

Identification of Chemical Constituents of *Oroxylum Indicum* (Bonglai) Hydrosol (Remaining Water After Oil Distillation) Extracted By Hydrodistillation Method

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ABSTRACT

Oroxylum Indicum (Indian Trumpet Flower) or called Bonglai in Malay is a medicinal plant widely used, especially in the Indian medicine system. *Oroxylum Indicum* can be extracted by the hydrodistillation method to obtain the essential oil and hydrosol. Nevertheless, the chemical constituents of the hydrosol of the leaves are yet to be determined as hydrosol is always discarded, leading to the wastage of products. Thus, this study investigated the chemical constituents of *Oroxylum Indicum* leaves hydrosol extracted by hydrodistillation by varying temperatures and determined the functional groups of the active constituents in the leaves for the benefit and usage in pharmaceutical industries. Hydro distillation is carried out at different temperatures to study the effect of temperatures on the active compounds in the hydrosol. The hydrosol sample of the leaves will be extracted by hydrodistillation method at temperatures of 50°C, 70°C and 80°C and separated via rotary evaporator, and later analyzed by GC-MS and FTIR analysis. This study will help us to identify the value and amount yield of the chemical constituents of *Oroxylum Indicum* leaves hydrosol which will be able to determine whether it will have significant values equal to the essential oil. From FTIR analysis, the functional groups for all samples are the same which are O-H stretch, H-bonded, N-H stretch and C=C stretch. The chemical constituents of *Oroxylum Indicum* hydrosol were determined by GC-MS analysis. The major components of hydrosol produced at 50°C are squalene (10.44%), 2ethylehexyl palmitate (8.56%), palmitic acid (7.50%), and di-n-2-propyl pentyl phthalate (1.69%), and at 70°C is acetic acid (5.88%) only, while at 80°C are only traces components respectively. This is due to most compounds contained may be decomposed during the preparation of samples prior to both analyses also the efficiency of the system and procedure during the extraction.

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1. Introduction

Oroxylum Indicum, (Indian Trumpet Flower) belongs to a member of the Bignoniaceae as it is characterized based on the brown bark and large pinnate leaves (Beck et al., 2001). This plant have been broadly practiced in traditional medication as folk and tribal medicinal gurus for various types of diseases. The leaves are used against diseases such as emollient, swelled spleen, stomachache, rheumatism, ulcers, jaundice, and soothing headaches (Bernstein, 1965). Studies shown that the medicinal properties of *Oroxylum Indicum* are antimicrobial, antioxidant, antidiabetic, hepato-protective, hepato-curative, anti-inflammatory, anti-carcinogenic, cradic-protective, immunomodulatory, nephron-protective, photocytotoxic and others from various parts of the plant (Driedger, 1998). The experimental studies of both *in vitro* and *in vivo* shown that the medicinal properties of *Oroxylum Indicum* leaves are antioxidant and high potential cytotoxicity towards

cancerous cell by the essential oil extracted from the fresh leaves of *Oroxylum Indicum* (Mellers, 2000). Thus, the essential oils extracted from medicinal and aromatic plants possess various types of therapeutic value which is important for medicinal application (Guignon, 1998).

Hydro distillation extraction method is used to extract and isolate essential oil from the *Oroxylum Indicum* leaves to identify the chemical constituents and properties. Nevertheless, there is still inadequate studies on the usage of hydrosol as it is commonly discarded after obtaining essential oil (Goleman, 2009). Hence, the chemical constituents and therapeutic of the hydrosol is remain unknown and leads towards the wastage of products (Goleman, 2009). Therefore, it is important to identify and study the benefits of the chemical components of hydrosol is yet to be determined as well as to fully utilized the extraction of the plant. This study will analyze the chemical properties of hydrosols from *Oroxylum Indicum* leaves to identify the chemical and therapeutic constituents that might be useful for medical usage as well as the marketability potential of the hydrosol leaves.

Thus, the aim of this study to identify the chemical constituents due to the variation of temperature and functional group of the active compounds. This is done by extracting the hydrosol by using hydro distillation process and analysis via GC-MS and FTIR analysis.

2. Literature Review

Oroxylum Indicum is native in Asia and commonly found in deciduous area of Southeast Asia which are in India, Sri Lanka, Peninsular Malaysia, China, Thailand, Philippines, and Indonesia (Harminder et al., 2011) (Ahad et al., 2012). Studies shown that the plant is mostly found in ravines, damp region and moist places in the forest (Harminder et al., 2011). *Oroxylum Indicum* is a small to medium sized plant and able to grow up to 10-12m (40 ft) with light, greyish brown, soft and spongy bark. The plant has large and wide leaf stalks (2 to 4-inch long, and 3 to 4-inch wide) with sharp edges for the leaflets. The leaves appears like a pile of broken limb bones as the leaves stalks wither and fall of the tree near the base of the trunk (Harminder et al., 2011). The flowers are purple with 1 ft long stalk and will usually appear in rainy season (Neelu et al., 2014). The fruits are 1 to 3-ft long with 2 to 4-inch wide with large, flat sword shaped. The seeds are numerous round, flat, thin with wide silvery papery wing (Harminder et al., 2011).

