

Identification on Chemical Constituent of *Alpinia purpurata* Hydrosol Extracted by Hydro Distillation Method

Shamalah Sivem¹, Mohd Aizudin Abd Aziz², Muhammad Auni Hairunnaja³ & Norasiha Hamid⁴
^{1,2,3}Universiti Malaysia Pahang & ⁴University College of Yayasan Pahang

ARTICLE INFO

Article history:

Received Dec 28, 2021

Revised Jun 16, 2022

Accepted Jul 25, 2022

Keywords:

Alpinia Purpurata,
Hydro Distillation,
Essential Oil,
Hydrosol

Conflict of Interest:

None

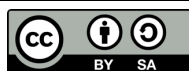
Funding:

Research Grant (UMP)
RDU190398.

ABSTRACT

Alpinia purpurata (Halia Bara) is a perennial herbaceous plant with virtually identical qualities to common ginger, but with smaller and more pungent rhizomes. It is widely known for ornamental purposes and is also applied in the medicinal field. Extraction of essential oil and hydrosol of *Alpinia purpurata* can be done through the hydro distillation method. The essential oil contains α -pinene, β -caryophyllene, geranial, neral and β -pinene that contribute to medicinal values while hydrosol's chemical components are yet to be identified. Hence, the primary objective of this study is to use the hydro distillation method to identify the chemical constituents and functional groups of active chemical compounds present in *Alpinia purpurata*'s rhizomes hydrosol. Hydro distillation is also done at different heating temperatures and distillation times to see what influence temperature and distillation time have on the components and active compounds in the hydrosol. The powdered sample is used for extraction at 60°C, 80°C, and 100°C, with distillation periods of 1, 1.5, 2, and 2.5 hours. Burette is used to extract essential oil from the hydrosol. To further investigate the hydrosol, gas chromatography-mass spectrometry (GC-MS) and Fourier-transform Infrared Spectroscopy (FTIR) are utilised extensively. The major chemical constituents detected in *Alpinia purpurata*'s hydrosol at 100°C for 2.5 hours of distillation time include 1-Dodecanamine (40.08 %) and functional groups present are O-H, N-H, C-H, and C=H stretching, according to GC-MS and FTIR data. The increased heating temperature and distillation time caused the denaturation of substances in the extracted hydrosol. The chemical constituents present in hydrosol are greatly different from the chemical constituents that are present in essential oil qualitatively and quantitatively.

Corresponding Author: Shamala Sivem, College of Engineering, Universiti Malaysia Pahang, 26300 UMP, Kuantan, Pahang, Malaysia. Tel. +6013 6615278 E-mail: shamalah98@gmail.com



© Shamalah Sivem, Mohd Aizudin Abd Aziz, Muhammad Auni Hairunnaja, Norasiha Hamid

This is an open-access article under the CC BY-SA 4.0 international license.

1. Introduction

Alpinia purpurata (Halia Bara) also known as red ginger, is same family of *Zingiber officinale* (Halia) and native to tropical and subtropical Asia (Victório, Kuster, & Lage, 2009). It resembles ordinary ginger in appearance, but it has smaller, more pungent rhizomes that are red on the outside with a yellow to pinkish cross-section, and a crimson base to its shoot. It is well-known for its attractive appearance and widely been used as decorative purposes. Other than that, it is also being used in various fields such as in medicines and vital source of raw materials in natural dye production. The rhizomes are used to treat headaches, rheumatism, sore throats, and kidney problems (Oirere, Anusooriya, Raj and Gopalakrishnan, 2015) while its leaves infusion is used to treat disturbs in respiratory tract treatment of cardiovascular diseases. Essential oils and hydrosol from *Alpinia purpurata* can be extracted by hydro distillation. It is a traditional and conventional method. Essential oils are concentrated liquids composed of a complex mixture of volatile chemicals that can be extracted from a variety of organs. Essential oils contain a variety of bioactive chemicals that have anti-oxidant and anti-microbial activities (Tongnuanchan & Benjakul, 2014). There are several studies that proved that the

essential oils that being extracted from several parts of *Alpinia purpurata* can be actually used for medicines, natural additives, antioxidants, antibacterial, anticancer and anti-inflammatory activities.

When there is a significant amount of essential oil present, the water condenses in the bottom of the container, and the essential oil and other volatile organic substances float on top of the water (Elham, 2020). Hydrosol or also known as distillate water which is the remaining water after extraction of essential oils being done. They are made by dispersing plant components in an industrial hydro distillation system. (Hamedi, Moheimani, Sakhteman, Etemadfarid & Moein, 2017). It possesses biological activities and have potential as global economic products of commerce (Rao B. 2013). It is used widely in perfume industries, cosmetics, food flavoring, aromatherapy and traditional therapies. However, the researchers tend to discard the hydrosol and focus on the extraction of essential oils which have market values as there is insufficient research being done regarding the uses of the *Alpinia purpurata's* hydrosol. Despite the fact that essential oils and hydrosol are prepared in the same technique, they are findable from different components (Smith & Richards, 2019). Inouye, Takahashi, and Abe (2008) compared the composition of 44 hydrolates to their essential oils and found that the primary components of oils and hydrolates differed in 42% of the cases, and that the main components of oils and hydrolates were much lower than those in essential oils. (D'Amato, Serio, López, & Paparella, 2018).

Therefore, it will be more beneficial by increasing the utilization of the hydrosol by conducting analysis on the chemical constituents and functional groups of active compounds that present in hydrosol using GC- MS and FTIR respectively, so that it can be used for many purposes in future. Besides that, the effects of the distillation time and heating temperatures on hydrosol produced from hydro distillation method are also studied.

2. Literature Review

Plumier was the first to classify the genus *Alpinia*, although it was named after Prospero Alpino, an Italian botanist who lived in the 16th century. The genus, the subclass Zingiberidae, and the order Zingiberales all belong to the division *Magnoliophyta (Angiospermae)* (Victorio, 2011). *Alpinia purpurata* has red bracts which gives a red-flowered inflorescence and its flowers are in white in colour. When *halia bara* is young, the petiole is crimson, and the lip is a fiery scarlet mottled with cream, unlike common ginger. The leaves are dark green in colour, sessile, alternate, and oblong in shape with a pointed apex (Chan & Wong, 2015). The leaves emerge from the stems in two rows, alternating left and right. Because of its beautiful and long-lasting flowers, it is widely grown as an ornamental plant in gardens. Other than that, most of the *Alpinia purpurata* organs such as rhizomes, leaves and flowers play different roles of benefits. Rhizome, for example, has a pungent odour and increases appetite, taste, and voice, while its leaves are used to make yellow dye. *Alpinia purpurata's* pharmacological activities include antioxidant, antibacterial, larvicidal, cytotoxic, and vasodilation (Chan & Wong, 2015). Therefore, the extraction of essential oil and hydrosol can be done by hydro distillation which will give two layers of essential oil and hydrosol. In hydro distillation, the plant components are put in a still compartment with water added for boiling purpose. Then, the vapor mixture of water and oil is then condensed by indirect cooling with water after direct steam is delivered into the sample (M. Selvamuthukumaran & Shi, 2017).

Essential oils are extremely concentrated liquids that contain complex mixtures of volatile components and have antimicrobial properties. They can be extracted from a variety of organs. It is a form of alternative medicine which makes use of plant extracts to support health and well-being. Hydrosol is made up of condensing water from the distillation process, as well as polar, oxygenated, odour-inducing, hydrophilic, volatile oil constituents that form hydrogen bonds with water (D'Amato, Serio, López, & Paparella, 2018). Vapour flows through sections of the plant during steam distillation, breaking the cells carrying essential oils and volatile substances with a low molecular weight. (Elham, 2020). The cooling process will cause the vapour to condense into water. When there is a significant amount of essential oil present, the water condenses in the bottom of the container. Meanwhile, the essential oil and other volatile organic substances float on top of the water. (Elham, 2020). The fragrance of the hydrosol obtained after hydro distillation varies based on the time it was collected throughout the distillation process. Hydrosols collected during the early part of the distillation are pleasant-scented, while those collected during the later part have grassy or vegetal notes due to variations in their composition brought out by low or high boiling terpenoids contained in the hydrosols (Rao B. R., 2013). Aromatic hydrophilic oil, essential oil components that escape from hydrosol, is estimated to be worth between \$50 and \$100 million in India (Rao, 2013). From 2019 to 2024, the hydrosols market is expected to develop at a substantial pace of 5.17 percent, reaching a market value of USD 437 million by the end of 2024. (Politi, et al., 2020).

Gas chromatography-mass spectroscopy (GC-MS) and Fourier-transform infrared (FTIR) spectroscopy are used to identify the chemical ingredients and functional groups found in *Alpinia purpurata's* hydrosol. GC-MS

is a gas chromatography that equipped with a mass spectrometry detector. It's used to separate chemical mixtures and identify individual molecular components. Before being delivered into the gas chromatography inlet port, the collected sample is usually diluted with a solvent. The liquid sample will evaporate at the heated intake, and the mobile phase (helium) as an inert gas will transports the sample through the column. Different chemicals in the sample react differently with the column's stationary phase. Therefore, the velocity at which the sample travels down the column will differ, resulting in compound separation. The separated compounds will enter the detector one by one and the retention time of the compounds is determined. Each isolated compound from gas chromatography will be broken down by mass spectrometry. To produce electrically charged particles or ions, a high-energy beam of electrons is sent across the sample molecule. After that, the ions are sorted by mass to charge ratio and relative abundance in a mass analyzer. The mass spectrometry will generate a mass spectrum, which will reveal the abundance of each identified fragment's mass to charge ratio.

