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Original Article

Comparison of Tongkat Ali Root Chemical Composition Extracted by Soxhlet, Conventional Steam and Microwave Assisted Extraction Techniques

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ABSTRACT

Eurycoma longifolia Jack (Tongkat Ali) roots are used in traditional medicines for its wide range of biological effects. The process of extracting out this essential oil from the plant requires a delicate and efficient method. In this research the extract of the essential oil of *Eurycoma longifolia's* root using Microwave Assisted Extraction (MAE), Soxhlet Extraction and Conventional Steam Extraction (CSE) methods, is characterized to identify the components of essential oil extracted and then to compare the yield percentage and components of the extracts from both methods. The extract obtained was characterized using gas chromatography mass spectroscopy (GC-MS) by comparing the compositions of components present in commercial Tongkat Ali oil stored in the GC-MS library. The findings showed that the MAE gives a maximum yield percentage of 5 % with six chemical components extracted in 20 min while SE and CSE gives high yield percentage of 28.3% in 3 h and 2.5 % in 6 h respectively with only three chemical components extracted. Therefore, MAE is the optimum method for extracting essential oil from Tongkat Ali with a high quality.

Key words: *Eurycoma longifolia* Jack, Microwave Assisted Extraction, Soxhlet Extraction, Conventional Steam Extraction (CSE), Essential oil.

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INTRODUCTION

Essential oils are made out of an extensive variety of bioactive chemical compounds. They are naturally found in flowers, leaves, stems, woods and roots of plants. Essential oils are widely used in in pharmaceuticals, perfumes, beverages and detergents.[1] In this research, *Eurycoma longifolia* "Tongkat Ali" (T.A.) was extracted using Microwave Assisted Extraction (MAE), Soxhlet Extraction (SE) and Conventional Steam Extraction (CSE) methods. Tongkat Ali is used for increasing testosterone levels in men. It has also been used as antimalarial, anti-pyretic, antiulcer, cytotoxic and aphrodisiac properties.[2] T.A. have also been used traditionally as antihypertensive and for fever reduction. Some people use it for body building to increase the muscle mass and strength.[3] The quality and safety associated problems with the discovered high activity profile drugs and the active essential oil core challenges for the researches.[3] The main causes for the disadvantages are the lack of high performance, dependable extraction method and procedures for establishing the purity and standards for the essential oil.[4] The process of extracting out this essential oil from the plant requires a delicate and efficient method. Studies have found that particle size of sample, ratio of sample to solvent, temperature and time of extraction had significant effects on the extraction yield of *Eurycoma longifolia* roots [5,6] The main objectives of this research paper are to extract the essential oil of *Eurycoma longifolia's* root using MAE, SE and CSE to characterize the components of essential oil extracted and then to compare the yield percentage and components of the extracts from all the methods. This study is scoped about investigating one factor which is the extraction time affecting the yield and composition extracted from the MAE, SE , CSE and essential oil matrix using rotary evaporator and using the proper tool (GC-MS) for the characterization of the components of essential oils.

MATERIALS AND METHODS

Preparation of Raw Materials

The Tongkat Ali root was purchased from a certified supplier in Malaysia, it was cut and dried in the conventional oven at 45°C for 30 min. The dried sample was stored in an airtight container in room temperature.

Experimental Procedure

Method 1: Microwave Assisted Extraction (MAE)

The microwave assisted extraction was performed using Ethos Microwave Extractor. According to our

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previous study,[7] The microwave extractor was equipped with a chiller and a controller to control the time of extraction and microwave power. The weight of sample (*Eurycoma longifolia* roots) was kept constant at 5 g throughout the experiment and was soaked with 10ml of methanol for 10 min. This was done to enhance the diffusion of the solvent into the sample and improves the mass transfer of active compounds into the solvent. Before the extraction was done, the extractor was switched on and the chiller temperature was ensured to be lower than 10°C. One parameter was studied in this experiment which was the time of extraction. For this parameter, volumes of solvent and microwave power were kept constant at 50 ml and 250W respectively. The time was varied as 10, 20, 30, 40 and 50 min.

