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Characterization of *Eurycoma longifolia* (Tongkat Ali) Essential Oils Extracted by Microwave Assisted Extraction

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ABSTRACT

The main objective of this research is to extract and characterize the essential oils of *Eurycoma longifolia* jack (Tongkat Ali) root using microwave assisted extraction (MAE) The effect of various process parameters such as extraction time (5 min, 10 min, 15 min, 20 min, 30min, and 45 min), microwave power (200W, 225W, 250W, 275W, 300W and 400W) and raw material to solvent ratio (RMTSR) (1:6, 1:8, 1:10 and 1:12) on the yield and its major constituents were investigated in this study to obtain the optimum conditions for the extraction process. The pure essential oil obtained was characterized using gas chromatography mass spectroscopy (GCMS) techniques. The findings showed that the optimal conditions for the microwave assisted extraction of *Eurycoma longifolia* are 10 minutes extraction time, 225W microwave power and 1:10 for (RMTSR) with the maximum yield percentage of 1.91% .

INTRODUCTION

In this study, the volatiles or essential oils from *Eurycoma longifolia* or more commonly known as "Tongkat Ali" are extracted using microwave assisted extraction. This plant is mostly found in South-East Asian countries such as Malaysia, Indonesia, Thailand, Myanmar, and Vietnam (Rajeev *et al.*, 2010). The root extracts are used in local traditional medicines for its exclusive antimalarial, anti-pyretic, antiulcer, cytotoxic and aphrodisiac properties (Jagananth, 2000). Microwave assisted extraction of essential oils from plant material has become one of the famous and efficient way to extract out the volatiles. Soxhlet extraction is the most conventional method and widely used in the field of extraction (Vivekananda *et al*., 2007). However, soxhlet extraction has a major drawback of having longer extraction period, requires larger amount of heat energy for its process and could be a threat to the environment as it consumes large amount of organic solvent in the evaporation and concentration of the extract (Zuloaga *et al.*, 1999). Microwave assisted extraction provides the best solution because it requires less consumption of solvent and less extraction time as the process occurs in elevated temperature (Balunkeswar et al., 2015).

Although the thorough statistic demand in international market is imprecise, the price of the dried *Eurycoma longifolia* roots are expected to be rated in between 20 to 25 US dollars/kg. Nevertheless, the water extract seemed to have a better market value with 26 US dollars per bottle of 60 capsules (Kaur *et al.*, 2003). The main objective of this study is to extract the essential oils of *Eurycoma longifolia*'s root using Microwave assisted Extraction (MAE) and to characterize the components of essential oils extracted from the roots of *Eurycoma longifolia*. This study is scoped about investigating the factors (temperature, microwave power, extraction time and solvent volume) affecting the yield and composition extracted from the microwave assisted extraction, separation of solvent and essential oil matrix using rotary evaporator and using the proper tool (GCMS) for the characterization of the components of essential oils.

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MATERIALS AND METHODS

Methanol of 98.99% was obtained from UMP Chemical Laboratory store.

The Tongkat Ali root was purchased from LCH Agriculture, Sungai Petani, Kedah, Malaysia, (the supplier did not indicate whether the root was collected from the wild or cultivated)

Experimental procedure

The Tongkat Ali root was cut into small pieces (1-2 cm) to increase the surface area of extraction. Then, the root was dried in the conventional oven to constant weight at 60-80 ºC for 2 hours in the interval of 30 minutes. The dried sample was sealed and stored under room temperature. The microwave assisted extraction was performed using Ethos Microwave Extractor. The weight of sample (*Eurycoma longifolia* roots) was kept constant at 3 grams throughout the experiment and was soaked with 10ml of methanol for 10 minutes. This was done to enhance the diffusion of the solvent into the sample and improves the mass transfer of active compounds into the solvent. Three parameters were studied in this study which were time of extraction, microwave power and raw material to solvent ratio. The time of extraction was the first parameter, the RMTSR ratio and microwave power were kept constant at 1:10 (g/ml) and 250W respectively. The time was varied at 5, 10, 15, 20, 30 and 45 minutes. The microwave power was the second parameter, the RMTSR ratio and time of extraction were kept constant at 1:10 (g/ml) and 10 minutes respectively and the microwave power was varied as 220W, 225W, 250W, 275W, 300W and 400W. Finally for raw material to solvent ratio, the extraction time and microwave power were kept constant at 10 minutes and 250W respectively. The RMTSR was varied as 1:6, 1:8, 1:10 and 1:12 (g/ml).