FTIR is a phenotypic method used for determining the absorption of infrared light by molecules such as lipopolysaccharides, lipids, carbohydrates, nucleic acids, and proteins, resulting in a distinct FTIR spectrum that reflects the sample's overall composition (Vogt, et al., 2019). The sample will be exposed to infrared radiation. The sample will absorb some of the radiation, while the rest will pass through. By absorbing IR light, molecules are stimulated to a higher vibrational state (Ramaiah, Bathia & Ramesh, 2017). A sample's fingerprint is defined as an infrared spectrum, with absorption peaks matching to the frequency of vibrations between the material's atoms (Nicolet & All, 2001). Different materials will have different infrared spectrum due to its atoms or molecules combinations.

The identification on components and functional groups of the essential oil in *Alpinia purpurata*'s rhizomes and leaves have been conducted by many studies. Sivasothy, et al (2011) mentioned, the essential oil extracted from *Alpinia purpurata* rhizomes and leaves was hydro-distilled for 4 hours in a Clevenger- type apparatus, and the extracted essential oil was analyzed d thoroughly by GC-MS using a fused-silica capillary column with dimensions of 30m x 0.25mm and a film thickness of 0.25m. Helium was employed as the carrier gas at a rate of 1.0 min-1. In the split mode, a 0.2L sample was injected. A split ratio of 50:1 and ionization voltage of 70 eV were maintained. The oil yields (w/w) on a fresh weight basis were 0.03 percent (leaves) and 0.02 percent (rhizomes), according to the research. The oil from the rhizomes of *halia bara* yielded 54 of recognizable constituents, accounting for 95.5 %, while the leaf oil yielded 46 identifiable constituents, accounting for 91.7 % (Sivasothy, et al., 2011). The main chemical constituents found in the essential oil of *Alpinia purpurata*'s leaves and rhizomes were α -pinene, α -pinene, neral, geranial, geranyl acetate, and β -Caryophyllene, according to the findings (Sivasothy, et al., 2011). The infrared spectrum of a pure compound extracted from *Alpinia purpurata* essential oil reveals that it comprises functional groups of C-N, C=O (amide), C=C (aromatic), C-O (ethers), and C-H by the maceration method with methanol as the solvent (Suzery, Yuyun, Ria, & Cahyono, 2019).

Additionally, phytochemical analysis of *Alpinia purpurata* n-hexane leaf extract revealed that it contains active elements such as alkaloids, terpenoids, flavonoids, steroids, cardioglycosides, tannins, carbohydrates, oil, and fats (Oirere E., Anusooriya, Raj, & Gopalakrishnan, 2015). The presence of bioactive compounds in leaf extract of *Alpinia purpurata* such as Methanesulfonate of [3R,4S]-3-Propargyloxy-4-[(R)-1-hydroxy- 3-phenyl-3-butenyl]-1-(p-methoxy phenyl)-2-azetidinone which is an intermediate in the semi-synthetic synthesis of paclitaxel (commercial chemotherapy), 4-Morpholinomethyl-7-methoxy coumarin (anti-cancer activity) and methenolone is used to treat aplastic anemia (Oirere E. , Anusooriya, Raj, & Gopalakrishnan, 2015). Despite the fact that essential oils and hydrosol are made in the same way, they contain different ingredients (Smith & Richards, 2019). Therefore, it is important to understand the uses of both hydrosol and essential oils. Because the chemical ingredients of *Alpinia purpurata*'s hydrosol have yet to be identified and scientifically examined, this is an excellent chance to investigate the chemical constituents of the hydrosol so that it can be fully utilized in the future.

3. Method

3.1 Preparation of *Alpinia purpurata* sample

Fresh rhizomes will be used in the study and can purchase from local market in Kuantan, Pahang. The fresh rhizomes were washed thoroughly under the tap water to remove any dirt and insects. Then, they were shade dried for days to remove moisture content. The dried sample will be powdered using grinder. 3g of the powdered sample is weighed and placed in a tea bag. Around 15 tea bags are prepared for the experiment. The tea bags are sealed at the end by stitching.

3.2 Extraction of essential oil and hydrosol of *Alpinia purpurata*

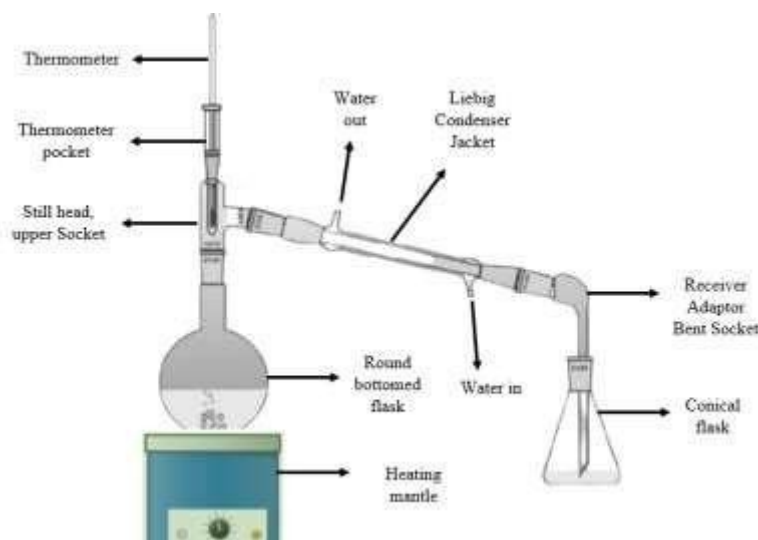


Figure 1. Quick Fit Hydro Distillation system

Figure 1 shows the Quick Fit Hydro Distillation system used for hydro distillation method. The tea bag was inserted in the flask with the round bottom. 500 mL of distilled water (solvent) is poured into the round bottomed flask that is linked to the Liebig condenser jacket. The solvent was heated to 100°C with a heating mantle before being hydro distilled for 1 hour. The time taken for the distillation is set after the temperature of heating is maintained at the desired temperature. The distilled water will vaporize and condense in the condenser, where the vapours will be collected in the conical flask. The hydro distillation extract, which comprised both essential oil and water, was separated using a burette. The processes were repeated with heating temperatures of 60°C and 80°C and distillation time at intervals of 1.5, 2 and 2.5 hours.

3.3. Analysis of determining the chemical constituents of *Alpinia purpurata*'s rhizomes hydrosol using GC-MS

GC-MS is an equipment that used to analyze the chemical components of the extracted hydrosol samples. In GC-MS, methanol was applied as the solvent, and the sample was diluted with methanol at a ratio of 1:9. (sample: methanol). An Agilent 7890A Gas Chromatograph with a 5975-MS System and a Carbowax-20M capillary column with a 50 m x 0.22 mm film thickness of 0.25 μm was used for the analysis. Helium acts as a carrier gas at a flow rate of 4.0 mL/min. The 1 μL sample was introduced to the column with the injector in the split mode. The Ionization voltage of 70 eV, ion source temperature of 230°C, and mass range of 50–600 U were significant MS operating parameters (Sivasothy, et al., 2011) (at Analytical Laboratory of FTKKP Lab, Universiti Malaysia Pahang).

3.4 Analysis of determining the functional groups of active compounds of *Alpinia purpurata*'s rhizomes hydrosol using FTIR

FTIR analysis was carried out using IR spectra to identify the characteristics and type of functional groups of the chemical constituents present in *Alpinia purpurata*'s hydrosol. Each hydrosol sample was examined using FTIR spectrometer (IS50, Thermoscientific, USA) within the wavenumbers of 4000–400 cm^{-1} , after a drop of the sample was placed on the universal attenuated total reflectance (ATR) accessory. (Oirere E. K., Anusooriya, Raj, & Gopalakrishnan, 2015).

4. Results and Discussion

4.1 GC-MS analysis

There is no hydrosol produced at 60°C for 1 and 1.5 hours of distillation time as the temperature is insufficient for the distilled water and the plant sample of *Alpinia purpurata* to undergo evaporation and condensation in a shorter period of time. The chemical composition of *Alpinia purpurata*'s hydrosol at different distillation periods and heating temperatures is shown in Tables 1, 2, 3, and 4. Based on the analysis results from GC-MS, it clearly shows that the chemical constituents obtained at every temperature and distillation time are different. The main element found in the hydrosol produced by hydro distillation at 60°C for 2 hours was octadecanoic acid, which has a peak area of 38.88 % and gradually drops to 28.30 % as the distillation period increases. Additionally, the peak area of octadecanoic acid reduces linearly as the distillation time increases at heating temperature of 80°C as in Figure 12.q The peak area initially was 15.28% at 1 hour and started to drop to

10.91%, 10.53% and finally to 10.18% at 2.5 hours. Hence, it is clearly shows that distillation time influences the decomposition activity of the constituents in the hydrosol. The longer the distillation time, the more the substances in hydrosol decomposed, thus the smaller the peak area.

