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Identification of the Chemical Constituents of *Curcuma caesia* (Black Turmeric) Hydrosol Extracted by Hydro-distillation Method

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Abstract. *Curcuma caesia* (black turmeric), a perennial herb that has a distinguishable bluish-black rhizome with a bitter and pungent smell and is widely used and extracted for its medicinal values. *C. caesia* extracted by hydro-distillation method produce essential oil and hydrosol. The essential oil of *C. caesia* is known for its high medicinal value, but the chemical constituent of the hydrosol is yet to be studied. Hence, this study will investigate the chemical constituent of the hydrosol of *C. caesia*'s rhizome extracted by hydro-distillation to comprehend the benefits and usages of the hydrosol produced for further research in pharmaceutical and natural products industries. Besides, hydro-distillation is carried out in different temperatures to study the effect of temperature on the active compounds in the hydrosol. Hydro-distillation of powdered rhizome is use to obtain the hydrosol of *C. caesia* at the temperature of 60℃, 80℃ and 100℃ before being separated by using a rotary evaporator. The sample is then analyze by using Gas chromatography–mass spectrometry (GC-MS) and Fourier transform infrared (FTIR). From FTIR analysis, the functional groups found in the hydrosol were OH, C=C and -NH groups. As the temperature increased, more components were decomposed. Hence, lesser functional groups were found in the hydrosol at 100℃ as compared to 60℃. Chemical constituents of the hydrosol of *C. caesia* were identified by GC-MS analysis, with camphor (0.57%) as the only major component at 100℃. Comparing the chemical constituents of the hydrosol at 60℃ and 80℃, the elevated temperature of hydro-distillation caused decomposition of the chemical constituents of the hydrosol due to changes of properties. The chemical constituents of the hydrosol of *C. caesia* were significantly different from the essential oil qualitatively and quantitatively, with the medicinal value of the hydrosol was uncertain due to the trace amount of camphor and other chemical constituents possessed in the hydrosol.

1. Introduction

Curcuma caesia (black turmeric) is a perennial herb which belongs to the family of Zingiberaceae, commonly found in north-east and central of India [1]. *C. caesia* is an endangered species which consists of high medical values and cosmetic applications, is economically vital to the native people as income [2]. *C. caesia* has a bluish-black rhizome with a bitter taste and pungent smell and is widely used in treating haemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrual disorder, anthelmintic, aphrodisiac, inflammation, and more [1]. Owing to its high medical and aromatic properties, it is also widely cultivated as a medicinal plant in many Southeast Asian countries [3].

C. caesia's therapeutic activities including antioxidant, antibacterial antipyretic, larvicidal, insecticidal, antimicrobial, wound healing, and anti-hyperglycemic were carried out by extracting the rhizome of *C. caesia*. The extracts were studied based on the chemical compositions of extractions [4]. The essential oil from different plants possesses significant biological properties. Hence, there have been several ongoing types of research to study the bioactive compounds present in the essential oils [5].

Hydro-distillation has been applied to extract the rhizome of *C. caesia* to study the properties of the essential oils [2]. The previous study of the usage of the hydrosol showed that it will lead to a waste as the hydrosol being discarded as a by-product as the chemical constituents, and the usage of the hydrosol remains unknown. By taking the example in the current aromatherapy industry, in the production of the essential oil, hydrosols are always discarded as a by-product in the distilling process, which is wastage for the plants as a single distillation process produces 10% of essential oil and 90% of hydrosol. Even though essential oil and hydrosol are produced in the same process, they are comprised of different components [7]. Since *C. caesia* is an endangered species, it could be an excellent way to maximise the utilisation of *C. caesia*. It can be done by analysing the chemical constituents of *C. caesia*'s rhizome hydrosol, in order to study further the biological properties and the medical usage of the hydrosol.

Hence, the chemical constituents in the hydrosol of the rhizome of *C. caesia* are identified by using GC-MS, and FTIR analysis and the effect of the temperature of the hydro-distillation process on the active compounds of the hydrosol are investigated.

2. Materials and method

2.1 Materials

Fresh rhizomes of *C. caesia* were collected from the supplier and left to dry for seven days at room temperature. The dried rhizomes were powdered using electric commercial herb grinder Swing powder machine Grain crusher 2500G Y and stored for extraction in a closed container.