Separation of the mixture of solvent from extract

After the extraction process was completed, the mixture of essential oil and solvent were separated from the solid by decantation and filteration. Then, rotary evaporator was used to separate the mixture of essential oil from solvent. After the evaporation of the alcohol was completed, the pure essential oil was transferred into a sample bottle for characterization.

GC-MS Analysis

The main task for the characterization was to identify the components produced through the MAE in order to compare with the components of the Tongkat Ali root produced through other extraction techinques

The components of the essential oil were quantified using gas chromatography mass spectroscopy (GCMS) of the Agilent model. This instrument functions by separating chemical mixtures (the GC component) and identifying the compositions at a molecular extend (the MS component). It is based on the principle that a mixture will separate into individual components when heat is applied (Neoh et al., 2011). One microliter aliquot of sample was injected at 1:100 split ratio into GCMS system consisting of an Agilent 6890N Gas Chromatograph coupled with an Agilent 5973i Mass Detector and 6890 series auto sampler. The separation was performed using capillary column with helium as the carrier gas. The oven programming was held initially at temperature of 60°C and later ramped at 5°C /min to 220°C and was held isothermally at this point for 20 minutes (Secilmis Canbay and Bardakc, 2011). As the separated components emerge from the column opening, they diverge into the MS. In order to identify the chemical constituents present in the extracted essential oil, their mass spectra was either compared with those of standard compounds from the National Institute of Standard and Technology (NIST) library data from GC-MS, database (Wiley/ NBS library) or with published mass spectra (Adams., 2001). Close similarity in retention time of the compound from both techniques were observed when the components were quantified.

Statistical analysis

Analysis of variance (ANOVA) was used to test the effects of extraction time, microwave power and raw material to solvent ratio (RMTSR) on the yield of essential oil extracted. The results of the two replicates for all the analysis done were expressed as mean and ANOVA. Significance is accepted at $P<0.5$.

RESULTS AND DISCUSSION

Effect of extraction time

The percentage yield of the Tongkat Ali essential oil was calculated at a constant microwave power of 250 W, and at raw material to solvent ratio of 1:10 based on average values obtained. The yield percentage was calculated using Equation 1.

$$\text{Yield Percentage, \%} = \frac{Essential\ oil\ weight\ after\ evaporation}{Raw\ material\ weight} \times 100 \quad \text{Eq.1}$$

Figure 1 illustrates the percentage yield of essential oil extracted from *Eurycoma longifolia* (Tongkat Ali) at different extraction time at a constant microwave power of 250 W, and at raw material to solvent ratio of 1:10. From the table and graph, it can be clearly seen that the amount of yield increases as the extraction time was increased from 5 min to 10 min. The yield obtained at 5 and 10 min are 1.5%, and 1.83% respectively. Apparently microwave assisted extraction was able to produce the highest yield of 1.83% when the extraction time is 10 min. Nevertheless, as the extraction time was increased beyond 10 min, the yield can be seen to be reduced. The same observation was reported for microwave assisted extraction of pectin from waste Citrullus lanatus fruit rinds (Prakash Maran *et al.,* 2014). The initial increase in extraction yield supports the fact that the more the time the more the thermal accumulation within the solvent (methanol) due to the absorption of microwave energy enhanced the dissolution process of essential oil into the solvent (Maran *et al.,* 2013). The decrease in yield after 10 min extraction time could

however be related with the possible degradation of the plant material as the extraction time increases (Chen et al., 2007).