The effect of heating temperatures on the chemical constituents that present in the hydrosol is studied. Based on Figure 13, the hydrosol produced at 60°C for 2.5 hours showed that acetic acid and octadecanoic acid had peak areas of 2.47% and 28.30% respectively. Subsequently, the peak areas reduced to 2.09% and 10.18% when the heating temperature is increased to 80°C and both of the chemical constituents absent at the temperature of 100°C. Besides that, oleic acid only presents at 60°C and fully denatured when the temperatures were increased to 80°C and 100°C for 2.5 hours distillation time. High temperatures boost the diffusion rate, solvent convection, and fuel viscosity decrease, all of which improve distillation efficiency. (Do, et al., 2019). However, at high temperatures, the substances in the hydrosol starts to decompose. The higher the heating temperatures, the lesser amount of the constituent present in hydrosol thus the smaller the peak area. At 100°C and 2.5 hours of distillation time, 1-Dodecanamine is identified as the major constituents with a peak area of 40.08%.

Other than that, there are some chemical constituents that also present in *Alpinia purpurata*'s hydrosol such as Vitamin E, γ -Sitosterol, β -Sitosterol and 1-Dodecanamine. Both n-Hexadecanoic acid and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- are having antioxidant and antimicrobial activities respectively (Falowo, Muchenje, Hugo, Aiyegoro, & Fayemi, 2017). Octadecanoic acid is used in soaps, detergents and cosmetics industries (George & Britto, 2016) while 1-Dodecanamine has a pharmacological action towards anti-Staphylococcal activity (Al-tameme & Hameed, 2015). Aside from that, β -Sitosterol is often used to treat heart disease, hypercholesterolemia, immune system modulation, rheumatoid arthritis, cancer prevention, tuberculosis (TB), hair loss, cervical cancer, and benign prostatic hyperplasia, among other conditions (Saeidnia, Manayi, & Gohari, 2014). Therefore, it would be great if we can utilize the hydrosol in future as it also has pharmacological actions.