2.2 Hydro-distillation

Hydro-distillation was carried out using a Soxhlet extractor Five grams of powdered sample was placed in a porous thimble which was then placed in a cylinder (Extractor). After that, 300 mL of distilled water which will act as the solvent was poured into a round bottom flask that was connected with the extractor and a condenser. The solvent was heated at 100°C by using heating device for two hours. The distilled water vaporised through the side tube into the condenser, where the vapours condensed and fell into the thimble, thus extracting all the volatile compounds from the powdered rhizome. As the level of the solvent and the extracted volatile compounds in the extractor and siphon rose, the siphon sucked up all the solvent from the thimble to the round bottom flask, and this process was continued for few cycles. The extract collected from hydro-distillation consisting the essential oil and hydrosol was separated using a rotary evaporator for 1 hour with the temperature set at 50°C. The processes were repeated with hydro-distillation process at 60°C and 80°C [8].

2.3 FTIR Analysis

The functional group of the hydrosol samples extracted at 60, 80 and 100℃ were analysed by using FTIR. One drop of each hydrosol samples was placed on the universal attenuated total reflectance (ATR) accessory and was analysed between wavenumbers of 4000 – 400 cm^{-1} using FTIR spectrometer (IS50, Thermo scientific, USA).

2.4 GC-MS Analysis

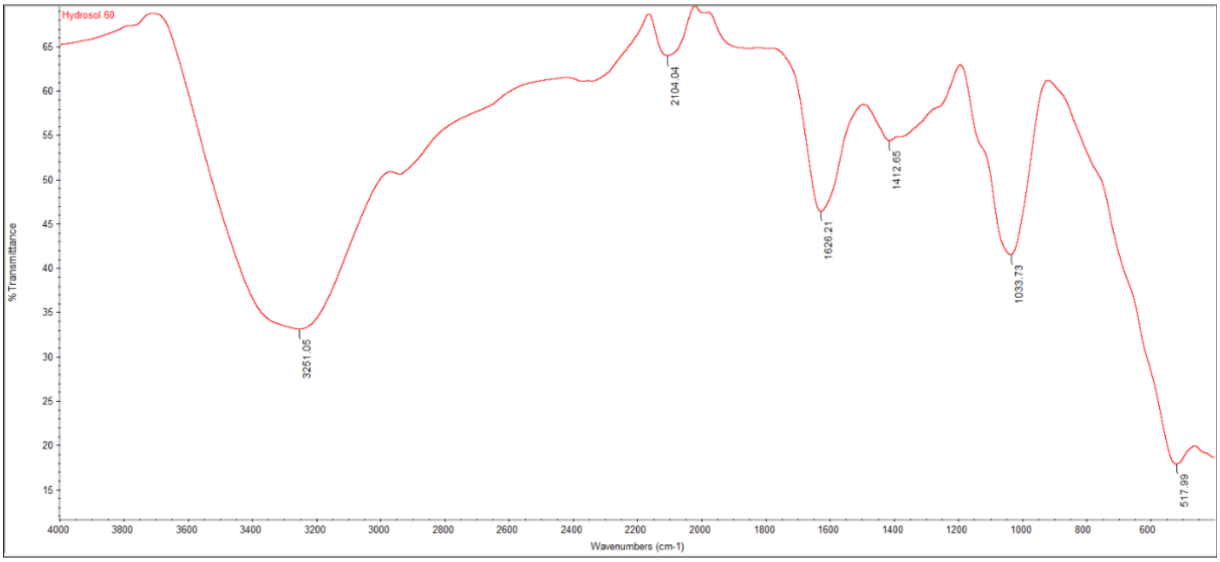
The chemical constituents of the hydrosol samples extracted at 60, 80 and 100℃ were analysed by using GC-MS method. Propanol was used as the solvent and was mixed with the samples at the ratio of 9:1 (Propanol:Sample). GC-MS analysis of the samples was done by using Gas Chromatograph 7890A

(Agilent) coupled with 5975-MS System, fitted with a Carbowax-20M capillary column, 50 m × 0.22 mm with a film thickness 0.25 µm. Helium was used as the carrier gas at a flow rate of 4.0 mL/min. 1 µl sample was injected in the split mode as stated in a study by Pandey et.al [9].