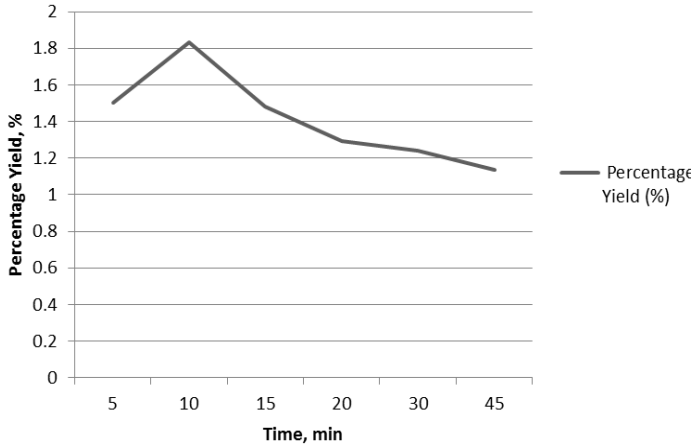

Fig. 1: Percentage Yield against Extraction Time.

Effect of microwave Power

The percentage yield of the Tongkat Ali essential oil is calculated at a constant extraction time of 10 min, and at raw material to solvent ratio of 1:10 based on average values obtained. The percentage yield was calculated using Equation 1.

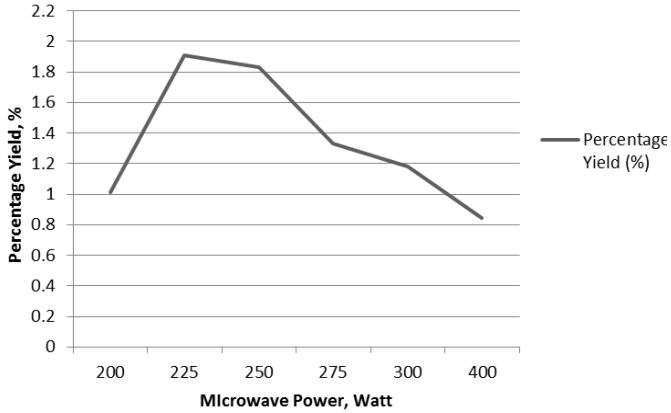

Fig. 2: Percentage Yield against Microwave Power.

Figure 2 illustrates the percentage yield of essential oil extracted from *Eurycoma longifolia* (Tongkat Ali) at different microwave power at a constant extraction time of 10 min, and at raw material to solvent ratio of 1:10. Six different microwave power levels were used in the extraction which were 200 W, 225 W, 250 W, 275 W, 300 W, 400 W. From Figure 2, it can be seen that when the microwave power increases, the percentage yield of extracted Tongkat Ali essential oil increased until 225W and started to decrease from 250W until 400W. It is observed that the highest yield of 1.91% which was obtained when Tongkat Ali essential oil extracted was conducted at 225 W for a period of 10 min. This is because higher microwave power would supply more energy to the immersed Tongkat Ali, produce rapid generation of heat, and subsequent formation of higher pressure gradient inside of the Tongkat Ali and methanol (solvent) such that the extraction is enhanced (Rezvanpanah *et al.,* 2008). However, for the higher microwave power, the yield of Tongkat Ali essential oil decreases. There is the possibility of a negative effect from higher microwave power, which might tend to overheat the product by elevating the temperature too high, leading to the compound breakdown or product damage (Ma *et al.,* 2009).

Effect of raw material to solvent ratio (RMTSR)

The Tongkat Ali essential oil was calculated at a constant extraction time of 10 min, and microwave power of 250 W based on average values obtained. The percentage yield is calculated according to Equation 1. Figure 3 illustrates the percentage yield of essential oil extracted from *Eurycoma longifolia* (Tongkat Ali) at different raw material to solvent ratio at a constant extraction time of 10 min, and microwave power of 250 W. Four different RMTSR were used in the extraction which are 1:6, 1:8, 1:10 and 1:12. From Figure 4.3 it can be clearly seen that the Tongkat Ali essential oil extraction increases as the RMTSR increases until it reached the maximum yield at 1:10 which is 1.83%. This is because the driving force during mass transfer within the solid is considered to be the concentration gradient, which is greater when the high solvent-to-solid ratio are used, resulting in an increase of the diffusion rate (Khanal et al., 2006). However at RMTSR of 1:12 the yield started to decrease because the raw material could not take up more solvent than the equilibrium point resulting with low mass transfer and diffusivity (Cacace and Mazza 2003).