Method 2: Soxhlet Extraction

The soxhlet extractor was manually set up and was equipped with a condenser, soxhlet extractor and a round-bottomed flask. The extraction was done by using 5 gm of the powdered roots and 10ml of methanol this was kept for 10 min One parameter was studied in this experiment which was the time of extraction. For this parameter was kept constant at 350 ml. The time was varied as 2 hr, 3 hr, 4 hr, 5 hr and 6 hr.

Method 3: Conventional Steam Extraction (CSE)

The preparation of Tongkat Ali sample was prepared same as in MAE. For CSE, the steam extraction was done using a 500ml steam generating flask, a 250ml round bottomed flask and a condenser. A 5g of Tongkat Ali roots were used throughout this experiment. The steam generator flask was filled with 450ml of distilled water and heated. The steam passing through the distillation flask containing the powdered roots will extract the volatile components which were then collected into the receiving flask for different time of extraction which is 2 hr, 3 hr, 4 hr, 5 hr and 6 hr.

Separation of the Mixture of Solvent and Extracted Oil

The mixture of essential oil and solvent were separated from solid by decantation method and filter paper. The rotary evaporator was used to obtain the finalized extract. This separation process helped to eliminate the remaining solvent from the essential oil and gave a pure residual oil. The extract from MAE and SE, were filled in the flask, placed in a water bath in the rotary evaporator unit. The rotation speed was kept constant for each separation carried out and the temperature was set slightly higher than the boiling point of the solvent (methanol) which is 75°C. This is done to ensure that the solvent will be completely evaporated from the essential oils. The rotation is set to level 3 and the aspirator vacuum was switched on. After the evaporation was completed, the rotation is stopped and the pure essential oil was transferred into a sample bottle for characterization. While for CSE, hexane was used to separate mixture of essential oil and water. Then, the mixture of hexane and essential oil was separated using rotary evaporator. The rotation is set to level 3 and the aspirator vacuum was switched on. After the evaporation was completed, the rotation is stopped and the pure essential oil was transferred into a sample bottle for characterization.

RESULTS AND DISCUSSION

All the yield percentage of the Tongkat Ali essential oil was calculated at a constant volume of solvent for all three methods.

Microwave Assisted Extraction (MAE)[7]

Figure 1 illustrates the percentage yield of essential oil extracted from *Eurycoma longifolia* Jack (Tongkat Ali) at different extraction time at a constant volume of solvent using Microwave Assisted Extraction (MAE). From the table and graph, it can be clearly seen that the amount of yield increases as the extraction time was increased from 10 min to 20 min. The yield obtained at 5 and 10 min are 4.2%, and 5% respectively. Apparently microwave assisted extraction was able to produce the highest yield of 5% when the extraction time is 20 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 biphenyl cyclooctene lignans from Schisandra chinensis Baill fruits.[8] The initial increase in extraction yield supports the fact that the more the time the more the thermal accumulation within the solvent due to the absorption of microwave energy enhanced the dissolution process of essential oil into the solvent.[9] The decrease in yield after 10 min of extraction time could however be related with the possible degradation of the plant material as the extraction time increases.[10]

Soxhlet Extraction

Figure 2 illustrate the percentage yield of essential oil extracted from *Eurycoma longifolia* Jack (Tongkat Ali) at different extraction time at a constant volume of solvent using Soxhlet Extraction Method. From the table and graph, it can be clearly seen that the amount of yield increases as the extraction time was increased from 2 h to 3 h. The yield obtained at 2 and 3 h are 6.2%, and 28.3% respectively. Apparently soxhlet extraction was able to produce the highest yield of 28.3% when the extraction time is 3 h.