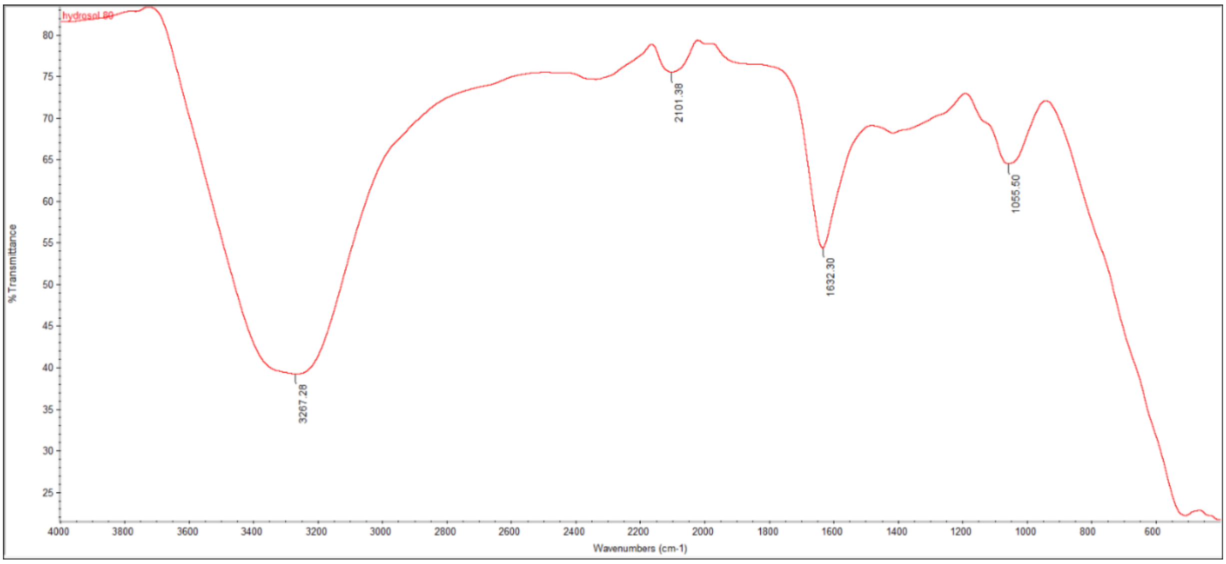
3. Results and discussion

3.1 FTIR Analysis

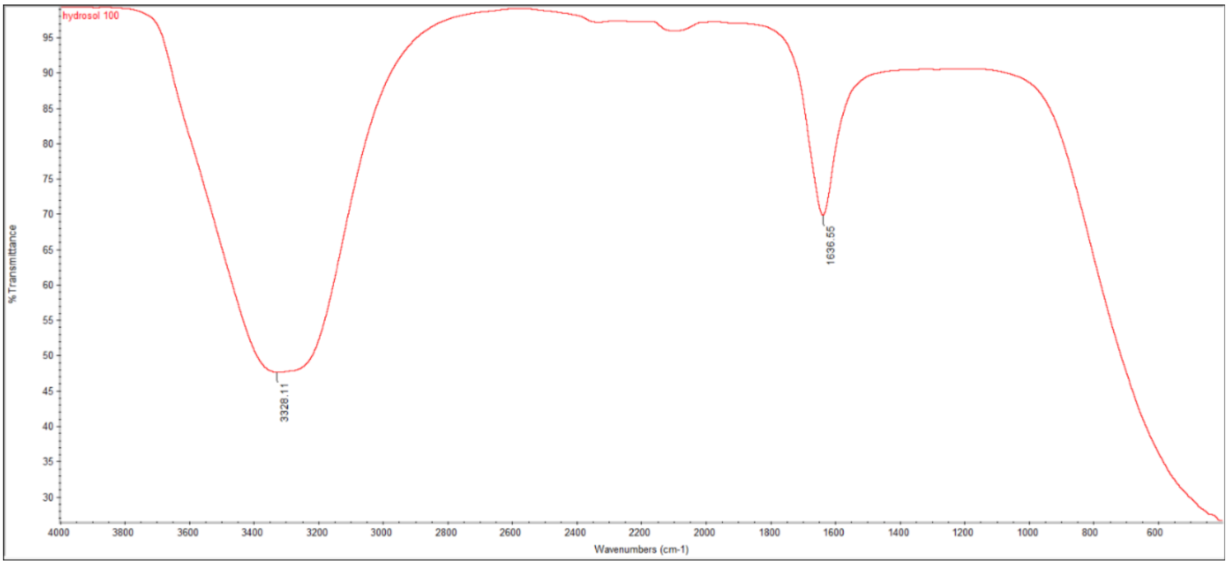
The analysis of the FTIR spectrum of the hydrosol of *C. caesia* produced from hydro-distillation at 60℃ as shown in Figure 1(a) showed the presence of OH, C ≡ C, C = C, C - C, -NH, CO, CN and C-Br groups. Hence, the hydrosol has compounds that are water, alcohol, carboxylic acid, amide, alkyne, alkene, amine, aromatic, ester, ether, aliphatic amines and alkyl halide from the properties obtain in FTIR. However, when the temperature increased to 80℃, the graph which is shown on Figure 1(b) showed that the O-H group at peak 1413 and peak 518 were present but insignificant, showing some alcohol, carboxylic acid and alkyl halide compounds were reduce due to high temperature. As the temperature increased to 100℃ as shown in Figure 1(c), more groups were absent, only O-H group at peak 3328 and C=C and N-H groups at peak 1637, which showed only water, alcohol, carboxylic acid, alkene, amide and amine compounds were present in the hydrosol from hydro-distillation at 100℃. The comparison of the hydro-distillation at 60℃, 80℃ and 100℃ was shown in Figure 2. Hence, based on the comparison, it can be concluded that as the temperature of the heating process in hydro-distillation increased, more compounds in the extract will be destroyed.