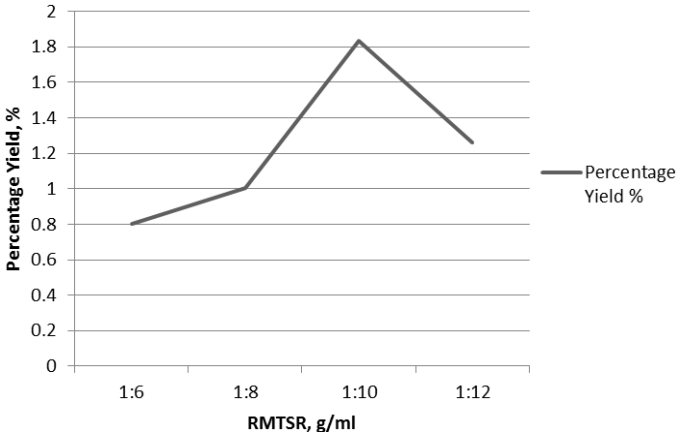

Fig. 3: Percentage Yield against RMTSR

GCMS Analysis

The Tongkat Ali essential oil obtained from the parameters of extraction time, microwave power and weight ratio were analysed using gas chromatography and mass spectroscopy in order to compare the extracted essential oil compositions with the commercial Tongkat Ali essential oil. Figure 4, Figure 5 and Figure 6 shows the peaks found in GCMS for the extraction time of 5 minutes, microwave power of 200W and raw material to solvent ratio of Tongkat Ali : MeOH at 1:6, respectively. Table 4, Table 5 and Table 6 summarises the components of essential oil

found in each of the samples for all the three parameters investigated in this study.

Table 1, 2 and 3 reveals the results and the components identified through GCMS. The components in the Tongkat Ali essential oil were detected at the retention time in the range from 1 to 20 minutes. The solvent, methanol was detected first at retention time of 1.922. There were 9 components detected in total. Generally, the components such as (3,5-Dimethoxy-4-hydroxyphenylacetic acid, Carbazole, Acetic acid, Butyrolactone and 3-methylbutanoic acid) were found in each and every samples of the parameters. The largest peak found is 3,5-Dimethoxy-4-hydroxyphenylacetic acid with retention time of 9.199. Some components that were detected at retention time of 10.133, 12.693 and 18.263 and were identified as (Nonanal, 2-Methylhexanol, 2(5H)-Furanone) were the least found components.

Statistical analysis

Based on the statistical analysis done using single factor ANOVA (analysis of variance), the p value obtained for the extracted essential oil (g) based on extraction time, microwave power and RMTSR are 0.001469, 0.000057 and 0.001549 respectively. Thus, all the results are valid and significant at $p<0.5$.

Table 1: Components Identified in Tongkat Ali Extract at Extraction Time Parameter.

No	Retention Time	Compound	Relative Peak (%) Extraction Time (min)					
			5	***10***	***15***	***20***	***30***	***45***
1	1.922	Methanol (Solvent)	20.59	47.94	29.03	18.46	9.41	15.19
2	6.612	2-Hexadecanol	~	2.40	1.68	5.10	~	0.55
3	9.199	3,5-Dimethoxy-4-hydroxyphenylacetic acid	19.38	7.59	3.24	9.88	17.76	18.16
4	9.405	Carbazole	14.87	3.69	0.81	6.46	11.76	9.69
5	10.133	Nonanal	~	~	~	11.15	~	~
6	11.803	Acetic acid	26.84	6.50	3.88	7.93	17.05	9.40
7	12.693	2-Methylhexanol	~	~	~	2.97	~	~
8	15.931	Butyrolactone	13.18	2.50	1.92	3.00	8.20	3.16
9	17.429	3-methylbutanoic acid	5.14	1.00	1.05	1.35	3.62	1.33
10	18.263	2(5H)-Furanone	~	~	13.18	~	1.10	~

(Source: Shafiqul Islam *et al.,* 2006 and Purwantiningsih *et al.,* 2011).

Table 2: Components Identified in Tongkat Ali Extract at Microwave Power Parameter.