The leaves of *Oroxylum Indicum* contains flavonoids, polyketides, and also essential oil (Ahad et al., 2012). The leaves are used traditionally by the medicinal *gurus* against diseases which are jaundice, emollient, that helps to soothe dry, itchy or scaly skin, treatment of swelled spleen, stomachache, rheumatism, besides soothes headaches and ulcers (Aparna & Srinivas, 2015). In Malaysia, the leaves are traditionally utilized to treat various diseases. The decoction of the leaves are suggested to treat gastralgia, appetite losses, rheumatism, wounds and as a remedy to relieve cholera, as well as fever. The boiled leaves of *Oroxylum Indicum* is utilized as application during and after delivery and to treat dysentery. The hot leaves also may be used to aid splenomegaly. It was found that the leaves are also applied to the cheek to aid toothache and as poultice to soothe headache (Herbal Medicine Research Centre, 2002).

Hydrosol, also known as hydrolates, are the byproducts of hydro distillation of plants that was yielded during the essential oil extraction process from aromatic plant contains a dribble of essential oils (Rao, 2013). Hydrosol contain tiny amounts of the loss essential oils constituents and some of water-soluble constituents of the essential oil and water-soluble constituents of plant from the extraction of the aromatic plant (Smail Aazza, 2011). It was found that the hydrosols acquire biological activities as it contains the traces of oxygenated fraction of the essential oils however in different quantities (Smail Aazza, 2011). According to (Rao, 2012), hydrosols can be utilized in many ways and it is safe towards human and animals. Hydrosols are usually used in perfumery, cosmetics, food and beverages industries as food flavoring, aromatherapy and also for traditional therapies (Rao, 2013). Hydrosol has been widely used in Europe as floral and herbal waters. Meanwhile, hydrosol has been used for food flavouring and medicinal purposes in Asia and Africa. It was found that hydrosols are also used as drinks and utilized in food products traditionally (Rao, 2012). Hydro distillation technique is a main method for the extraction process. Meanwhile, High Pressure Liquid Chromatography, Gas Chromatography Mass Spectrometry and Fourier Transform Infrared Spectroscopy are the analysis equipment involved for this study.

Hydro distillation is one of the traditional practices to extract essential oil from aromatic and medicinal plants that has been developed since the middle ages (Reddy, 2019). The essential oil obtained by the method is the evaporation of the heating of water and other solvent with plant parts supervised by the liquefaction of the vapors in the condenser (Rassem et al., 2016). The setup of this method consists of a condenser and a decanter to collect the condensate formed and discrete essential oils from water (Rassem et al., 2016). This method is a popular method of extraction and this method has been used by many researchers to extract essential oils from aromatic plant (Rassem et al., 2016). This is due to the method is easy to handle and relatively low

expenses for the equipment setup (Dilworth et al., 2017). This is due to It is found that one of the extractions conducted was the extraction of essential oils from *Oroxylum Indicum* fresh leaves (Zaghloul et al., 2015).

High Pressure Liquid Chromatography is a typical analytical chemistry instrument that is used to separate, determine, and quantify the analytes contents in the liquid analytes (Bhardwaj et al., 2015). This instrument has been used in various types of industry which are in pharmaceutical, biochemistry, food and beverages testing, environmental analysis and others (Sabir et al., 2016). High Pressure Liquid Chromatography predominantly uses a column which holds the packing material (stationary phase), a pump that moves the mobile phases through the packed column and also a detector that displays the retention of the molecules. Retention time differs based on the interactions between the stationary phase, the analyzed molecules and also the solvents used

Gas Chromatography Mass Spectrometry is a frequently used analytical method to identify and quantify the organic compounds in a sample. This technique has been used extensively in environmental science, forensics, health care, medical, biological research and others (Sparkman et al., 2011). GC is utilized to separate the volatile and thermally stable components in the sample and the GC-MS will determine the fragments of analyte based on the mass (Gokhale et al., 2017). According to Sparkman et al. (2011) and Emwas et al. (2015), the determination of the chemical constituents is based on the mass spectrum and the retention time. The separated components from gas chromatograph will be subjected to the ionization chamber before being separated on the basis of their mass-to-charge ratio (m/z) by the mass analyzer and identification of the m/z values by the detector (Emwas et al., 2015).