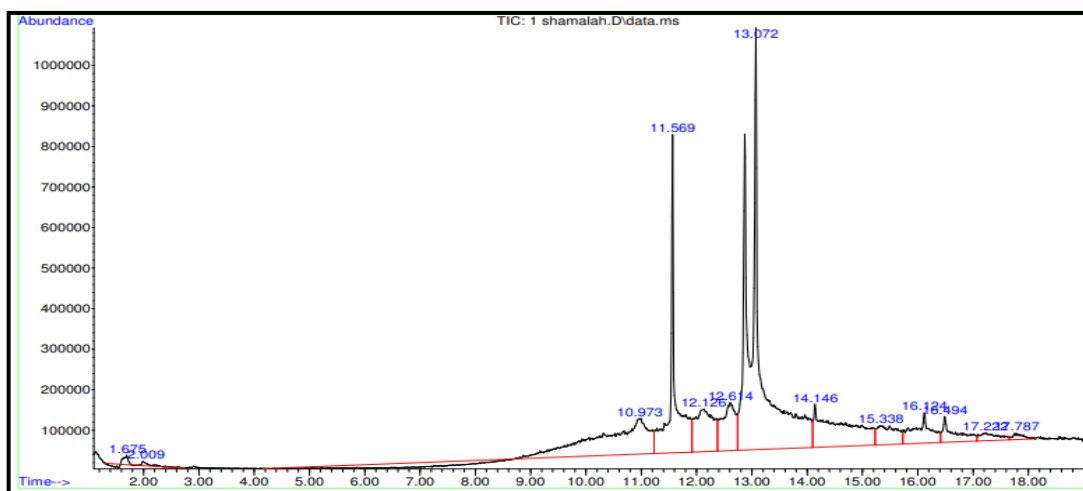


Figure 2. Peaks formed from GC-MS analysis for hydrosol at 60°C for 2 hours

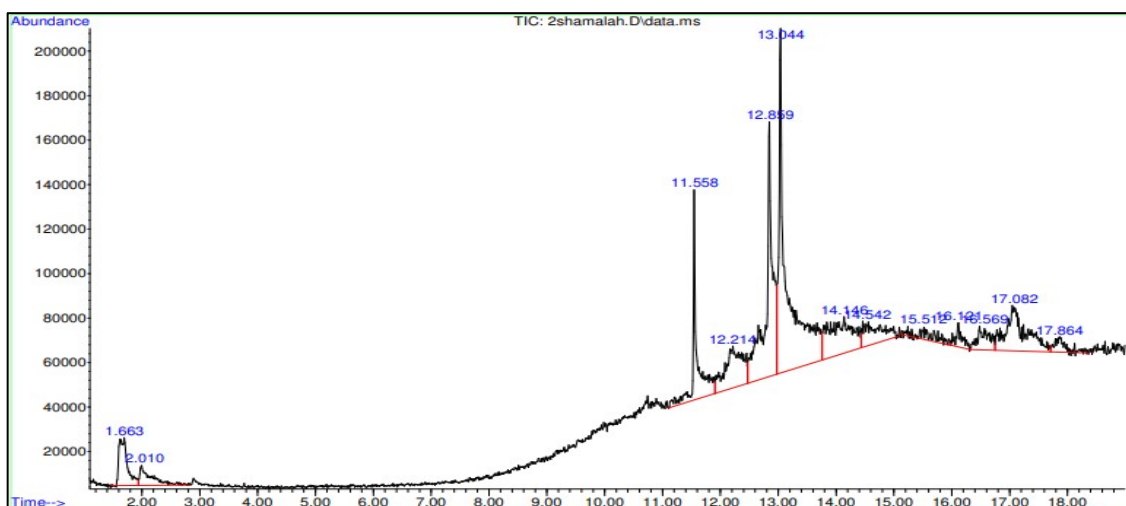


Figure 3. Peaks formed from GC-MS analysis for hydrosol at 60°C for 2.5 hours

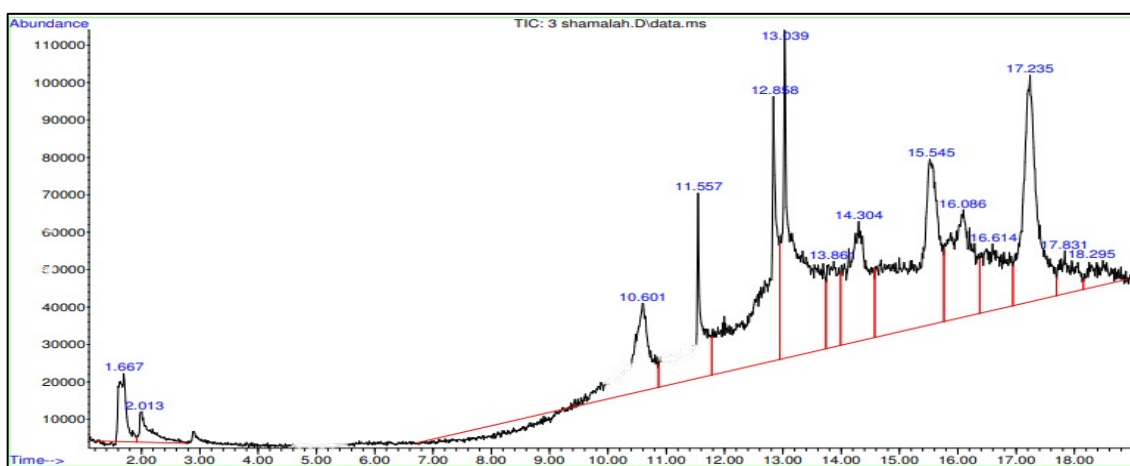


Figure 4. Peaks formed from GC-MS analysis for hydrosol at 80°C for 1 hour

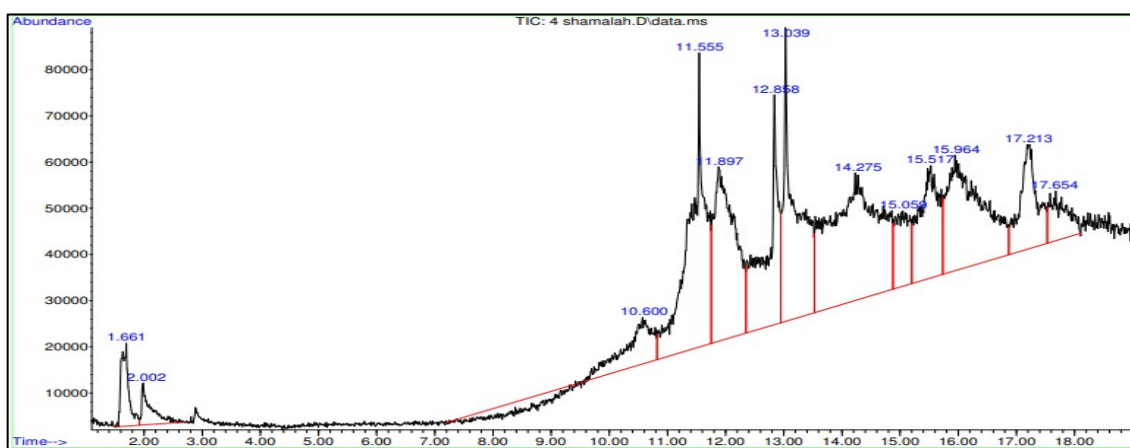


Figure 5. Peaks formed from GC-MS analysis for hydrosol at 80°C for 1.5 hours



Figure 6. Peaks formed from GC-MS analysis for hydrosol at 80°C for 2 hours

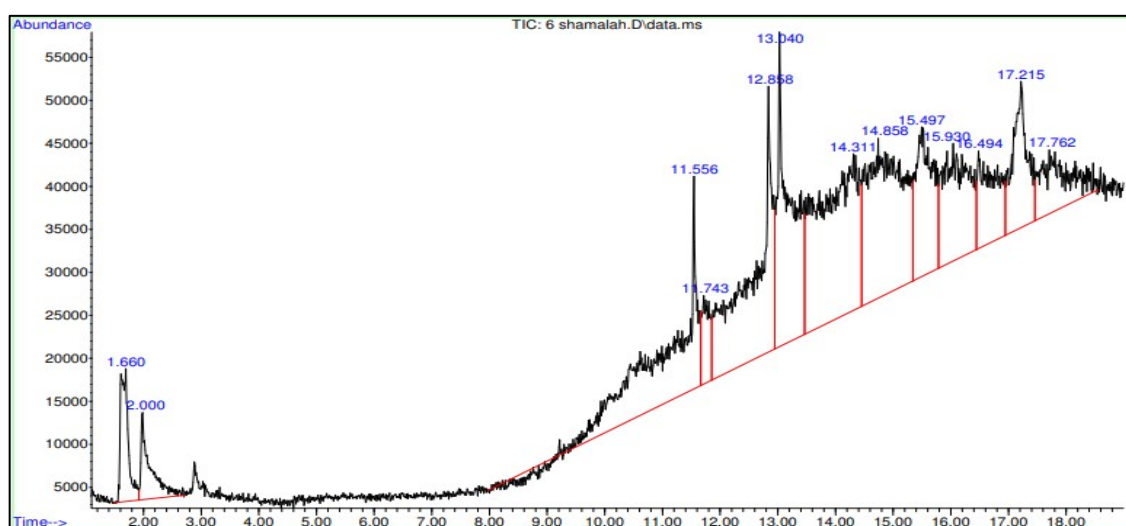


Figure 7. Peaks formed from GC-MS analysis for hydrosol at 80°C for 2.5 hours



Figure 8. Peaks formed from GC-MS analysis for hydrosol at 100°C for 1 hour.



Figure 9. Peaks formed from GC-MS analysis for hydrosol at 100°C for 1.5 hours

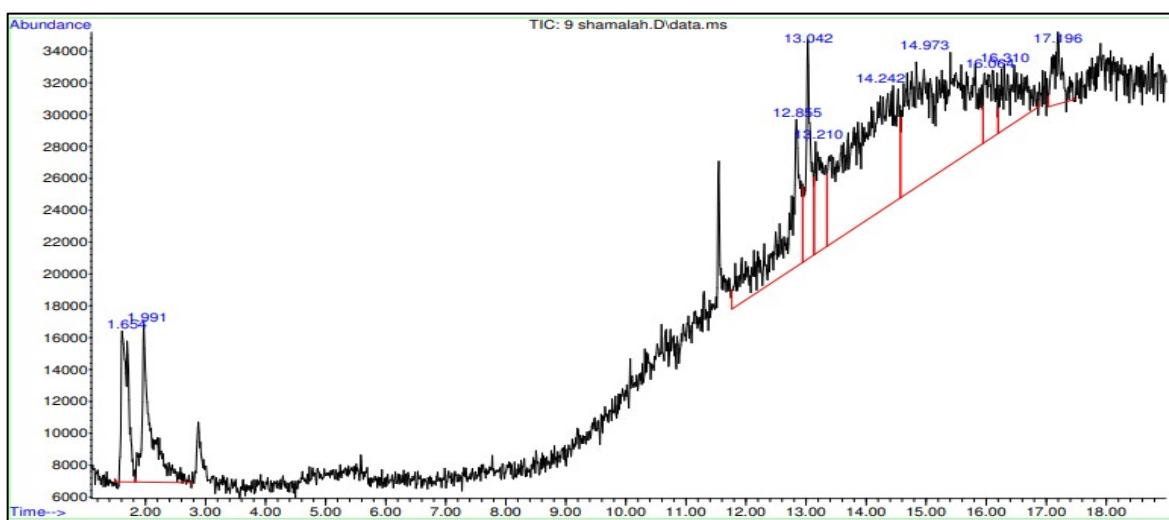


Figure 10. Peaks formed from GC-MS analysis for hydrosol at 100°C for 2 hours

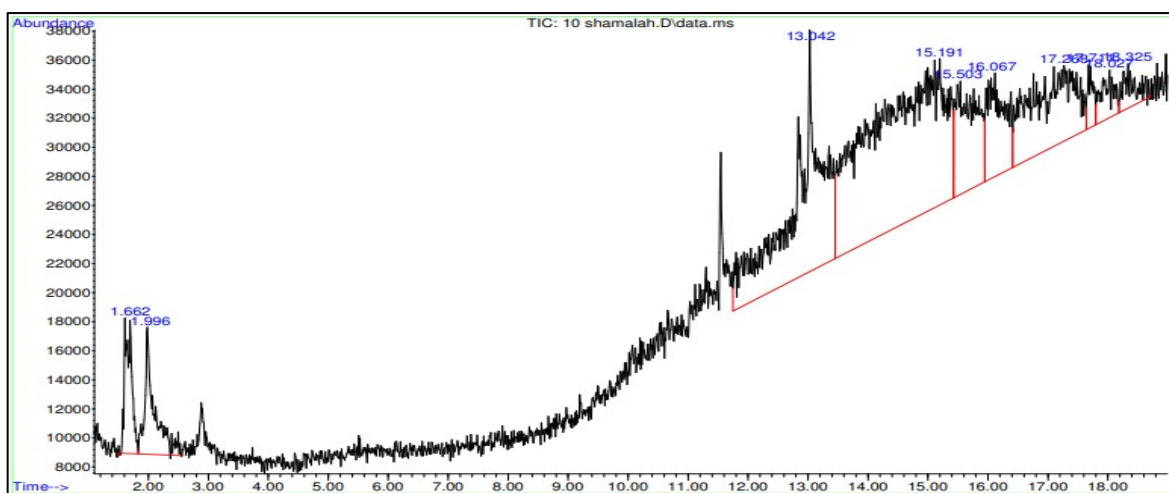


Figure 11. Peak formed from GC-MS analysis for hydrosol at 100°C for 2.5 hours

Table 1. Chemical composition of hydrosol of *Alpinia Purpurata* at 1 hour distillation time

No	Compound	Retention Time		Peak Area (%)	
		80 C	100 C	80 C	100 C
1	Acetic acid	2.016	2.005	1.2	2.89
2	Octanoic acid, decyl ester	10.605	-	3.34	-
3	n-Hexadecanoic acid	11.555	-	6.07	-
4	Octadecanoic acid	13.04	13.04	15.28	18.01
5	2-Ethylacridine	14.3	-	8.77	-
6	Tetrasiloxane, decamethyl-	15.55	15.839	17.08	6.38
7	Silane, trimethyl [5-methyl-2-(1methylethyl)-phenoxy]-	16.084	-	8.64	-
8	Hexacosanoic acid	17.238	-	11.67	-
9	Cyclotrisiloxane, hexamethyl-	-	11.555	-	10.74
10	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	-	14.215	-	14.7
11	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	-	17.185	-	12.2
12	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester)	-	18.157	-	17.24

Table 2. Chemical composition of hydrosol of *Alpinia Purpurata* at 1.5 hours distillation time.

No	Compound	Retention Time		Peak Area (%)	
		80 C	100 C	80 C	100 C
1	Acetic acid	2.006	2.006	1.24	4.53
2	γ -Sitosterol	11.555	-	13.75	-
3	β -Sitosterol	11.897	-	11.81	-
4	n-Hexadecanoic acid	12.858	-	8.33	-
5	Octadecanoic acid	13.04	-	10.91	-
6	Indole-2-one, 2,3-dihydro-N-hydroxy-4methoxy-3,3-dimethyl-	14.279	-	19.5	-
7	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	15.967	-	12.25	-
8	2-Octenoic acid	-	12.858	-	14.97
9	Tetrasiloxane, 1,7-diallyloctadecyl	-	14.226	-	22.08
10	N-(3-Methylbutyl) acetamide	-	15.529	-	20.17
11	Cyclotrisiloxane, hexamethyl-	-	16.415	-	5.34

Table 3. Chemical composition of hydrosol of *Alpinia Purpurata* at 2 hours distillation time.

No	Compound	Retention Time			Peak Area (%)		
		60 C	80 C	100 C	60 C	80 C	100 C
1	Acetic acid	2.005	2.006	1.995	0.31	1.69	7.08
2	Ethyl 9-hexadecenoate	10.968	-	-	10.94	-	-
3	1,5,9-Undecatriene,2,6,10-	12.121	-	-	-	7.23	-
4	Octadecanoic acid	13.072	13.04	-	38.88	10.53	-
5	n-Hexadecanoic acid	15.337	11.555	-	3.48	6.01	-
6	Oleic acid	16.127	12.858	-	4.21	9.33	-
7	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	17.227	14.888	-	1.3	8.61	-
8	Tetrasiloxane, decamethyl	-	14.268	-	-	14.69	-
9	Stigmast-4-en-3-one	-	15.518	-	-	33.52	-
10	Cyclotrisiloxane, hexamethyl	-	17.217	16.309	-	6.6	5.12
11	N-(3-Methylbutyl) acetamide	-	-	12.858	-	-	10.77
12	Acetamide, N-butyl	-	14.973	14.973	-	-	28.13

Table 4. Chemical composition of hydrosol of *Alpinia Purpurata* at 2.5 hour distillation time.