No	Retention Time	Compound	Relative Peak (%) Microwave Power (Watt)					
			200	***225***	***250***	***275***	***300***	***400***
1	1.922	Methanol (Solvent)	51.20	39.97	47.94	38.16	38.00	52.53
2	6.612	2-Hexadecanol	~	~	2.40	~	~	~
3	9.199	3,5-Dimethoxy-4-hydroxyphenylacetic acid	6.15	6.99	7.59	16.09	3.67	7.63
4	9.405	Carbazole	3.60	6.06	3.69	8.79	3.17	5.32
5	10.133	Nonanal	~	~	~	~	1.01	~
6	11.803	Acetic acid	4.41	5.11	6.50	23.97	6.74	3.19
7	12.693	2-Methylhexanol	~	~	~	~	~	~
8	15.931	Butyrolactone	1.82	1.65	2.50	8.77	2.37	1.76
9	17.429	3-methylbutanoic acid	1.00	1.02	1.00	4.21	3.07	1.09
10	18.263	2(5H)-Furanone	~	~	~	~	~	~

(Source: Shafiqul Islam *et al.,* 2006 and Purwantiningsih *et al.,* 2011).

Table 3: Components Identified in Tongkat Ali Extract at RMTSR Parameter.

No	Retention Time	Compound	Relative Peak (%) RMTSR (g/ml)			
			1:6	***1:8***	***1:10***	***1:12***
1	1.922	Methanol (Solvent)	41.59	4.25	47.94	4.13
2	6.612	2-Hexadecanol	~	~	2.40	0.93
3	9.199	3,5-Dimethoxy-4-hydroxyphenylacetic acid	8.83	18.04	7.59	4.61
4	9.405	Carbazole	4.80	9.67	3.69	2.52
5	10.133	Nonanal	~	0.31	~	10.48
6	11.803	Acetic acid	7.98	24.56	6.50	3.58
7	12.693	2-Methylhexanol	~	~	~	32.57
8	15.931	Butyrolactone	2.86	12.99	2.50	0.96
9	17.429	3-methylbutanoic acid	1.11	6.07	1.00	0.42
10	18.263	2(5H)-Furanone	~	2.31	~	~

(Source: Shafiqul Islam *et al.,* 2006 and Purwantiningsih *et al.,* 2011).