Fourier Transform Infrared Spectroscopy is one of the extensively used approaches as it is highly sensitive, reliable, nondestructive, and rapid detection technique which is beneficial to determine the functional groups of chemical compounds. FTIR has the ability to detect various wavelengths that is used to identify the presence of carboxylic acid, amines, amides, amino acids, aromatic compounds and others in the plant sample (Bhat et al., 2015).

Based on the research by Sonowal et al. (2020), FTIR analysis on the fresh leaves of *Oroxylum Indicum* was conducted based on the peak the values to characterize and identify the presence of chemical constituents in the leaves. The FTIR spectrum of the plant sample has been plotted and recorded by ATR (Attenuated Total Reflectance) technique in a FTIR spectrometer with a range of 4000 cm^{-1} and 400 cm^{-1} . The spectrum was obtained by keeping the sample over the ATR probe lens. Based on the study the peaks observed in FTIR spectrum of *Oroxylum Indicum* are at 3219 cm^{-1} , 2344 cm^{-1} , and 1282 cm^{-1} respectively. This is a great opportunity to look into the chemical components of the hydrosol so that it may be fully utilised in the future because the chemical components of the hydrosol from *Oroxylum Indicum* still not yet been identified and properly analysed extensively.

3. Method

3.1 Materials

Oroxylum Indicum leaves were collected and left to dry for seven days at room temperature. The dried leaves were powdered using a grinder and stored for extraction and further analyses. The leaves were dried to minimize the loss of medicinal compounds in the leaves and increasing the yield of the product obtained (Gibbs & Huang, 1991).

3.2 Hydro Distillation

There are two parts in Hydro distillation which it was carried out by using Soxhlet apparatus extraction system and distillation process by separating hydrosol of the samples by rotary evaporator. 1.8425 g of *Oroxylum Indicum* powder was placed into a thimble. 500 mL of distilled water which act as solvent was poured into a round bottom flask and connected with Soxhlet extractor and condenser. The sample was heated at 50°C to break down the molecules of the sample for 3 hours. The extraction temperature was set to boiling point of water which is 100°C for 1 hour (McLuhan, 1970). The volatile compounds from the sample was extracted as the vapors condensed and fell into the thimble when the distilled water was vaporized through the side tube into the condenser. The siphon sucked up all solvent from thimble to the attached round bottom flask as the solvent level and extracted volatile compounds rose. This ensures all volatile compounds came into the flask along with solvent. The process was repeated for a few cycles.



Figure 1. Extraction of the samples by Soxhlet extraction system

Then, distillation process performed by rotary evaporator was used for the separation of essential oil from hydrosol (McLuhan, 1970b). Temperature of the rotary initially was set to 50°C for 1 hour. The processes were repeated with hydro distillation process at 70°C and 80°C. The hydrosol obtained will be measured and further analyzed by GC-MS and FTIR analysis.

3.3 FTIR

Lastly, a few drops of the hydrosol samples at 50°C, 70°C, and 80°C were analysed accordingly on the attenuated total reflection (ATR) for infrared spectrometry analysis. The FTIR spectra of plant samples were recorded by ATR technique in a FTIR spectrometer (MacIntyre, 2002). FTIR spectra scanned with a range of 4000- 400 cm^{-1} (MacIntyre, 2002). The spectrum of the sample was obtained during the analysis by keeping the sample over the lens of the ATR probe. The functional group of the active compounds in *Oroxylum Indicum* leaves will be determined.

3.3.1 GC-MS analysis

Then, the hydrosol samples from *Oroxylum Indicum* leaves extracted earlier at 50°C, 70°C, and 80°C will be injected into the Gas Chromatography Mass Spectrometry after obtained it from hydro distillation method. The analysis was carried out on a Agilent 19091J-433: 4294.60089 system. HP-5 5% Phenyl Methyl Siloxan (30m x 250 μm x 0.25 μm) was applied for the analysis. As for the operating conditions of the column, the oven temperature was set at 50°C to 300°C at 8°C/min with holding time for 5 minutes where the final temperature maintained for 36.25 minutes. Helium gas was used as the carrier gas of the analysis at a constant flow rate of 1ml/min. The injector temperature was kept at 250°C, split ratio of 10:1, temperature of ion source at 230°C, scan mass range m/z 40-600, the temperature of transfer line at 300°C. The identified chemical constituents in the hydrosols will be recorded and further analyses for the functional group in FTIR.