No	Compound	Retention Time			Peak Area (%)		
		60 C	80 C	100 C	60 C	80 C	100 C
1	4,9-Decadienoic acid, 2-nitro-,ethyl ester	1.664	-	-	4.4	-	-
2	n-Hexadecanoic acid	11.555	11.555	-	8.3	11.33	-
3	Oleic acid	12.858	-	-	18.09	-	-
4	Octadecanoic acid	13.04	13.04	-	28.3	10.18	-
5	2-Ethylacridine	16.565	-	-	2.78	-	-
6	Vitamin E	17.078	-	-	9.47	-	-
7	Silicic acid, diethyl bis (trimethylsilyl)-ester	-	14.311	-	-	16.41	-
8	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	-	15.935	-	-	7.6	-
9	Indole-2-one,2,3-dihydro-N-hydroxy- 4--dimethyl-	-	16.49	-	-	4.35	-
10	Tetrasiloxane, decamethyl-	-	17.217	-	-	5.51	-
11	Ethylamine	-	-	1.995	-	-	4.19
12	1-Dodecanamine	-	-	15.187	-	-	40.08
13	N-(3-Methylbutyl) acetamide	-	-	17.27	-	-	12.06

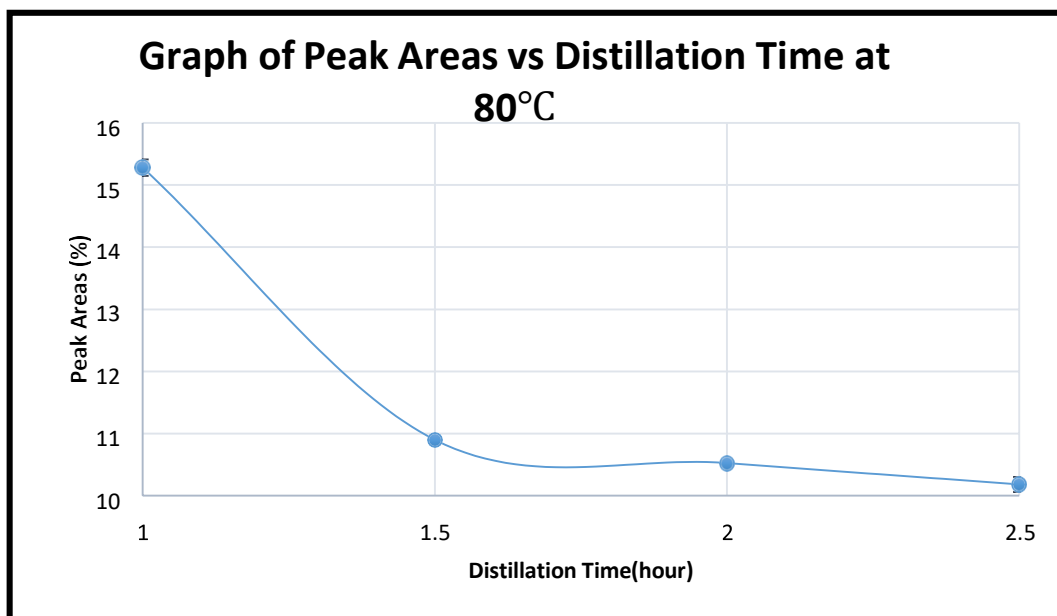


Figure 12. Graph of Peak Areas VS Distillation time at 80°C.

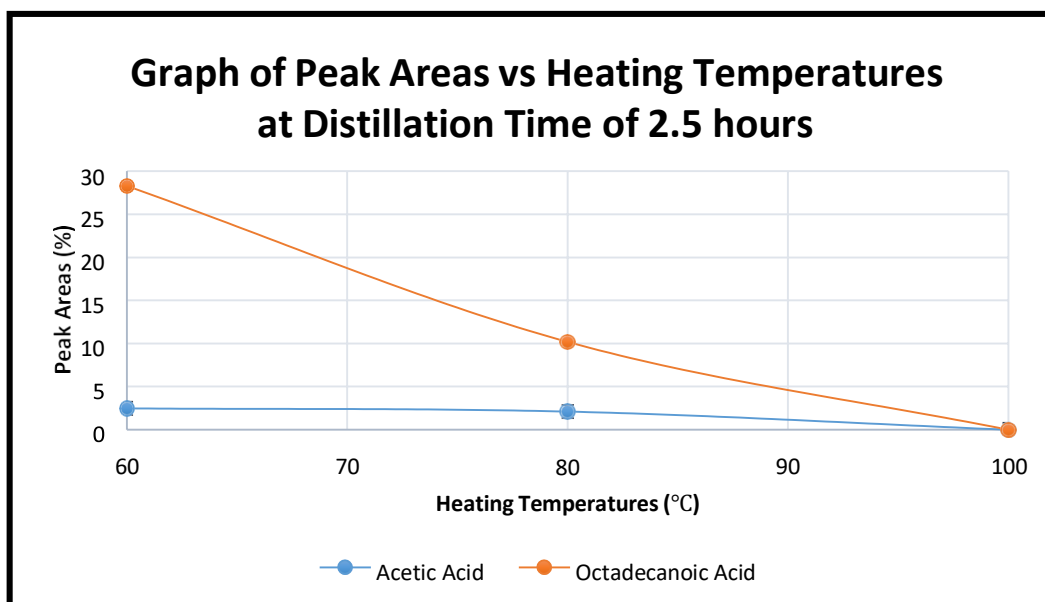


Figure 13. Graph of Peak Areas VS Heating Temperature at Distillation Time of 2.5 hours.

4.2 FTIR Analysis

There is no hydrosol produced at 60°C for 1 and 1.5 hours of distillation time as the temperature is insufficient for the distilled water and the plant sample of *Alpinia purpurata* to undergo evaporation and condensation in a shorter period of time. The kinetic energy of liquid molecules is directly proportional to the temperature of the sample. Evaporation takes longer at lower temperatures due to a lower kinetic energy of liquid molecules.

Table 5 summarizes the results of the FTIR spectrum analysis of the hydrosol produced by hydro distillation at various distillation times and heating temperatures. O-H, N-H, C-H, C-C, and C=C stretching were identified in hydrosol produced at 60°C for 2 hours. Therefore, it contains of alcohol, aliphatic primary amine, secondary amine, alkyne, alkene, conjugated alkene, amine and cyclic alkene. However, when the distillation time is increased to 2.5 hours, the C=C stretching peak was absent. Meanwhile, the stretching that present at 60°C for 2 hours is weak, hence it is easily get denatured due to continuous exposure to the same temperature for 2.5 hours. Other than that, hydrosol produced at heating temperature of 80°C for 1 hour consists of O-H, N-H, C-H, C≡C, C=C, C-N and C-O stretching while the hydrosol collected at 2.5 hours does not have C≡C, C-N and

C-O stretching. Based on the FTIR results obtained for 100°C of distillation time of 1 and 1.5 hours, it shows the presence of three peaks and it reduces to two peaks for 2 and 2.5 hours respectively. Therefore, the longer the distillation time, the lesser the peaks that produced.

Based on the obtained results, hydrosol produced at distillation time of 1 hour at 80°C consists more peaks compared to hydrosol produced at 100°C. Hydrosol collected at 80°C consist of alcohol, aliphatic primary amine, secondary amine, alkyne, alkene, conjugated alkene, amine, cyclic alkene and ester. Other than that, the hydrosol collected at 60°C for 2 hours has four peaks while at 80°C and 100°C have three and two peaks respectively. When the heating temperature exceeds the optimum temperature of the extraction, it can cause activity of denaturation to take place. At higher temperature, some of the substances in hydrosol decomposed and it affects the quality of the hydrosol. Therefore, the higher the temperature of heating, the more the substances denatured in the hydrosol.

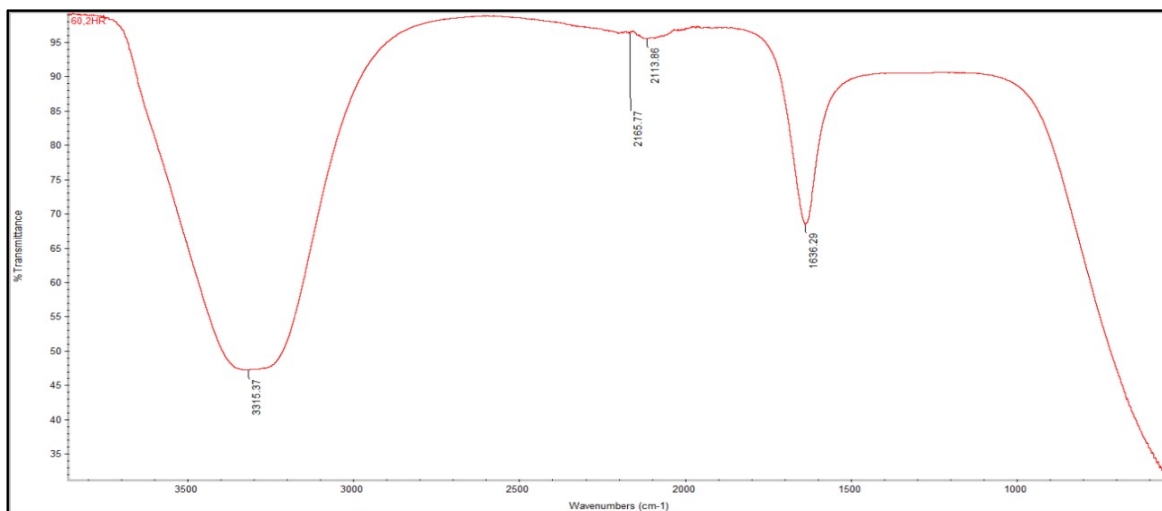


Figure 14. FTIR spectra showing % transmission against wavenumber for hydrosol at 60°C for 2 hours