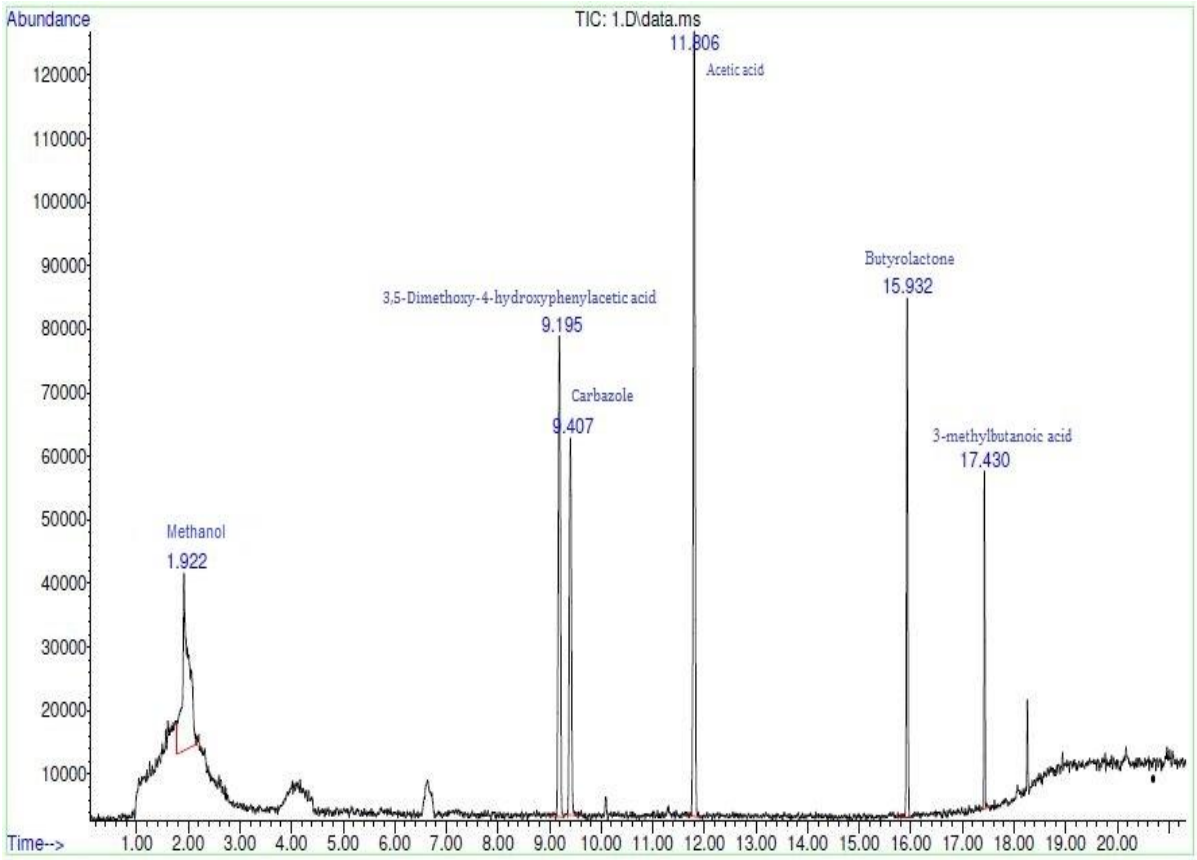

Fig. 4: GCMS of 5 minutes Extraction time.

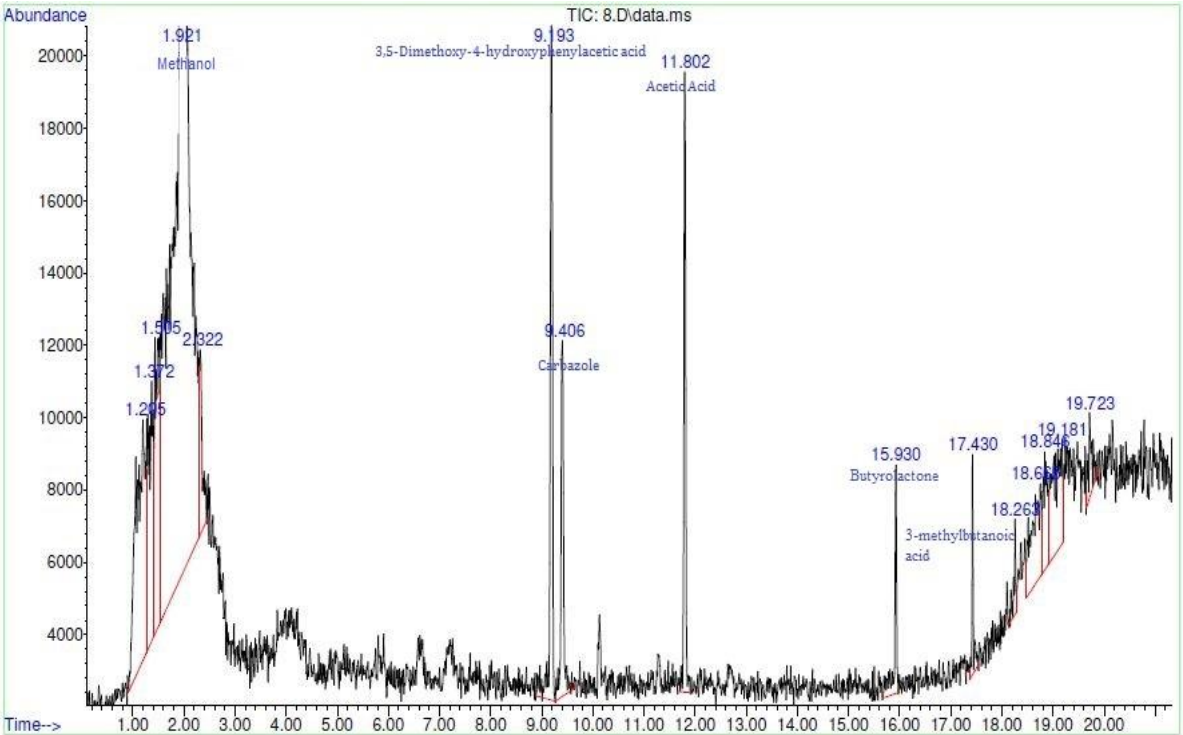

Fig. 5: GCMS of extract using Microwave Power of 200W.