4. Results and Discussion

4.1 FTIR Analysis Results

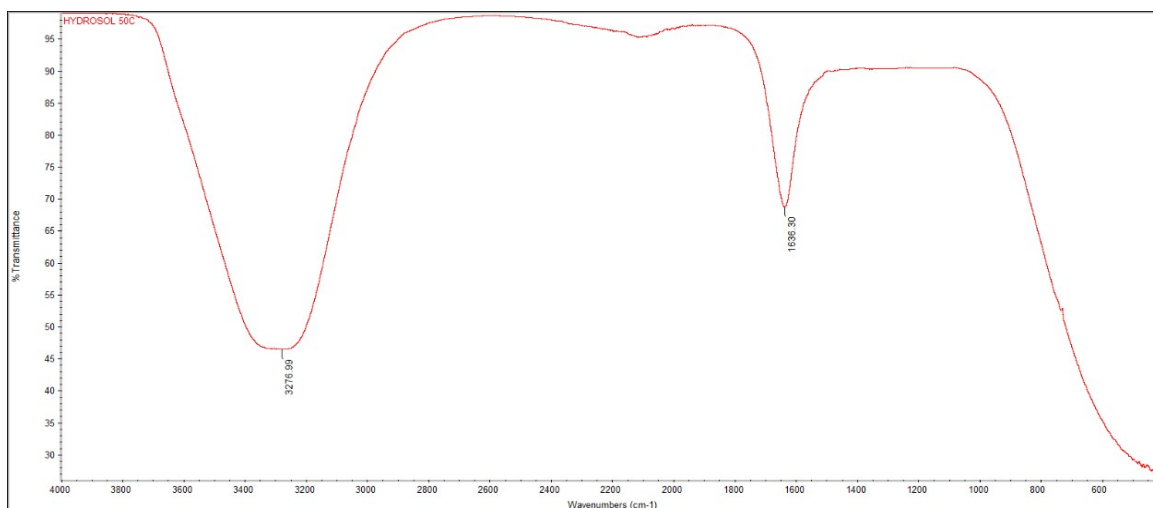


Figure 2. FTIR Spectra shows % transmission against wavenumbers for hydrosol at 50°C

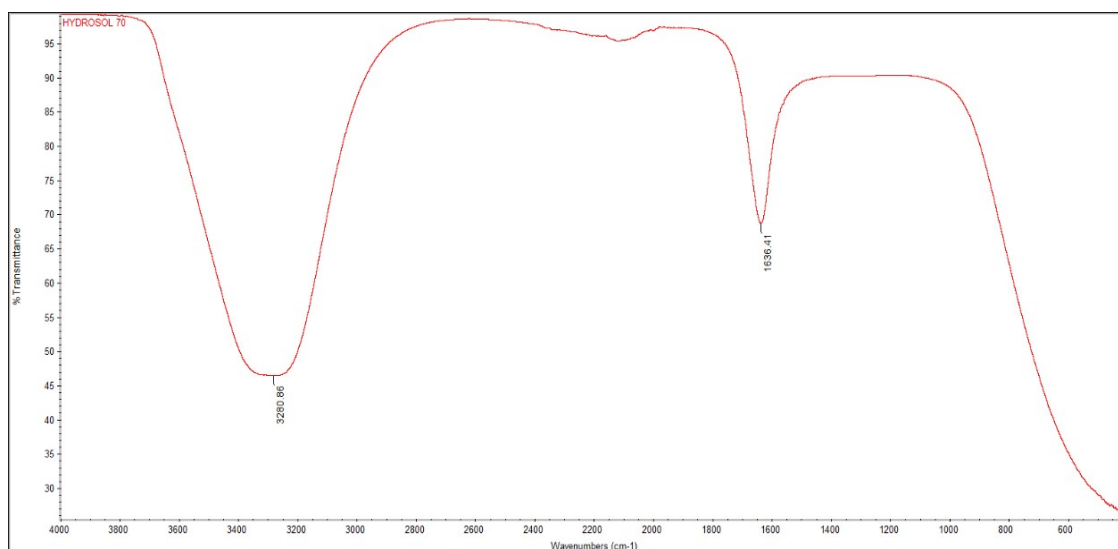


Figure 3. FTIR Spectra shows % transmission against wavenumbers for hydrosol at 70°C