(a)



(b)



(c)

Figure 1. FTIR Spectra showing % transmission against wavenumbers for hydrosol at (a) 60℃ (b) 80℃ (c) 100℃.

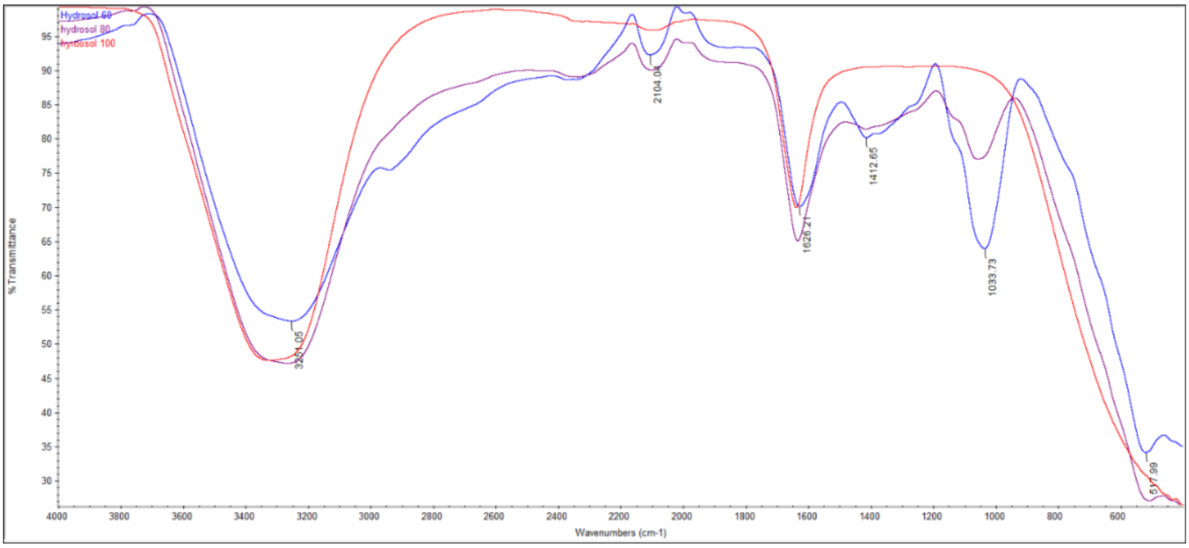


Figure 2. FTIR Spectra comparison for hydrosol at 60, 80 and 100℃.