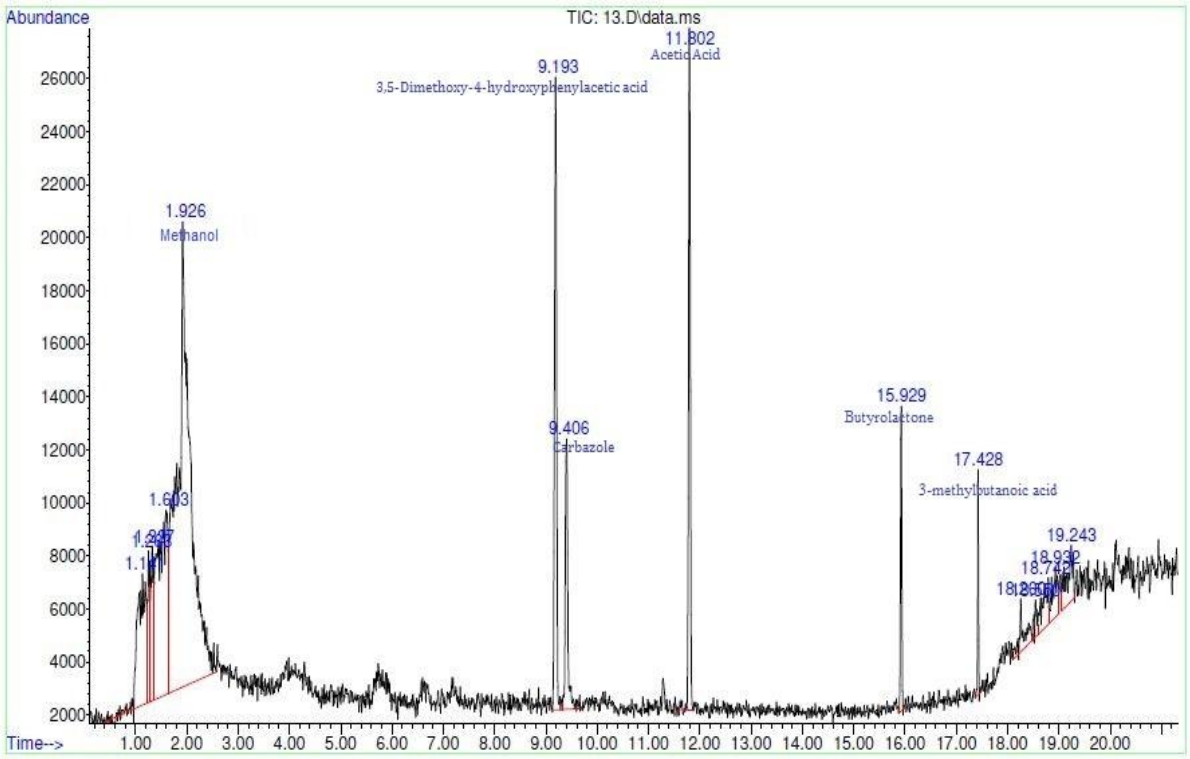

Fig. 6: GCMS extract weigh to solvent of 1:6.

CONCLUSION

The essential oil of *Eurycoma longifolia* has been extracted using Microwave assisted Extraction (MAE) and the components of the extracted essential oil were characterized and quantified using GCMS, showing similarities to previous publication results .Three parameters were investigated in this study namely extraction time, microwave power and raw material to solvent ratio (RMTSR). The optimum conditions for the microwave assisted extraction of *Eurycoma longifolia* are 10 minutes for extraction time, 225W for microwave power and 1:10 for RMTSR with the maximum yield percentage of 1.83%, 1.91% and 1.83% respectively.

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Conflict of Interests: There are no conflicts of interest.

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