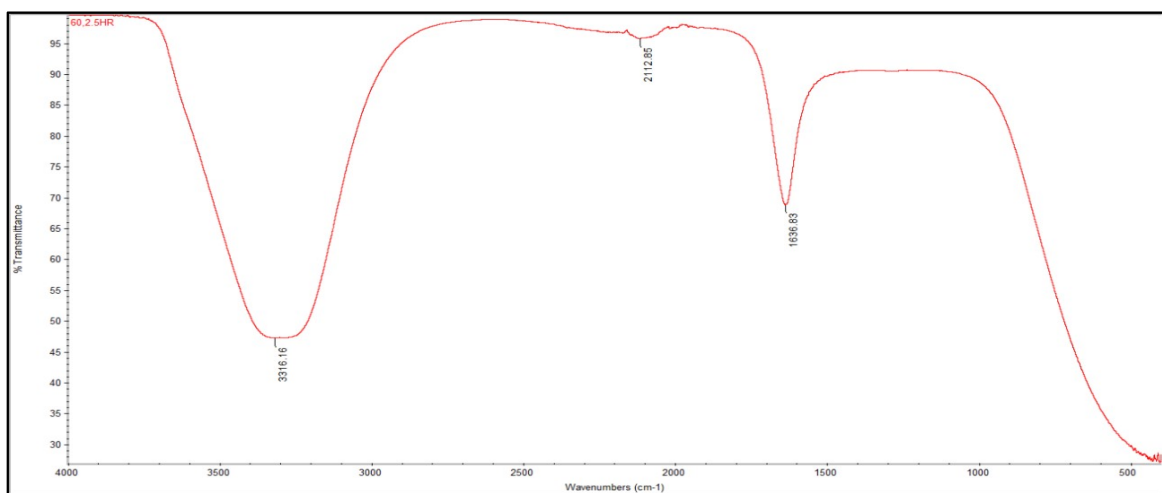


Figure 15. FTIR Spectra showing % transmission against wavenumber for hydrosol at 60°C for 2.5 hours

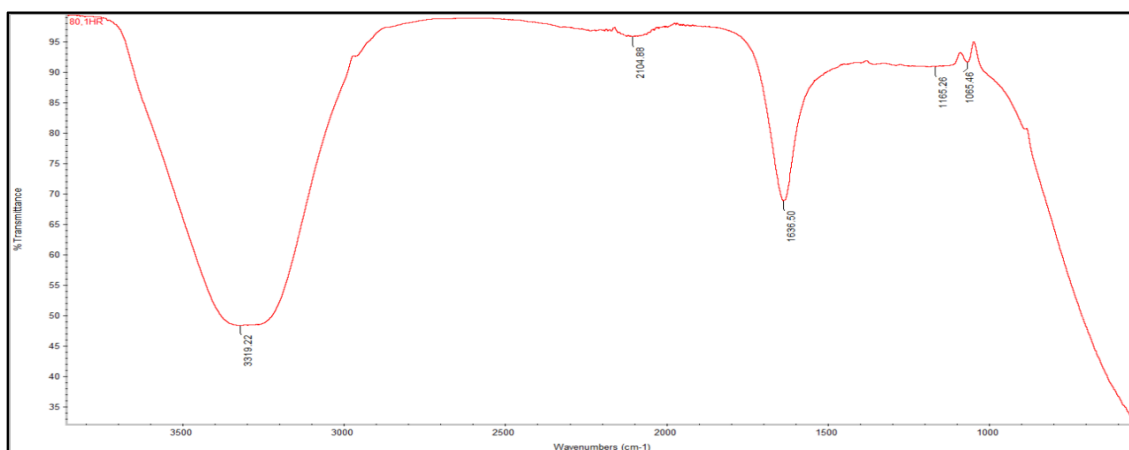


Figure 16. FTIR Spectra showing % transmission against wavenumbers for hydrosol at 80°C for 1 hour

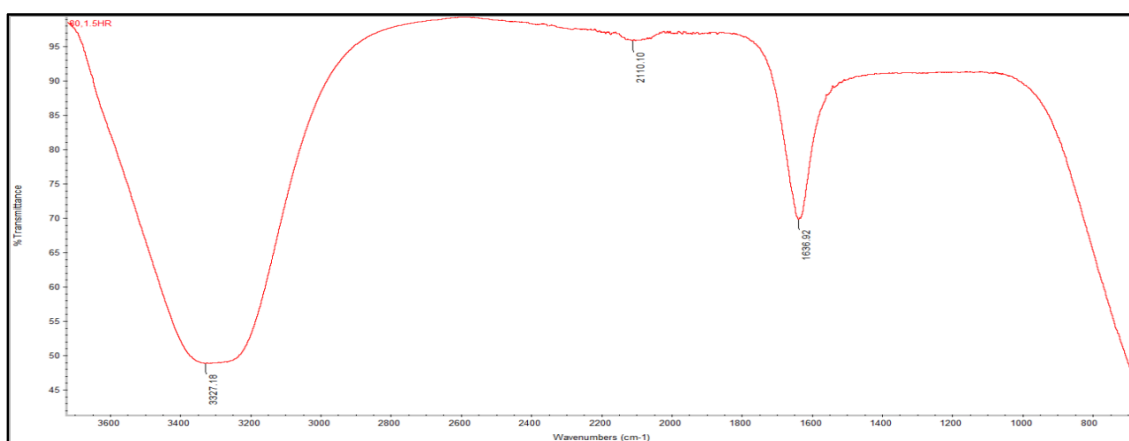


Figure 17. FTIR Spectra showing % transmission against wavenumbers for hydrosol at 80°C for 1.5 hours

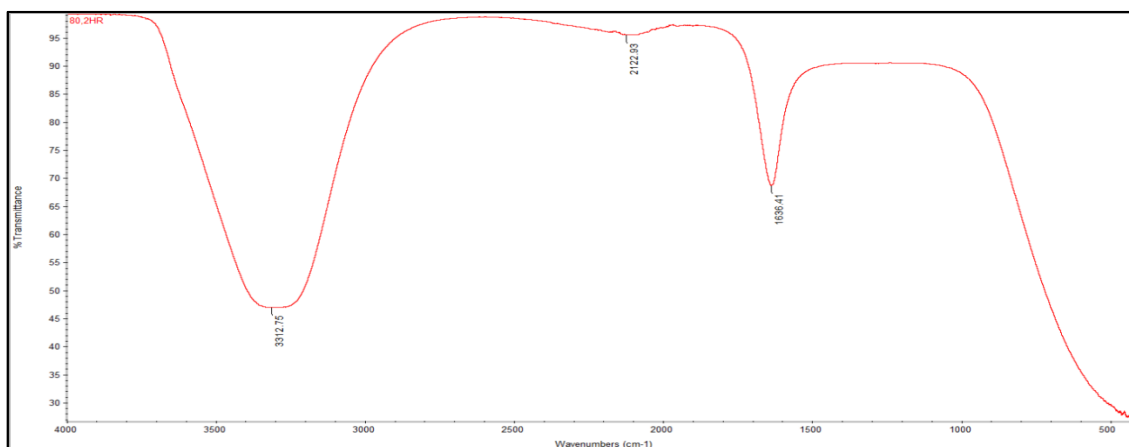


Figure 18. FTIR Spectra showing % transmission against wavenumber for hydrosol at 80°C for 2 hours

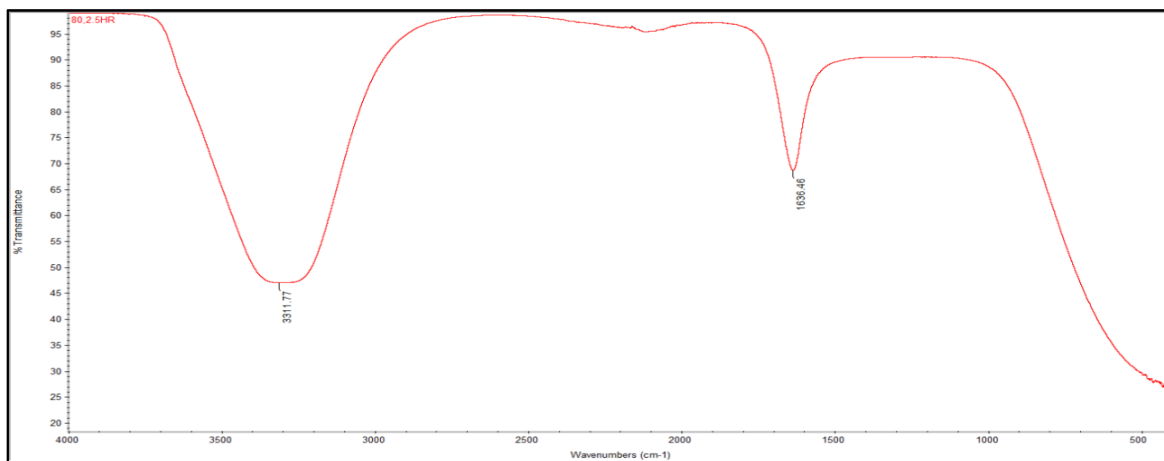


Figure 19. FTIR Spectra showing % transmission against wavenumber for hydrosol at 80°C for 2.5 hours