3.2 GC-MS Analysis Results

Based on the analysis from GC-MS analysis, the chemical constituents of the hydrosol of *C. caesia* produced from hydro-distillation at 60, 80 and 100°C were determined. The constituents identified for hydro-distillation at 60, 80 and 100°C were listed in Table 1. For the hydrosol produced from hydro-distillation at 60°C, the major components identified were oleic acid with the peak of (12.66%), n-hexadecanoic acid (8.45%), eicosanoic acid (0.44%), camphor (0.43%) and N-[4-bromo-n-butyl-2-Piperidinone (0.43%). Following the increased of temperature to 80℃, the peak area of oleic acid and n-hexadecanoic acid dropped to 0.03% and 0.13% respectively, making the major components of hydrosol of *C. caesia* produced at 80°C were camphor (0.57%) and n-hexadecanoic acid (0.13%). Other components that were found in hydrosol at 60◦C such as N-[4-bromo-n-butyl-2-piperidinone, eicosanoic acid, (Z)-9-octadecenal, 1,5-Dimethyl-7-oxabicyclo[4.1.0] -heptane and docosanoic acid were also absent due to the increased of temperature making the properties of the hydrosol to change. Further increasing of temperature to 100°C, the peak area of n-hexadecanoic acid was further dropped to 0.02%. Hence, the major components of the hydrosol of *C. caesia* produced at 100℃ was camphor (0.57%), and the other trace components were similar with 80℃, which were 1,3,5-trimethyl-benzene (0.02%), 1-tridecene (0.02%), 2,4-bis(1,1-dimethylethyl)-phenol (0.03%), (E)-5-octadecene (0.01%), n-hexadecanoic acid (0.02%) and oleic acid (0.02%). It can be concluded that the increased temperature of hydro-distillation will affect the chemical constituents of the hydrosol where some of the chemical constituents found will be decomposed.

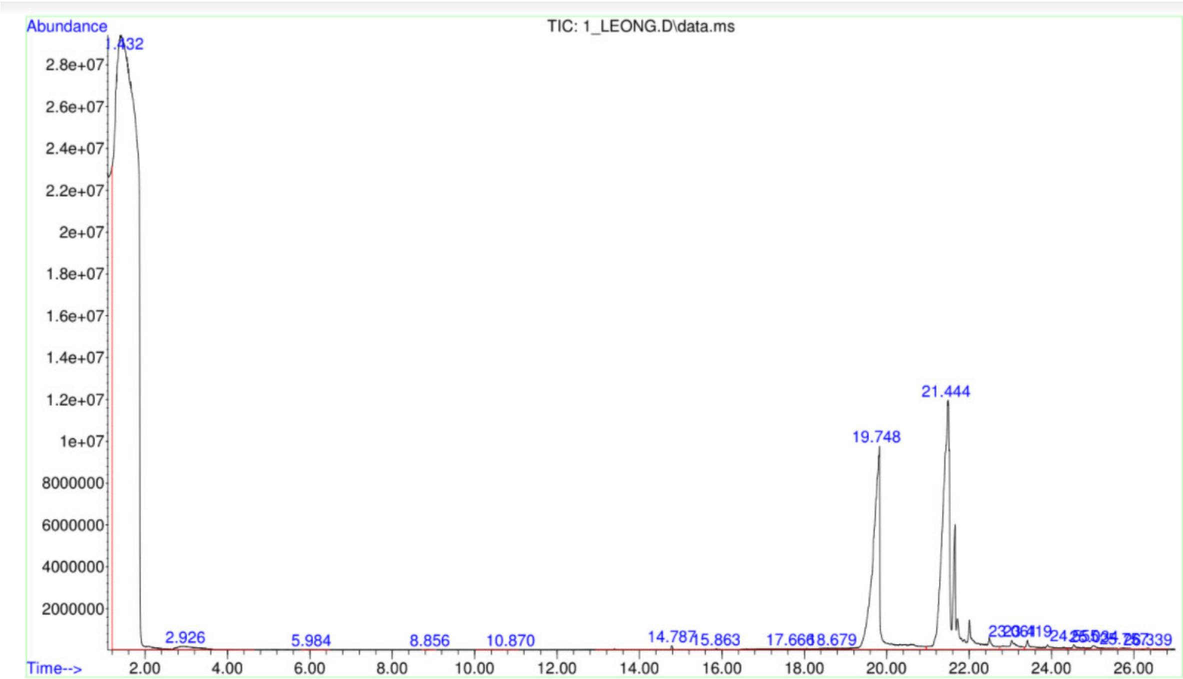


Figure 3. Peaks formed from GC-MS analysis for hydrosol at 60°C.

