals can be soluble in different solvents, such as water, ethanol, and methanol. The solubility of a phytochemical can be used to identify its type. For example, alkaloids are typically soluble in acidic solvents, while flavonoids are typically soluble in organic solvents and chemical reactions where phytochemicals can undergo a variety of chemical reactions. For example, tannins react with iron to form a blue-black precipitate.

Table 1 shows the phytochemicals presence in the HW and EE extract of *Peperomia pellucida*. Previous studies have reported that the presence of bioactive compound in *Peperomia pellucida* such as the phenolic compounds (Anokwuru et al., 2011), flavonoids (Rao et al., 2016), alkaloids, tannins and saponins (Benhammou et al., 2013) are responsible for the antioxidant properties of the plant.

Table 1: Phytochemical Screening of Hot Water (HW) And Ethanol Extracts (EE) From *Peperomia pellucida* Leaves

Extracts	Phytochemical Test			
	Flavonoid	Phenolic	Resin	Steroid
HW	+	+	-	-
EE	+	+	+	-

It was found that the most prominent bioactive compound such as flavonoid and phenolic are presence in both HW and EE extract, however resins only presence in EE extract. Both HW and EE extract does not contained steroid. The presence of flavonoids and phenolics shows that *Peperomia pellucida* has potential as an antioxidant agent.

Total Phenolic and Flavanoid content

Phenolics, also known as phenols, are a group of chemical compounds that contain a hydroxyl group (-OH) attached to an aromatic ring. Phenolic compounds are widely found in plants, where they play important roles in protecting the plant from pests and diseases. *Peperomia pellucida* is a good source of phenolics, which are a group of plant compounds that have been shown to have a number of health benefits (Silalahi, 2022). Flavonoids are a large group of phenolic compounds that are found in fruits, vegetables, and tea. Some examples of flavonoids include quercetin, catechin, and anthocyanins. Phenolic acid which previously detected in *Peperomia pellucida* including caffeic acid, ferulic acid and p-coumaric acid (Ref). These phenolic acids have been shown to have anti-hypertension (Kurniawan et al., 2016), anti-diabetes (Susilawati et al., 2018) and anti-osteoporosis (Kartika et al., 2021). Results obtained showed that leaves extract from *Peperomia pellucida* isolated from USIM's environment also contained a significant phenolic and flavonoid content. The flavonoid content was found higher (3.94 g/L) as compared to the phenolic content with 2.34 g/L. The high concentration of phenolic and flavonoid in the *Peperomia pellucida* leaves extract showed the potential as an antioxidant agent.

Table 2: Total phenolic and flavonoid content of *Pepperomia pellucida* leaves hot water extract.

Antioxidant analysis	Total content (g/L)[a]
Phenolic content	2.34 ± 0.08
Flavanoid content	3.94 ± 0.05

[a]*Value is the reading of triplicate data.*

ATR-FTIR

Attenuated Total Reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy is a powerful, rapid, and easy-to-use technique for phytochemical analysis. ATR-FTIR is a non-destructive technique, which means that the sample can be reused after analysis. ATR-FTIR works by passing a beam of infrared radiation through a high-refractive-index crystal, such as diamond or germanium. When the infrared radiation encounters the sample, it is attenuated, or weakened. The amount of attenuation depends on the functional groups present in the sample. ATR-FTIR can be used to identify and quantify a wide range of phytochemicals in plant material, including alkaloids, flavonoids, tannins, terpenoids, and carbohydrates. The functional groups presence in Peperomia pellucida is represented in Figure. 1. The absorbance peak spectrum obtained was in the range of 4000-600 cm^{-1}. The infrared absorption spectra are interpreted in Table 2.

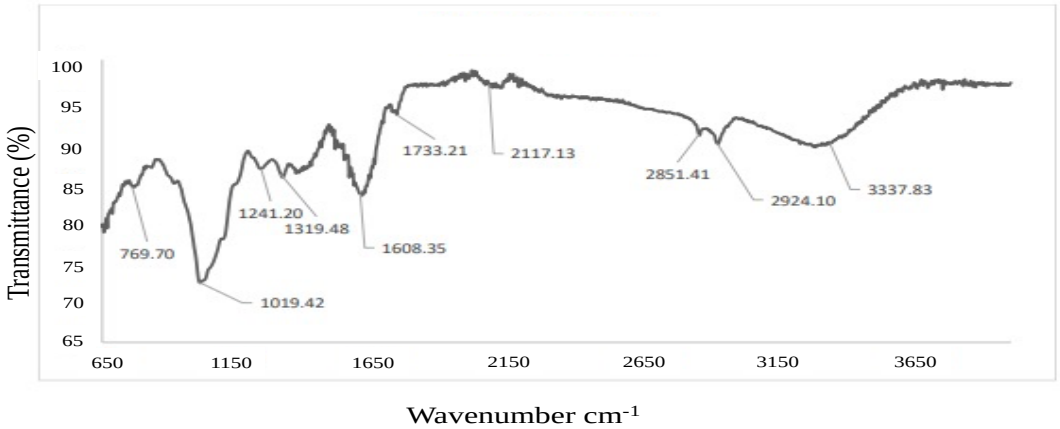

Figure 1: ATR-FTIR peak of *Peperomia leaves* extract.

