

Identification of functional group present in *Andrographis paniculata* (kalmegh) leaves by FTIR analysis

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Abstract. This present study aims to analyze the functional group present in the *Andrographis paniculata* leaves or also known as kalmegh by using Shimadzu Fourier Transform Infrared (FTIR) with the absorbance range of 500 cm⁻¹ - 4000 cm⁻¹. *Andrographis paniculata* (Nees) is a valuable traditional medicinal plant and it has many important bioactive compounds. In this study, kalmegh leaves powder was soaked in two different solvent which were 80% aqueous methanol and distilled water. After soaked for 24 hours, the soaked kalmegh leaves was extracted using rotary evaporator to get the crude extract before being subjected to Fourier Transform Infrared (FTIR) spectroscopy for characterization of functional group contains in the leaves. Results obtained from this present research show six major compound group which are alkene, amine, aromatic, nitro, and alcohol. This six functional group were identified from various wavenumber of bending and stretching type of alkene group (894.97 cm⁻¹ and 1645.10 cm⁻¹), stretching type of amine (1080.11 cm⁻¹), stretching type of aromatic group (1409.96 cm⁻¹), stretching type of nitro group (1543.05 cm⁻¹), stretching type of alcohol group (3323.35 cm⁻¹) and phenol group (3749.62 cm⁻¹). This study attempts to display the use of FTIR while simultaneously creating interest among related researchers in natural plant analysis.

1. Introduction

Natural plant resources are widely distributed around the world for several thousands of years as traditional medicine [1]. The important value of these medicinal plant lies in its great importance for the individual and community's health care. Also known as herb, these materials have been a crucial catalyst for the development of disease resistant drug to counter against countless type of negative metabolic activities in human body [2]. Based on a report released by the world health organization (WHO), up to 80% of the human population in the third world countries rely on medicine fabricated from plant for their health needs [3]. The active bio-constituents that contribute to the beneficial properties of these plants are many i.e. flavonoids, tannins, steroids and polyphenols [4]. Furthermore, some of this group such as flavonoids have been extensively studied and confirmed to possess the capability to act as antioxidant, antibacterial, antiviral and anti-carcinogen agent on multiple condition [5]. Due to this reason, it is estimated that approximately 25% of commercialized medicine incorporated plant extract as the ingredient.



Generally, there are two type of metabolite present in plant which are primary and secondary metabolites. Both are classified based upon their function in plant metabolism [6]. Primary metabolites directly affect the physiological function in plant and also commonly called as central metabolites. Conversely, secondary metabolite does not directly entail in the growth of plant but it still plays a significant role in combating diseases [7]. Therefore, a comprehensive analysis on the constituent is of utmost importance since through the understanding of compound present will the researchers be able to fully utilize the potential of each plant.

An example of such plant is kalmegh (*Andrographis paniculata*) where it is categorized as an herbaceous plant originated from the family of Acanthaceae. It is also commonly known as the 'king of bitter' due to the extremely bitter taste. Furthermore, the plant can be easily found growing in southeastern Asia like Indonesia, India, Sri Lanka and Pakistan. In this region, kalmegh can be found in moist shady place such as at the hills, village, forest and also along the roadside. It is an annual herbs plant that has feature characteristics which is branched, growing 1-2 meters straight upward, have sharply quadrangular stems and also have dark green color leaves. The seeds are small in size with an ovoid shape and yellow, green and brown in color [8]. Kalmegh is one of a wonderful herbal plant that have many benefit for medicinal purposes since the plant possesses the properties of analgesic, anti-inflammatory, antidiabetic, antifertility, antibacterial, antipyretic and antihepatotoxic [9,10]. Kalmegh leaves produce a variety of compounds such as flavonoid and andrographolide that show lower side effect when compares to other commercialize medicine for the same purpose since it contains phytochemical constituent possessing the capability to do so [11].

Up till now, several techniques have been introduced for analytical process of plant extract. They are UV spectrometry [12], gas chromatography [13], thin layer chromatography (TLC) [14], and nuclear magnetic resonance (NMR) and high performance liquid chromatography (HPLC) [15]. Although all of the method mention are accurate and highly valued for their precise performance, but they consume too much time and require several reagents to operate. Thus, this study tries to implement a simple and less time consuming technique to accomplish the same task.

Fourier Transform Infrared Spectroscopy is one of the popular techniques used for physiochemical analysis to determine unknown composition of compound by identifying their functional groups [16]. The technique makes used of the intensity of absorption spectra and frequency by measuring the effectiveness of photon energy transferred and vibrational frequency of molecules when exposed to infrared light source [17]. The advantage of this technique is that it is cheaper since it does not require reagents to work, less time consuming, highly accurate in its determination and eco-friendly. At present, the application of FTIR have been successfully applied in various field peculiarly in medicine for the identification of Pharmacopoeia [18].

2. Materials and methods

Material: Distilled water was taken from Physical Laboratory University Malaysia Terengganu, Methanol (Sigma Aldrich), Kalmegh leaves powder was plucked from kalmegh tree that buy from botanist at Selangor.