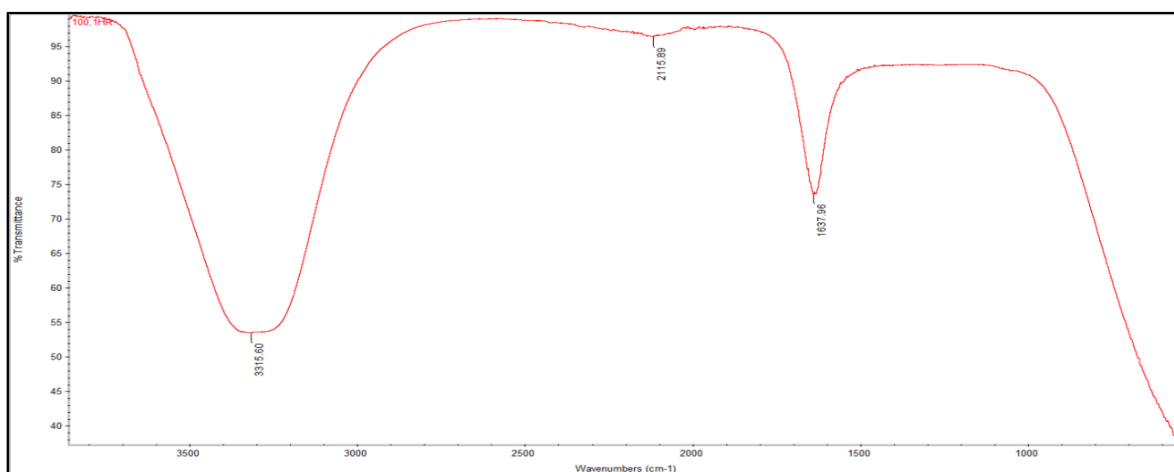


Figure 20. FTIR Spectra showing % transmission against wavenumbers for hydrosol at 100°C for 1 hour

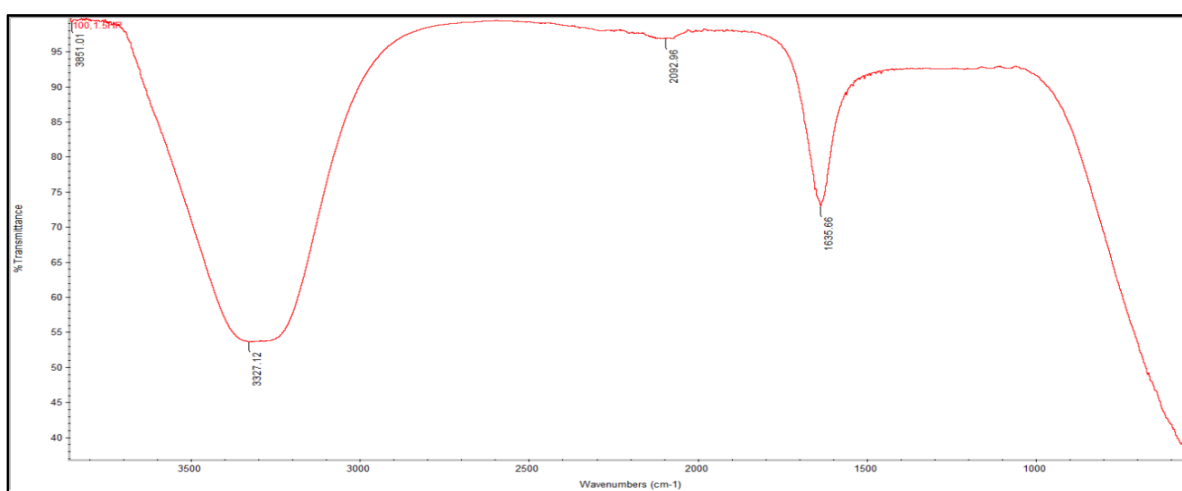


Figure 21. FTIR Spectra showing % transmission against wavenumbers for hydrosol at 100°C for 1.5 hours

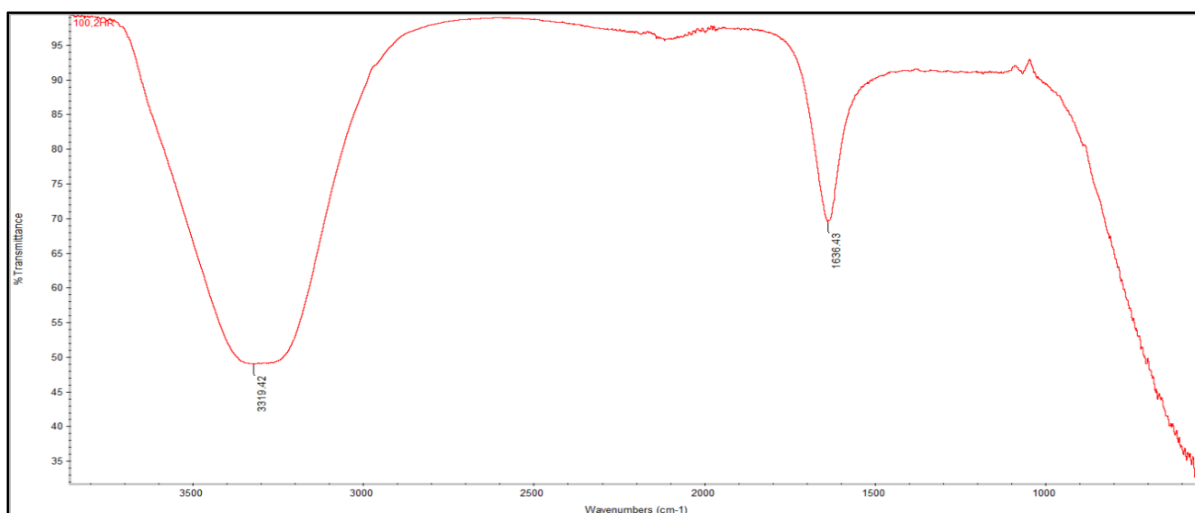


Figure 22. FTIR Spectra showing % transmission against wavenumbers for hydrosol at 100°C for 2 hours

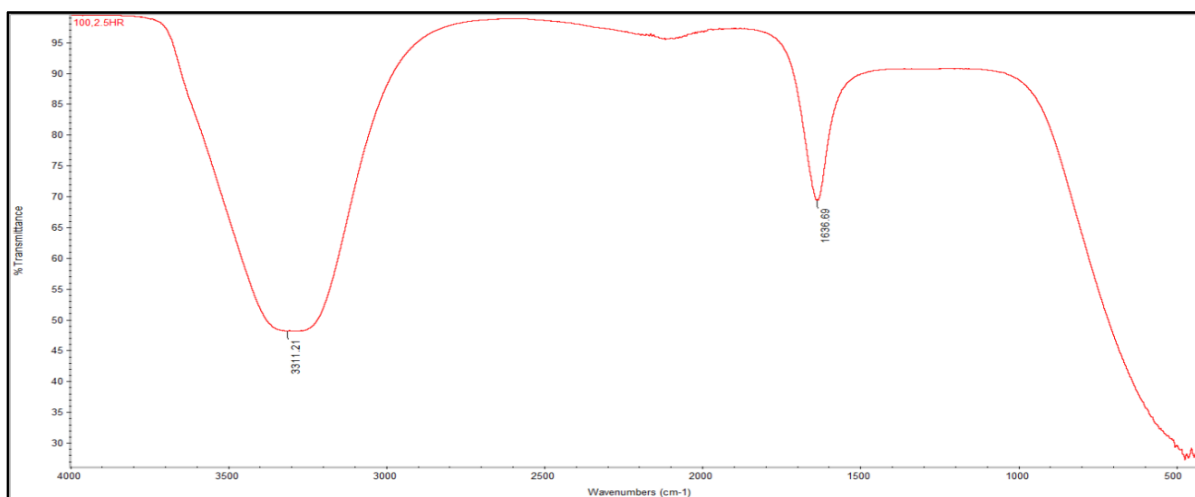


Figure 23. FTIR Spectra showing % transmission against wavenumbers for hydrosol at 100°C for 2.5 hours

Table 5. Functional groups of hydrosol for each peak of *Alpinia Purpurata* from FTIR Spectra at 60°C, 80°C and 100°C.

Heating temperature °C	Distillation time (hour)	Wavenumber (cm ⁻¹)	Group	Compound Class
60	2	3315.37	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne
		2165.77	C≡C stretching	Alkyne
		2113.86	C≡C stretching	Alkyne
	2.5	1636.29	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene
		3316.16	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne
		2112.85	C≡C stretching	Alkyne
		1636.83	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene

80	1	3319.22	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne	
		2104.88	C≡C stretching	Alkyne	
		1636.50	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene	
		1165.26	C-N stretching, C-O stretching	Amine, ester, tertiary alcohol	
		1065.46	C-O stretching, C-N stretching	Alcohol, amine	
	1.5	3327.18	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne	
		2110.10	C≡C stretching	Alkyne	
		1636.92	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene	
	2	3312.75	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne	
		2122.93	C≡C stretching	Alkyne	
		1636.41	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene	
	2.5	3311.77	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne	
		1636.46	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene	
	100	1	3315.60	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne
			2115.89	C≡C stretching	Alkyne
1637.96			C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene	
1.5		3327.12	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne	
		2092.96	C≡C stretching	Alkyne	
2		1635.66	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene	
		3319.42	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne	
2.5		1636.43	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene	
		3311.21	O-H stretching, N-H stretching, C-H stretching	Alcohol, aliphatic primary amine, secondary amine, carboxylic acid, alkyne	
		1636.69	C=C stretching, N-H bending	Alkene, conjugated alkene, amine, cyclic alkene	

* Functional groups and compound classes were identified based on the peak from IR Spectroscopy

Table (IR Spectrum, 2019).