Table 2: Spectral Characteristics of *Peperomia pellucida* Leaves

Functional group assignment	Wavenumber (cm^{-1})	Wavenumber (cm^{-1}) (Spectroscopic Tools)	Predicted compound
OH	3337.83	3200-3550	Alcohol
-C-H_2	2924.10	~2900	Alkanes
C-H	2851.41	2850-2990	Alkanes
C=C	2117.13	2100-2250	Alkyne
C=O	1733.21	1720-1740	Aldehydes
C=C	1608.35	1440-1625	Aromatic
C-F	1241.20	1000-1400	Alkyl
NO_2	1319.48	1300-1400	Nitro
C-F	1019.42	1000-1400	Alkyl
C-Cl	769.70	600-840	Alkyl

Results in Table II demonstrated the functional group which consists of hydroxyl group (alcohol), alkanes, alkynes, benzoid compounds (aromatic), aldehydes and alkyl. Flavonoids are polyphenols demonstrated by two benzene rings joined by a linear carbon chain (Asep et al., 2019). FTIR spectrophotometry was used to identify the benzenoid chemicals, which corroborated the results of the phytochemical screening that identified flavonoids and phenols. The predominant functional groups of bioactive chemicals were thought to be the alkanes and phenols found in the mixture (Khan et al., 2018).

Antibacterial Test

Microbes, sometimes referred to as microbial pathogens, are the source of many human diseases. According to Zubair et al. (2015), Peperomia leaves have been traditionally used to treat diarrhoea because of their antibacterial property. It has been reported that the ethanolic extract of *Peperomia pellucida* leaves has an inhibitory ability against acne-causing bacteria (*Propionibacterium acnes*). Meanwhile, in this study, the hot water extracts from *Peperomia pellucida* have shown a significant antibacterial activity against several bacteria. The HW extracts of *Peperomia pellucida* leaves were further tested their antibacterial properties against two Gram-positive bacteria

(*Staphylococcus aureus* and *Streptococcus* sp.) and three Gram-negative bacteria (*E.coli*, *Salmonella* sp. and *Ralstonia* sp.) as shown in Table 3.

Table 3: Average Zone of Inhibition of HWE Against Different Bacteria[a]

Bacterial strain	Average zone of inhibition (mm)
Ralstonia sp.	15 ± 1.4
E. coli	1.0 ± 0.2
Salmonella sp.	12.5 ± 1.5
Streptococcus sp.	0.75 ± 0.10
Staphylococcus aureus	3.5 + 0.50
Penicillin Streptomycin	10 ± 0.1

[a]*Sterile distilled water as negative control.*
[b]*Penicillin-Streptomycin as positive control (100 µg/mL); Each value is the mean ± three replicates.*
Average zone of inhibition is the value of total diameter – disc (5 mm).

From the results, it was clearly shown that the highest zone of inhibition of HW leaves extracts are against *Ralstonia* sp. (15 ± 1.4) followed by *Salmonella* sp. with 12.5 ± 1.5. The zone inhibition of Gram-positive bacterial were low as compared to Gram-negative bacterial. The lowest zone of inhibition revealed by *Streptococcus* sp. with 0.75 ± 0.10. The bioactivity of *Peperomia pellucida* extract as an antimicrobial is influenced by various factors such as concentration, compounds used for extraction, organs used and types of microbes (Silalahi, 2022). The significant antibacterial effect demonstrated by HW extracts of *Peperomia pellucida* is successfully added the medicinal value of Malaysian wild herbs.

4. Conclusion

Flavonoid and phenolic phytochemical substances were found in the hot water and ethanol extracts. Alcohol's hydroxyl group, alkanes, aromatic benzoid compounds, aldehydes, alkyl, carboxylic acid, and alkynes were the main functional groups found. The results of this study are noteworthy because they highlight the potential of *Peperomia pellucida* which is grown on the USIM campus, as a source of antioxidants for use in nutraceuticals and pharmaceutical products.

Acknowledgement

This work was supported by the Universiti Sains Islam Malaysia under Geran Sepadan USIM/MG/IIUM-UM-UITM/KGI/SEPADAN-K/72422. We want to thank Kolej PERMATA Insan for providing space of Al Razi Halal Action Laboratory to conduct the experiment.

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