Conventional Steam Extraction (CSE)

Figure 3 shows the percentage yield against extraction time for CSE. From the graph above, it can be clearly seen that the percentage yield increase as the extraction time increase from 2 hr to 6 hr. The percentage yield at the first 2 h is 2.0%. As the time of extraction increase, the percentage yield of essential oil of Tongkat Ali also increases. The

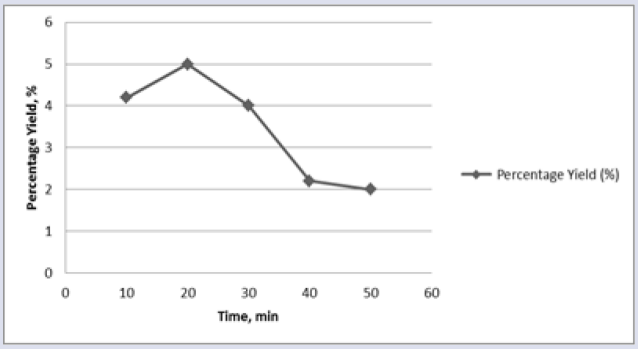

Figure 1: Graph of Percentage Yield against Extraction Time (Microwave extraction).

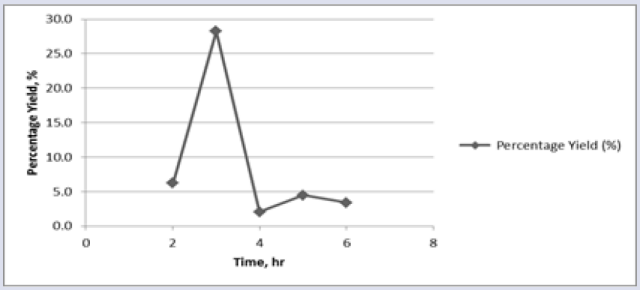

Figure 2: Graph of Percentage Yield against Extraction Time (Soxhlet extraction).

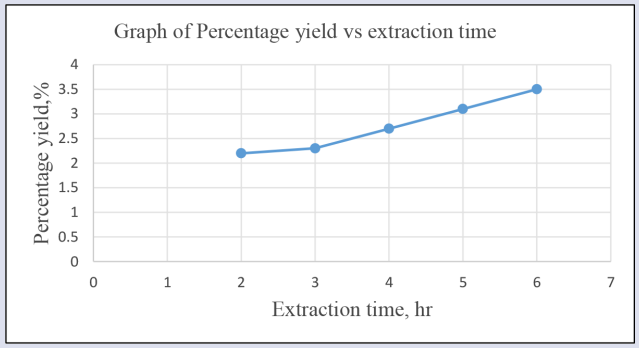

Figure 3: Graph of Percentage Yield against Extraction Time for CSE.

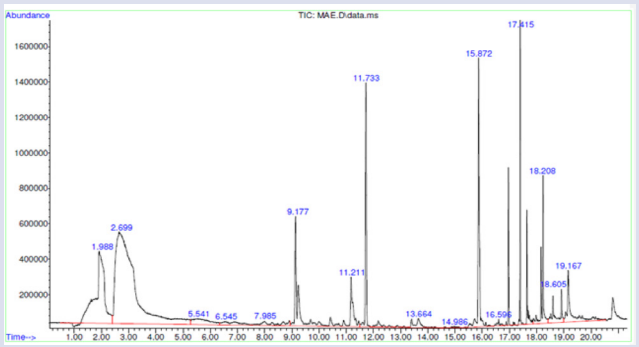

Figure 4: GC-MS Peaks at Extraction of 20 min.

Table 1: Components Identified in Tongkat Ali Extract at Extraction Time Parameter for MAE Source: (Kardono *et al*. 1991).

		Relative Peaks (%)				
		Extraction Time (min)				
No	Components	10	20	30	40	50
1	Methanol (Solvent)	98.47	98.26	92.95	98.43	98.6
2	Phenol	0.21	0.13	0.14	0.1	0.09
3	Pentafluoropropionic acid	0.13	0.06	0.04	-	-
4	Acetaldehyde	0.01	0.01	0.04	0.01	0.01
5	Benzaldehyde	-	-	-	-	0.06
6	Acetic acid	0.13	0.03	-	0.05	-
7	2-propyl tetradecyl ester	0.12	0.07	0.04	-	-
8	2-Trifluoromethylbenzoic acid	-	0.08	-	0.06	0.06
9	1-Propanol	-	-	-	0.01	-
10	1-Hexadecanol	-	-	-	0.03	-

Table 2: Components Identified in Tongkat Ali Extract at Extraction Time Parameter for Soxhlet Extraction. Source: (Kardono *et al*. 1991).