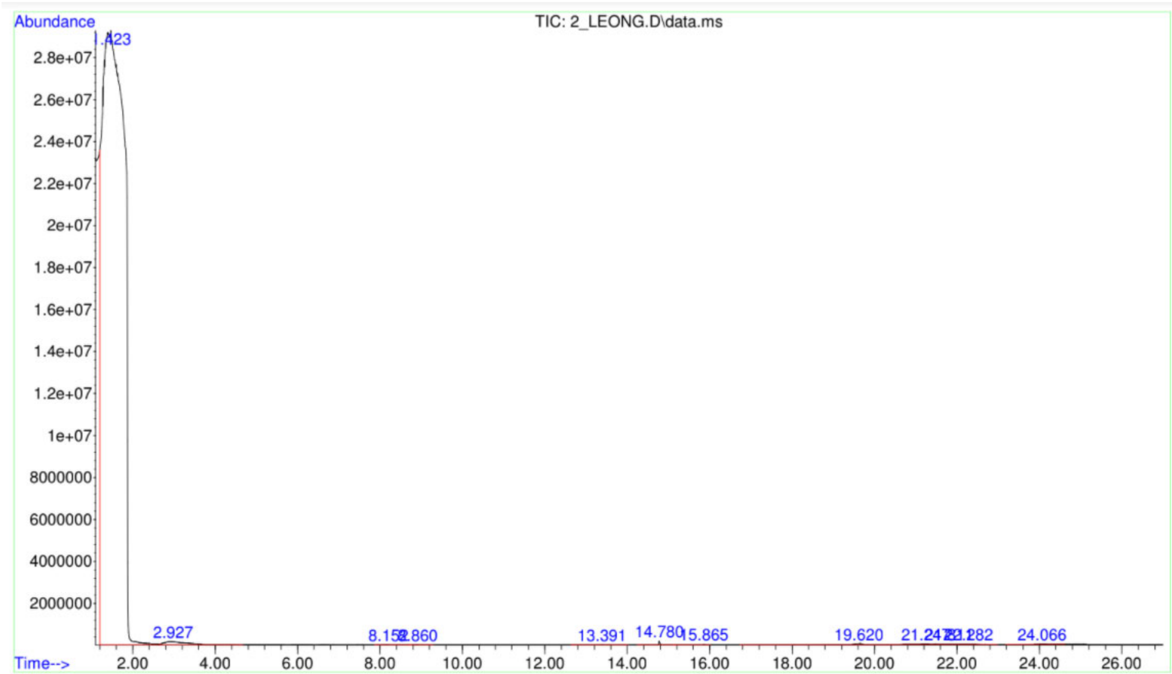


Figure 4. Peaks formed from GC-MS analysis for hydrosol at 80°C.