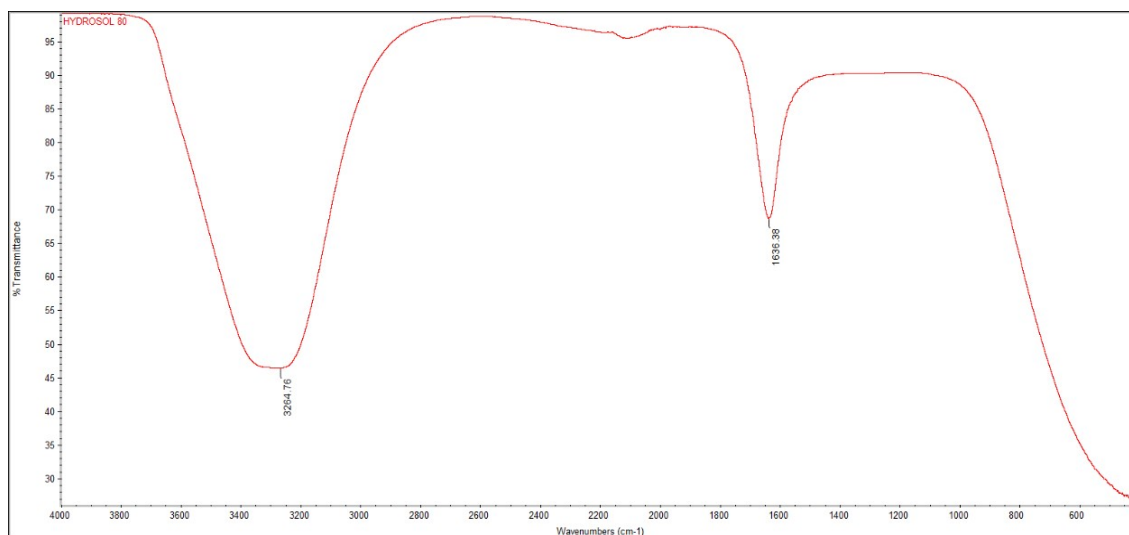


Figure 4. FTIR Spectra shows % transmission against wavenumbers for hydrosol at 80°C

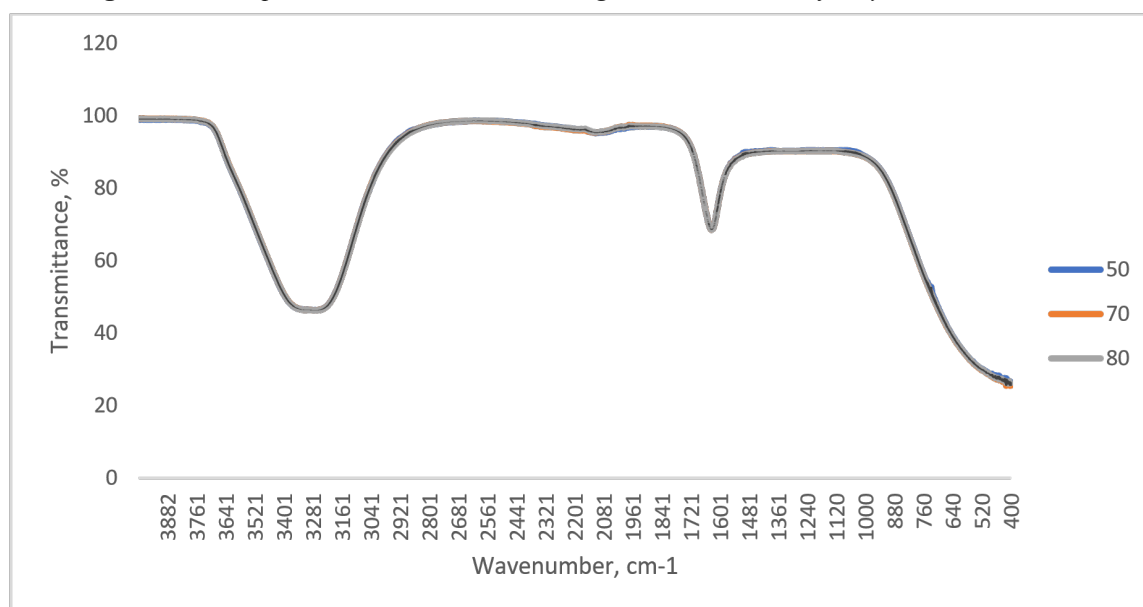


Figure 5. FTIR Spectra comparison of hydrosol at 50°C, 70°C and 80°C

Table 1. The Functional Group of Each Peak from FTIR Spectra

Temperature (°C)	Peak (cm ⁻¹)	Group	Compound Class
50	3276	O-H stretch, H- bonded, N-H stretch	Alcohols and primary amides
	1636	C=C stretch	Alkenes
70	3280	O-H stretch, H- bonded, N-H stretch	Alcohols and primary amides
	1636	C=C stretch	Alkenes
80	3264	O-H stretch, H- bonded, N-H stretch	Alcohols and primary amides
	1636	C=C stretch	Alkenes

Functional group and compound class in the table above were identified according to the peak from IR Spectroscopy Table (Klimoski & Palmer, 1993).

The analysis results of the FTIR spectrum of the hydrosol of *Oroxylum Indicum* leaves produced from hydro distillation at 50°C, 70°C and 80°C shows not much difference as shown in Figure 5 and there are no significant differences between each illustrated lines. The obtained data shown the same functional group and compounds class which are O-H stretch, H- bonded, N-H stretch and C=C stretch with suggested compounds of alcohols, primary amides and alkenes respectively. Based on the result obtained, most of the compounds in hydrosol extracted may be destroyed during the preparation of the samples prior to FTIR analysis. This is due to the chemical constituents of the sample may be decomposed because of the temperature during the extraction process that results in loss of volatile chemical compounds (Gilbert et al., 2004). Other than that, the loss of chemical constituents of hydrosol sample is may be due to the efficiency of the system and procedure during hydro distillation extraction.