2.1. Preparation of Plant Extract

The leaves material for this study were collected from plantation in Selangor, Malaysia. The kalmegh leaves were plucked and collected early in the morning to ensure the freshness of the leaves. Then, it was washed properly for 3-4 times under running tap water to remove the residue before dried under room temperature and stored in air tight polyethylene bags. Later, the leaves were crushed into finely powder form by using grinder. For the extraction, 20g of the leaves powder was weighed before soaked in 400 ml of distilled water (aqueous extract) and 80% of aqueous methanol with the ratio of powder and solvent is 1:20. The extracts were soaked and shook by using shaker for 24 hours which is the optimal time for the extract and solvent to reach the equilibrium state. After 24 hours, the extract was filtered using vacuum pump using Whatman No.1 filter paper. The filtrate extract then was evaporated

using rotary evaporator at 40 °C water bath and cooling compressor at 10 °C in order to get the dried crude. The dried crude then stored into the freeze dryer to ensure all the water is removed and stored properly before use it for FTIR analysis.

2.2. Identification of Functional Group

Fourier transform infrared (FTIR) spectrometry was used to analyze and characterize the functional group present in the leaves [19]. A small amount of crude extract was placed at the germanium piece of infrared spectrophotometer with the absorbance collected over the wave number ranged from 500 cm^{-1} to 4000 cm^{-1} . The spectra were compared with previous journal as reference. The data collected from the FTIR analysis were plotted by using OriginPro 2016 32 bit software. The peak present on the graph represent the functional group in both methanol and aqueous kalmegh leaves extract base on the wavelength value. This method are taken from [20] with a slight modification.

3. Results and Discussion

The infrared spectroscopic analysis gives the information about the possible functional groups of active principles. The functional group analysis of the dried powder, methanol and aqueous extract of kalmegh leaf was performed by using solid FT-IR spectroscopic analysis using KBr disc method. Figure 1 shows the graph of FTIR spectrum of kalmegh leaves extract. Results obtained for both solid and liquid form was compared. Graph plotted in Figure 1 have been used for comparison of different extracts using methanolic and aqueous. The analysis of the functional group was made by compared the result of absorption peak produced with the previous research. All the functional group represented by the peak absorption was recorded in Table 1.

Table 1. Wavenumber and functional group of kalmegh leaves for aqueous, methanolic and dried powder extract

Wavenumber (cm^{-1})	Functional Group
894.97	Alkene =C-H bending
1080.11	Amine C-N stretching
1409.96	Aromatics C=C stretching
1543.05	Nitro N-O, stretching
1645.10	Alkenes C=C stretching
3323.35	Alcohol O-H stretching
3749.62	Phenol O-H stretching

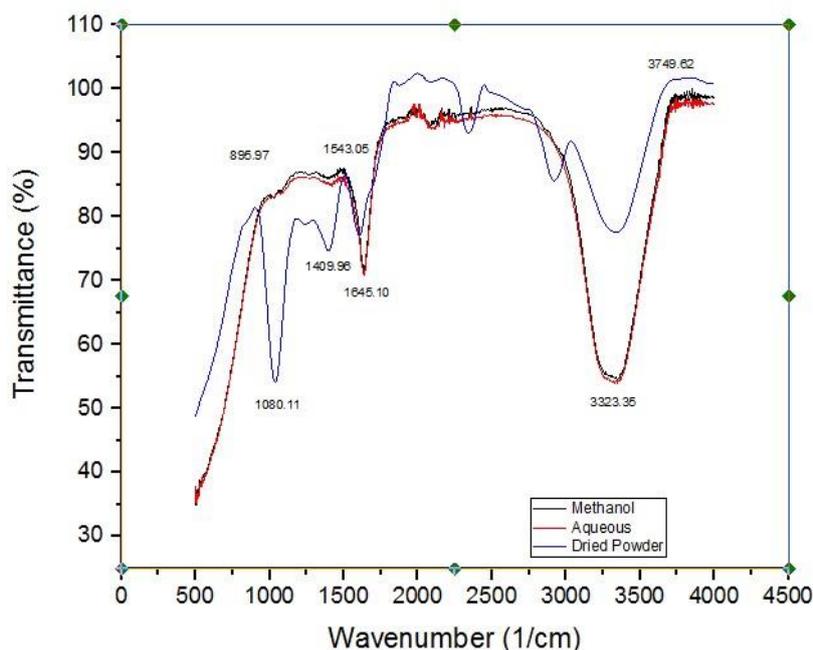


Figure 1. FTIR spectrum from different solvent of kalmegh leaves extract

Based on Figure 1 and Table 1, major compound that have been found contained in the kalmegh leaves extract are some specific functional group which are alkene, amine, aromatic, nitro, alcohol and phenol. At wavenumber 3323 cm^{-1} which is the broadest peak produced represent a strong absorption of alcohol group with stretching vibration. The appearance peak at 1645.10 cm^{-1} represent the alkenes stretching group while strong bond at 1543.05 cm^{-1} wavenumber was reveals a nitro asymmetric stretch. According to peak value 1409.96 cm^{-1} , this represent C=C stretching which is called as an aromatics group. The spectrum obtained from wavenumber 1080.11 cm^{-1} represent a stretching vibration of amines group while at frequency 894.97 cm^{-1} shows another alkene group but with bending vibration. At peak 3749.62 cm^{-1} stretching mode display the presence of phenol compound [12]. From Figure 1, the wavelength intensity of the methanol and aqueous extract is almost similar with slight difference, but the dried powder frequency have a huge difference of frequency.

4. Conclusion

The preliminary result of FTIR give an idea to extend this research for their further research. In this present study, preliminary screening confirmed the presence of six functional group present in kalmegh leaves extract. Hence the kalmegh leaves extract need to be further manipulated to investigate bioactive compound content in kalmegh leaves that maybe it can be used for various field applications that give many benefit in future. Purification and chromatography techniques are required to isolate bioactive compound in future work

5. References

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