	Relative Peaks (%)				
	Extraction Time (h)				
Components	2	3	4	5	6
Methanol (Solvent)	99.11	78.53	99.23	76.61	53.05
Vanillin	-	2.22	-	-	-
Acetaldehyde	-	0.43	0.01	0.83	1
Benzaldehyde	-	0.87	-	-	-
Acetic acid	-	-	0.02	-	-
2-Trifluoromethylbenzoic acid	-	-	0.03	-	-
1-Propanol	-	-	0.01	-	-
Dimethyl Ester	0.15	-	-	1.27	10.71

percentage yield of essential oil in CSE increases as time of extraction increases. The percentage yield for CSE at 2hr, 3hr, 4hr, 5hr and 6 hr. were 2.2%, 2.3%, 2.7%, 3.1% and 3.5 respectively. The same observation was reported from the steam distillation of Patchouli or Pogostemon Cablin (Blanco) sample.[11] In the event of increasing the extraction time, some chemicals decreased, some others increased, and at the same time there were also some components that were not affected. It was also found that the method of sample preparation did influence the amount of oil extracted.[11]

GC-MS Analysis

The Tongkat Ali essential oils obtained from the parameters of extraction time was analyzed using gas chromatography and mass spectroscopy in order to obtain the chemical components found in the extract.

Table 3: Components Identified in Tongkat Ali's roots extract at different extraction time for CSE.

		Relative peaks (%)				
		Extraction Time (hr)				
No	Compound	2	3	4	5	6
1	Hexane (solvent)	62.65	91.02	92.16	76.75	42.33
2	Furanone	~	~	1.28	~	~
3	Acetic acid	~	~	~	~	1.04
4	2-Propenoic acid	~	~	~	~	1.51
5	1-methylundecyl ester	8.37	~	~	~	1.51
6	Acetaldehyde	2.21	1.84	~	~	~

Table 4: Comparison of Essential Oil chemical composition extracted from MAE, SE and CSE methods.

	Composition (%)		
Components	MAE	SE	CSE
Phenol	**0.13**	**-**	**-**
Pentafluoropropionic acid	0.06	-	-
Acetaldehyde	0.01	0.43	-
Benzaldehyde	-	0.87	-
Acetic acid	0.03	-	1.04
2-propyl tetradecyl ester	0.07	-	-
2-Trifluoromethylbenzoic acid	0.08	-	-
2-Propenoic Acid			1.51
1-methylundecyl ester			1.51
1-Propanol	-	-	-
1-Hexadecanol	-	-	-
Vanillin	-	2.22	-
Dimethyl Ester	-	-	-

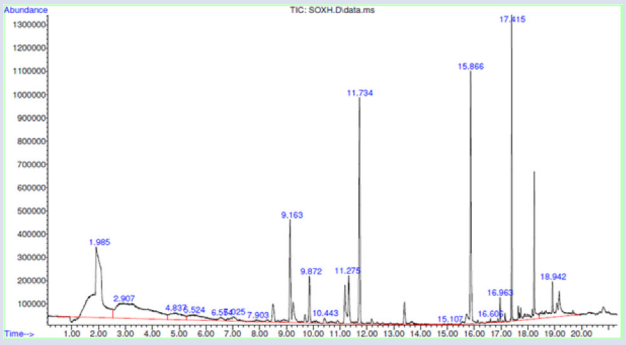

Figure 5: GC-MS Peaks at Extraction of 3 h.

Microwave Assisted Extraction

Figure 4 shows the peaks found in GC-MS for the extraction time of 20 min. Table 1 summarizes the components of essential oils found in each of the samples for all the five different extraction time investigated in this study.

Table 1 reveals the results and the components identified through GC-MS. The components in the Tongkat Ali essential oil were detected at the retention time in the range from 1 to 20 min. The solvent, methanol was detected in the first peak. There were nine components detected in total. Generally, the components such as Phenol, Pentafluoropropionic acid, Acetaldehyde, Acetic Acid, 2-propyl tetradecyl ester and2-Trifluoromethylbenzoic acid were mostly found in each and every sample of the parameters. Some components such as Benzaldehyde, 1-Propanol and 1-Hexadecanol were the least found components.