3.2 GC-MS Analysis Results

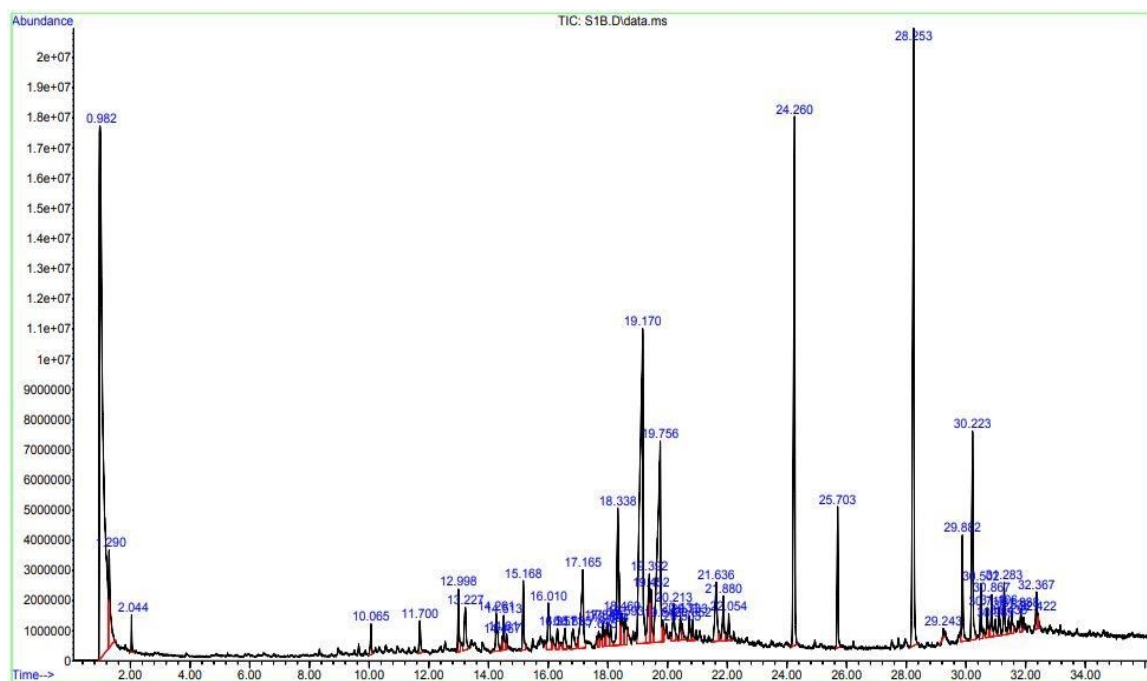


Figure 6. Peaks formed from GC-MS analysis for hydrosol at 50°C

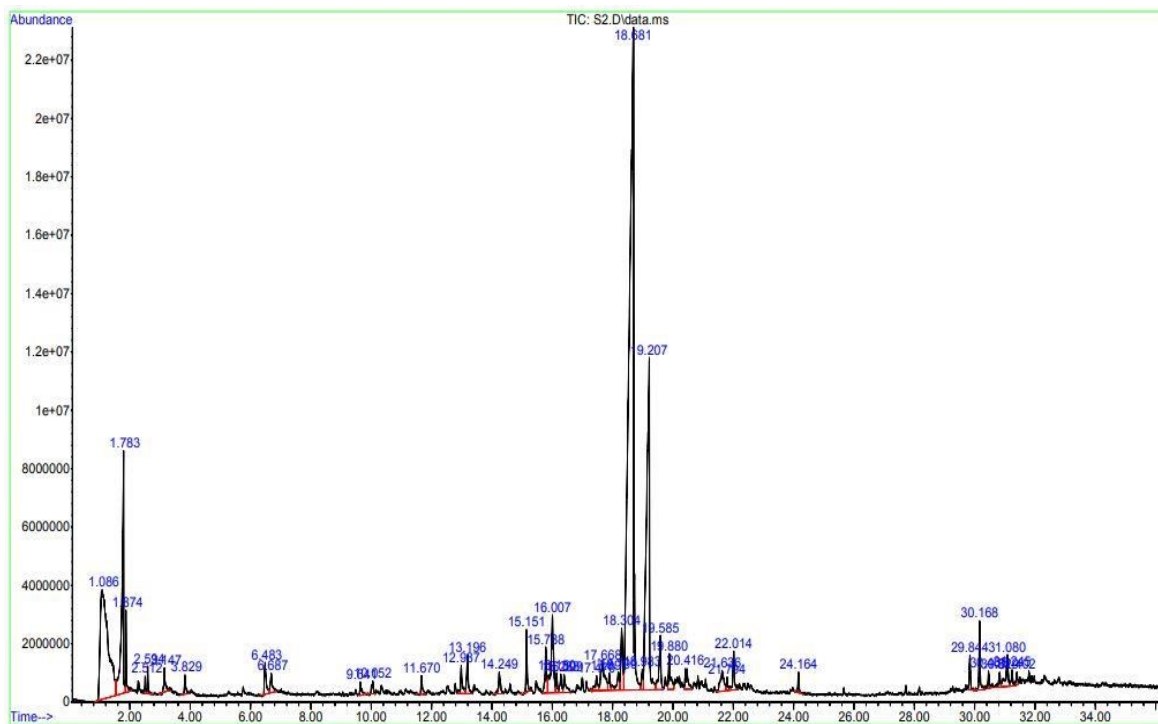


Figure 7. Peaks formed from GC-MS analysis for hydrosol at 70°C

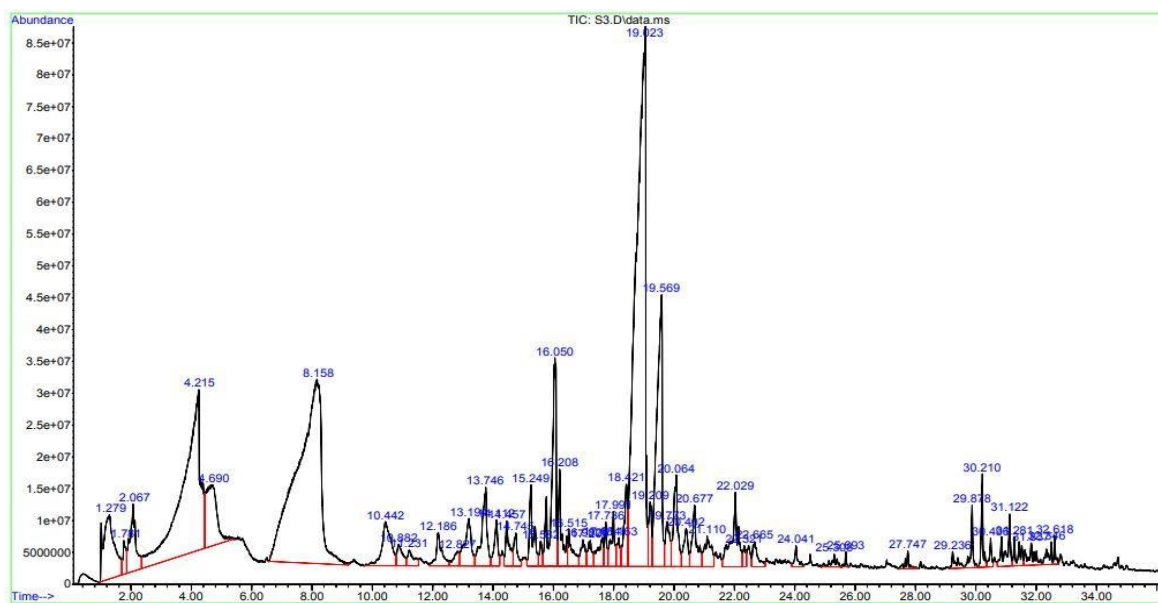


Figure 8. Peaks formed from GC-MS analysis for hydrosol at 80°C

Table 2. Chemical composition of the hydrosol of *Oroxylum Indicum* from hydro distillation at 50°C, 70°C, and 80°C

Temperature (°C)	Retention time	Peak area (%)	Species	Common Name
50	19.759	7.50	n-Hexadecanoic acid	Palmitic acid
	24.257	8.56	Hexadecenoic acid, bis (2-ethylhexyl) ester	2-ethylehexyl palmitate
	25.701	1.69	Phthalic acid, di (2-propylpentyl) ester	di-n-2-propylpentylphthalate
	28.252	10.44	Squalene	Squalene
70	1.786	5.88	Acetic acid	Acetic acid
	6.481	0.75	Phenol, 2-methoxy-	2-Methoxyphenol
	12.989	0.65	Benzaldehyde, 3,4dimethoxy-	Veratraldehyde
	21.794	0.26	Octadecanoic acid	Stearic acid
80	13.913	1.36	Benzaldehyde, 3,4-dimethoxy-	Veratraldehyde
	14.745	0.49	Benzoic acid, 3,4dimethoxy-methyl ester	Methyl veratrate
	15.585	0.27	Benzeneacetic acid, 4hydroxy-3-methoxy-, methyl ester	Acetic acid
	19.771	0.82	n-hexadecanoic acid	Palmitic acid
	32.616	0.18	Hexadecenoic acid, hexadecyl ester	Cetyl palmitate