5. Conclusion

In this research, the chemical constituents and functional groups of the hydrosol of *Alpinia purpurata* produced at 60°C, 80°C and 100°C for 1, 1.5, 2 and 2.5 hours were analyzed using GCMS and FTIR respectively. Octadecanoic acid (15.28 %), tetrasiloxane, decamethyl (17.08 %), and hexacosanoic acid (11.67 %) are the major components of hydrosol synthesized at 80°C for 1 hour. The peak area of octadecanoic acid reduces to 10.18% when the distillation time is increased to 2.5 hours while hexacosanoic acid started to disappear when the distillation time is increased to 1.5 hours. Besides that, when the temperature is increased to 100°C and 2.5 hours of distillation time, both acetic acid and octadecanoic acid are absent due to the denaturation of components at higher temperatures. Hence, the major components at 100°C and 2.5 hours of distillation time is 1-Dodecanamine with a peak area of 40.08%. Moreover, the chemical constituents obtained at every temperature and distillation time are qualitatively and quantitatively different.

The functional groups that identified in the *Alpinia purpurata*'s leaves hydrosol at every heating temperature and distillation times were different. Hydrosol collected at 80°C for 1 hour consists of O-H, N-H, C-H, C≡C, C=C, C-N and C-O stretching which indicates the presence of alcohol, aliphatic primary amine, secondary amine, alkyne, alkene, conjugated alkene, amine, cyclic alkene and ester. When the temperature is increased to 100°C, the decomposition of functional groups occurs thus C-N and C-O stretching is absent. When the distillation time is increased to 2.5 hours, there is only two peaks produced which are 3311.77 and 1636.46 cm⁻¹ compared to 80°C at 1 hour consists of five peaks. Therefore, the functional groups of *Alpinia purpurata*'s hydrosol is greatly influenced by the heating temperatures and distillation time. Hence, it is very crucial to determine the optimum heating temperature and distillation time to obtain high quality of hydrosol.

Acknowledgements

The researchers are grateful to Universiti Malaysia Pahang for funding this work under grant number RDU190398.

References

- Hamed, A., Moheimani, M. S., Sakhteman, A., Etemadfar, H., & Moein, M. (2017). An Overview on Indications and Chemical Composition of Aromatic Waters (Hydrosols) as Functional Beverages in Persian Nutrition Culture and Folk Medicine for Hyperlipidemia and Cardiovascular Conditions. *Journal of Evidence-Based Complementary and Alternative Medicine*, 22(4), 544-561. doi: <https://doi.org/10.1177/2156587216686460>
- Al-tameme, H., M. H., & Hameed, I. (2015). Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. 7(10), 238-252. doi: <https://doi.org/10.5897/JPP2015.0361>
- Chan, E., & Wong, S. (2015). Phytochemistry and pharmacology of ornamental gingers, *Hedychium coronarium* and *Alpinia purpurata*: A review. *Journal of Integrative Medicine*, 13(6), 368-379. doi: [https://doi.org/10.1016/S2095-4964\(15\)60208-4](https://doi.org/10.1016/S2095-4964(15)60208-4)
- D'Amato, S., Serio, A., López, C. C., & Paparella, A. (2018). Hydrosols: Biological activity and potential as antimicrobials for food applications. *Food Control*, 86, 126-137. doi: <https://doi.org/10.1016/j.foodcont.2017.10.030>
- Do, D. N., Pham, N. T., T. D., Bach, L., Nguyen, D., Q. T., & Nhan, L. T. (2019). Kinetic Modeling of Hydrodistillation of Essential Oil Production from Orange (*Citrus sinensis*) Peels on Pilot Scale. *Asian Journal of Chemistry*, 31(10), 2298-2302. doi: <https://doi.org/10.14233/ajchem.2019.22145>
- Elham, E. (2020). How to spot a fake hydrosol. Retrieved from <https://formulabotanica.com/how-spot-fake-hydrosol>.
- Falowo, A. B., Muchenje, V., Hugo, A., Aiyegoro, O., & Fayemi, P. (2017). Actividades antioxidantes de extractos de hoja de *Moringa oleifera* L. y *Bidens pilosa* L. y sus efectos en la estabilidad oxidativa de ternera cruda picada durante el almacenamiento en frío. *CYTA. Journal of Food*, 15(2), 249-256. doi: <https://doi.org/10.1080/19476337.2016.1243587>
- George, M., & Britto, S. (2016). Phytochemical, Antioxidant and Antibacterial Studies on The Leaf Extracts of *Curcuma Amada* Roxb. 8(2).
- IR Spectrum. (2019). Retrieved from Sigma-Aldrich: <https://www.sigmaaldrich.com/technicaldocuments/articles/biology/ir-spectrum-table.html#ir-table-bY>

- Selvamuthukumar, M & Shi, J. (2017). Recent advances in extraction of antioxidants from plant by-products processing industries. *Food Quality and Safety*, 1(1), 61-81. doi: <https://doi.org/10.1093/fqs/fyx004>
- Nicolet, T., & All, C. (2011). Introduction to Fourier Transform Infrared Spectrometry. Retrieved from <https://www.chem.uci.edu/~dmitryf/manuals/Fundamentals/FTIR%20principles.pdf>
- Oirere, E. K., Anusooriya, P., Raj, C. A., & Gopalakrishnan, V. K. (2015). Phytochemical analysis of n-hexane leaf extract of *Alpinia purpurata* (Vieill.) K. Schum using UV-VIS, FTIR and GC-MS. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(8), 387-389.
- Politi, M., Menghini, L., Conti, B., Bedini, S., Farina, P., Cioni, P. L., ... & De Leo, M. (2020). Reconsidering hydrosols as main products of aromatic plants manufactory: The lavender (Lavandula^x intermedia) case study in Tuscany. *Molecules*, 25(9), 2225. doi: <https://doi.org/doi:10.3390/molecules25092225>
- Ramaiah, G. B., Bathia, D. & Ramesh, K. P. (2017). Structural analysis of merino wool, pashmina and angora fibers using analytical instruments like scanning electron microscope and infra-red spectroscopy. *International Journal of Engineering Technology Science and Research*, 4(8), 112-125.
- Rao, B. (2012). Hydrosols and water-soluble essential oils of aromatic plants : Future economic products. *Indian Perfumer*, 56(3), 29-33.
- Rao, B. R. (2013). Hydrosols and Water-Soluble Essential Oils: Medicinal and Biological Properties. *Recent Progress in Medicinal Plants Essential Oils*, 1, 120-140.
- Saeidnia, S., Manayi, A., & Gohari, A. (2014, February). The Story of Beta-Sitosterol- A Review. doi: <https://doi.org/10.9734/EJMP/2014/7764>
- Sivasothy, Y., Chong, W., Hamid, A., I. E., Sulaiman, S., & Awang, K. (2011). Essential oils of *Zingiber officinale* var. *rubrum* Theilade and their antibacterial activities. *Food Chemistry*, 124(2), 514-517. doi: <https://doi.org/10.1016/j.foodchem.2010.06.062>
- Smith, G., & Richards, S. (2019). Lavender Essential Oil vs Lavender Hydrosol. Retrieved from <https://www.lavenderwind.com/2019/lavender-essential-oil-vs-lavender-hydrosol>.
- Suzery, M., Yuyun, R., Ria, S., & Cahyono, & B. (2019). Alkaloids piperine in dichloromethane fraction of red galangal rizhome (*Alpinia purpurata*) Alkaloids piperine in dichloromethane fraction of red galangal rizhome (*Alpinia purpurata*). doi: <https://doi.org/10.1088/1757-899X/509/1/012076>.
- Tongnuanchan, P., & Benjakul, & S. (2014). Essential Oils: Extraction, Bioactivities, and Their Uses for Food Preservation. *Journal of Food Science*, 79(7). doi: <https://doi.org/10.1111/1750-3841.12492>
- Victorio, C. (2011). Therapeutic value of the genus *Alpina*, Zingiberaceae. *Brazilian Journal of Pharmacognosy*, 21(1), 194-201. doi: <https://doi.org/10.1590/S0102-695X2011005000025>
- Victório, C. P., Kuster, R., & Lage, C. (2009). Production of rutin and kaempferol-3-Oglucuronide by tissue cultures of *Alpinia purpurata* (Vieill.) K. Schum. *Latin American Journal of Pharmacy*, 28(4), 613-616.
- Vogt, S., Löffler, K., Dinkelacker, A. G., Bader, B., Autenrieth, I. B., Peter, S., & Liese, J. (2019, November 10). Fourier-Transform Infrared (FTIR) Spectroscopy for Typing of Clinical *Enterobacter cloacae* Complex Isolates. *Frontiers in Microbiology*, 1-11. doi: <https://doi.org/10.3389/fmicb.2019.02582>