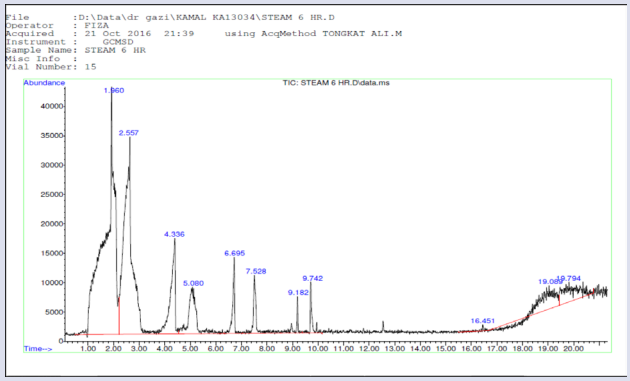

Figure 6: GC-MS Peaks at Extraction of 6 h.

Soxhlet Extraction Method

Figure 5 shows the peaks found in GC-MS for the extraction time of 3 h. Table 2 summarizes the components of essential oils found in each of the samples for all the 5 different extraction time investigated in this study.

Table 2 reveals the results and the components identified through GC-MS. The components in the Tongkat Ali essential oil were detected at the retention time in the range from 1 to 20 min. The solvent, methanol was detected first peak. There were seven components detected in total. Generally, the components such as Acetaldehyde and Dimethyl Ester were mostly found in each and every sample of the parameters. Some components such as Vanillin, Benzaldehyde, Acetic acid, 2-Trifluoromethylbenzoic acid and 1-Propanol were the least found components.

Conventional Steam Extraction (CSE)

Figure 6 shows the peaks found in GC-MS for the extraction time of 6 h. Table 3 summarizes the components of essential oils found in each of the samples for all the five different extraction time investigated in this study.

Figure 6 and Table 3 list the composition and peaks of essential oils of Tongkat Ali obtained by CSE according to the results of GC-MS. Five components of essential oils in Tongkat Ali have been identified. The

components identified were Furanone, Acetic acid, acetaldehyde, 2-propenoic acid and1-methylundecyl ester. From the Table 3, the highest extraction by CSE occurred at 6 hr. which capability of extracting 3 components. There were 1.04% acetic acid, 1.51% propionic acid and 1.51% 1-methyldecyl ester. While at 3 hr. and 4 hr. were the lowest extractions by CSE as it only capable of extracting 1.84% acetaldehyde and 1.28% furanone respectively. There were less component found is CSE. This may be due to the sample was heated for longer extraction time. Sample of Tongkat Ali cannot sustain to high temperature as the component inside easily breakdown into other component that is not part of chemical composition of essential oil in Tongkat Ali.[12]

Comparison of Essential Oil chemical composition extracted from MAE, SE and CSE Methods

Table 4 shows the list of chemical components based on the highest yield using MAE, SE and CSE methods with the extraction time of 20 min 3 h and 6 h respectively, There are total six components that have been identified in the GC-MS analysis for MAE method whereas for SE and CSE method there only three components found. There is no common component found in all three methods. This is probably due to the amount of heat used during the extraction for each method. For SE and CSE methods, plenty of heat is been used which may have caused degradation of components.[10] Even though SE method gives the highest percentage of yield compared to MAE and CSE methods, the number of components that are identified in MAE are higher. This shows that MAE has a higher quality of extraction with a shorter period of time which is 20 min compared to SE and CSE.

CONCLUSION

The analysis concluded that MAE has a better extraction time and yield compared to SE and CSE. Although some of the components of the oils obtained are similar, they do differ quantitatively. MAE method offers many important advantages over SE and CSE methods including high extraction yield within a short period of time, highest composition of Tongkat Ali's component and shorter extraction time. Therefore, MAE is the optimum method for extracting essential oil from Tongkat Ali with a high quality.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest.

ABBREVIATIONS

CSE: Conventional Steam Extraction; **MAE:** Microwave Assisted Extraction; **GC-MS:** Gas Chromatography Mass Spectroscopy; **T.A:** Eurycoma longifolia "Tongkat Ali".

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