Based on the analysis result obtained from GC-MS analysis, the chemical constituents of the hydrosol of *Oroxylum Indicum* produced from hydro distillation at 50°C, 70°C and 80°C were determined. The constituents identified for hydro distillation at 50°C, 70°C and 80°C were listed in Table 2 respectively. The major components identified for the hydrosol produced from hydro distillation at 50°C at obvious peaks were squalene (10.44%), 2-ethylehexyl palmitate (8.56%), palmitic acid (7.50%), and di-n-2propylpentylphthalate (1.69%). Following the increased of temperature to 70°C, the major component identified at obvious peak is acetic acid (5.88%), and other traces amount which are 2-methoxyphenol (0.75%), veratraldehyde (0.65%), and stearic acid (0.26%), which is different compared to the hydrosol obtained in at 50°C. As the temperature increases to 80°C, there is no significant components at obvious peak, instead the traces amount found were veratraldehyde (1.36%), palmitic acid (0.82%), methyl veratrate (0.49%), acetic acid (0.27%) and cetyl palmitate (0.18%). The result obtained shown that the increase of temperature of hydro distillation will affect the chemical constituents of the hydrosol where the chemical constituents decomposed (Gilbert, 2004). Apart from that, the identified components with low peak area such as stearic acid (0.26%) is identified as the traces amount of the hydrosol due to the data of component may be overlapped with other components. Besides that, as compared to the results from essential oil and hydrosol in Table 2 of *Oroxylum Indicum* components respectively, the chemical constituents of the essential oil is a total opposite quantitatively and qualitatively from hydrosol in Table 2 where the components contained in the essential oil does not present and different from hydrosol (Mellers et al., 2000). This may be happened due to the efficiency of the system during hydro distillation extraction as well as the nature of the components itself which is either hydrophobic or hydrophilic (Goleman, 2009). The chemical constituents of essential oil of *Oroxylum Indicum* is hydrophobic, which is non-polar, where the components does not mixed with water. However, it is possible for the hydrophobic components contained in the essential oil present in the hydrosol as hydrosol contained traces amount of essential oil despite being rich of hydrophilic components which are polar components (Goleman, 2009). Furthermore, according to the result obtained, the most significant chemical constituents identified in the

hydrosol of *Oroxylum Indicum* is beneficial for medicinal purposes which are squalene, and fatty acids (acetic acid and palmitic acid). Squalene has been utilized for breast and skin cancer therapy as it have a high anti-tumor activity (Cress, 2009). As for acetic acid, it has been utilized as an antiseptic agent which commonly used to disinfect wounds (Kubrick, 1980). However, palmitic acid pose an adverse effects towards chronic diseases such as obesity or diabetes for adults, even though it supports in cellular functions an essential components for cell membranes as well as secretory and transport lipids (Anderson et al., 2007). Palmitic acid is commonly used as intermediates for emulsifier, emollients and lubricants in cosmetic industries (American Psychological Association, 1972).

5. Conclusion

In this study, both functional groups and chemical constituents of *Oroxylum Indicum* leaves hydrosol yielded from hydro distillation at 50°C, 70°C and 80°C were determined by FTIR and GC-MS analysis respectively. The functional groups and compounds class identified for all samples are the same which are O-H stretch, H-bonded, N-H stretch and C=C stretch with suggested compounds of alcohols, primary amides and alkenes respectively. This shown that there no significant differences shown between the variation of temperatures. This is due to most compounds contained may be destroyed during the preparation of samples prior to FTIR analysis as well as the efficiency of the system and procedure during the extraction. GC-MS analysis was performed to determine the chemical constituents of the *Oroxylum Indicum* leaves hydrosol from hydro distillation at 50°C, 70°C and 80°C. The major components of the hydrosol produced at 50°C at obvious peaks were squalene (10.44%), 2-ethylehexyl palmitate (8.56%), palmitic acid (7.50%), and di-n-2-propylpentylphthalate (1.69%). As for temperature at 70°C, the major component identified at obvious peak is acetic acid (5.88%), and other traces which are 2-methoxyphenol (0.75%), veratraldehyde (0.65%), and stearic acid (0.26%). As for temperature at 80°C, there is no significant components at obvious peak, instead the traces amount found were veratraldehyde (1.36%), palmitic acid (0.82%), methyl vertrate (0.49%), acetic acid (0.27%) and cetyl palmitate (0.18%). The analysis shown that the increase of temperature of hydro distillation will affect the chemical constituents of the hydrosol where the chemical constituents decomposed. Besides, the chemical constituents of hydrosol were total opposite quantitatively and qualitatively from the essential oil. However, the medicinal value of hydrosol was identified based on the major components identified in each samples such as squalene, acetic acid and palmitic acid.

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