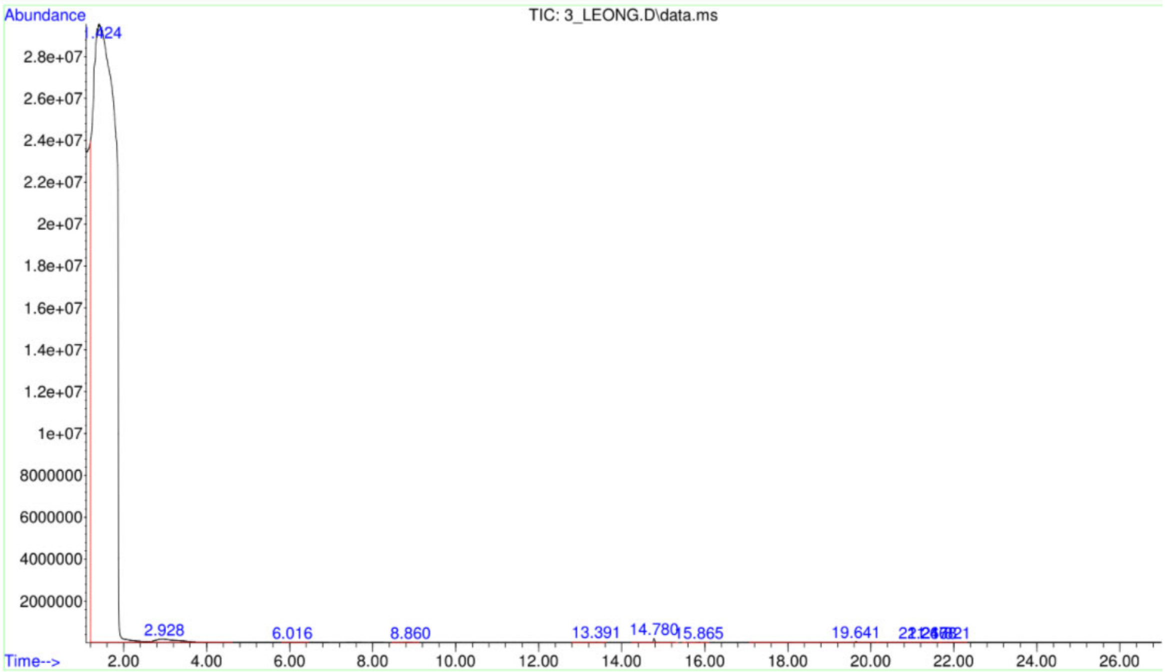


Figure 5. Peaks formed from GC-MS analysis for hydrosol at 100°C.

Table 1. Chemical composition of the hydrosol of *C. caesia* from hydro-distillation at 60, 80 and 100°C.

No	Compound	Retention time			Peak Area (%)		
		60°C	80°C	100°C	60°C	80°C	100°C
1	Camphor	2.924	2.930	2.930	0.43	0.57	0.57
2	1,3,5-trimethyl-benzene	5,987	8.863	6.017	0.02	0.01	0.02
3	1,2,3,5-tetramethyl-benzene,	8.857	-	-	0.01	-	-
4	2,4-bis(1,1-dimethylethyl)-phenol	14.790	14.778	14.778	0.05	0.04	0.03
5	(E)-5-Octadecene	15.866	15.866	15.866	0.06	0.06	0.01
6	n-Hexadecanoic acid	19.751	19.618	19.642	8.45	0.13	0.02
7	Oleic acid	21.442	21.811	21.267	12.66	0.03	0.02
8	N-[4-bromo-n-butyl-2-Piperidinone	23.062	-	-	0.43	-	-
9	Eicosanoic acid	23.418	-	-	0.44	-	-
10	(Z)-9-Octadecenal	24.554	-	-	0.15	-	-
11	1,5-dimethyl-7-Oxabicyclo[4.1.0]heptane	25.756	-	-	0.05	-	-
12	Docosanoic acid	26.342	-	-	0.02	-	-
13	1-Tridecene		13.388	13.388		0.01	0.01

4. Conclusion

In this study, the functional groups and chemical constituents of the hydrosol of *C. caesia* produced from hydro-distillation at 60, 80 and 100°C were determined by FTIR and GC-MS analysis respectively. The functional groups identified for hydrosol of hydro-distillation at 60°C were O-H, CΞC, C=C, C-C, N-H, C-O, C-N and C-Br groups. However, due to the decomposition occurred as temperature increased, only O-H, C=C and N-H groups were present for hydrosol of hydro-distillation at 100°C, which means the hydrosol on consists of water, alcohol, carboxylic acid, alkene, amine and amide compounds. GC-MS analysis was further performed to identify the chemical constituents of the hydrosol of *C. caesia* from hydro-distillation at 60, 80 and 100°C. The major components of the hydrosol produced from hydro-distillation at 60°C, were oleic acid with peak of (12.66%), n-hexadecanoic acid (8.45%), eicosanoic acid (0.44%), camphor (0.43%)

and N-[4-bromo-n-butyl-2-Piperidinone (0.43%). The major components hydrosol produced from hydro-distillation at 80℃ were camphor (0.57%) and n-hexadecanoic acid (0.13%). Lastly for hydrosol produced from hydro-distillation at 100℃ was camphor (0.57%). The analysis showed that the increased temperature of hydro-distillation caused the chemical constituents of the hydrosol decomposed. Besides, the chemical constituents of the hydrosol of *C. caesia* were significantly different from the essential oil qualitatively and quantitatively. Also, the chemical constituents found in hydrosol were vastly different from essential oil qualitatively and quantitatively, with the medicinal value of the hydrosol was uncertain due to the trace amount of camphor and different chemical constituents possessed in the hydrosol.

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