Determination of Medicinal Compounds in *Persicaria odorata* Using Solvent Extraction

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***Abstract* - In this study, essential oil was extracted from *P. odorata* leaves using three solvents (hexane, acetone and ethanol). Soxhlet extractor was used as a method of extraction. The extract obtained was then analyzed using GC-MS and FTIR to determine the presence of medicinal compounds in the oil. The result shows that extraction using hexane as solvent produced the highest yield of extract while acetone is able extract the most bioactive compounds from the dried plant sample (13 compounds) compared to other solvents. GC-MS analysis identified the presence of curlone (11.074%), turmerone (9.94%), squalene (9.94%) and limonene oxide (3.52%) as the major bioactive compounds in the oil. The results of FTIR analysis confirmed the presence of alcohols, phenols, aldehydes, nitro compounds and cellulose.**

Keywords— Persicaria odorata, essential oil, Soxhlet, medicinal compounds, bioactive.

I. INTRODUCTION

Persicaria odorata is an herbaceous plant which is native to South East Asia that grows best in tropical condition and can stand up to 15 to 30 cm in height [1]. P.odorata is known for the strong aroma it releases when the leaves are bruised. In Malaysia, people often include them in their famous Laksa dish hence giving the leaves its local name in Malaysia which is Laksa leaves. In addition for being an important ingredient in Asian kitchen, *P.odorata* leaves were believed to have many benefits in health and well-being. The leaves have been widely used in the field of medicines, cuisine, pharmacy and cosmetics [2] and also believed to treat dandruffs and stomach indigestion [3] as well as a good source of protein (Thomson et al. 2013).

The oil extracted from P.odrata leaves was reported to contain aliphatic aldehydes(85%), alcohols(11%), sesquiterpene(3%) and terpenes and carboxylic acids (1%)[4]. *P.odorata* oil were believed to poses antimicrobial properties due to the presence of aldehydes and terpenes [5].

The soxhlet extractor was first invented in 1879 by Frans Ritter von Soxhlet. Soxhlet extractor was proved to be more efficient that continuous extractor and chemist started to proposed new design in regard to Soxhlet's original design [6].

Soxhlet extraction was reported to be the most conventional and consist of a simple distillation process. It was the oldest known method to be used to conventional solid sample extraction[7].The main advantage of this method is that only one batch of solvent is required and it can be recycled. In fact, the sample is continuously in contact with the solvent. [8].

METHODOLOGY

A. Materials

Persicaria odorata leaves were purchased from local market in Wangsa Maju, Kuala Lumpur.

B. Pre-extraction

P.odorata leaves are separated from its stem then washed thoroughly to remove any dirt and damaged leaves. The leaves are dried in the oven at 40℃ for 24 hours, then grinded into smaller pieces using electric blender [9]. 10g of grinded plant sample is placed in a porous thimble made out of stacked up filter paper and placed in the main extraction chamber [10].

C. Soxhlet Extraction

200 ml of selected solvent is placed in the distillation flask. Thimble filled with sample and condenser were assembled and the heat is supplied to the system. The system was heated to reflux for six hours. After 6 hours, the extract was allowed to cool to room temperature before being concentrated in a rotary evaporator.

D. Analysis

The oil obtained were weighted and the percentage of oil yield were calculated using formula [11].

$$\%\ \boldsymbol{yield\ of\ plant\ extracts} = \left(\frac{\boldsymbol{weight\ of\ plant\ extract}}{\boldsymbol{initial\ weight\ of\ sample}}\right) \times \mathbf{100\%}$$

The extract with highest oil yield were send to GC-MS and FTIR for further analysis.

II. RESULTS AND DISCUSSION

A. The percentage of oil yield from extraction process

Table 1 Average oil yield using different solvent

Solvent	**Hexane**	**Ethanol**	**Acetone**
Sample weight (g)	10.41	9.73	9.42
Oil weight (g)	1.80	1.06	1.43
Average oil yield (%)	17.30	10.98	15.16

The plant extract recovered from leaves of *P.odorata* which extracted by hexane, ethanol and acetone showed a yield of 17.30%, 10.98% and 15.16%, respectively as represented in Table 1. Colour of the plant extract extracted by acetone and ethanol was light and dark green respectively. However, plant extracts which using a hexane as a solvent showed a clear light yellowish-green.

B. GC-MS Analysis

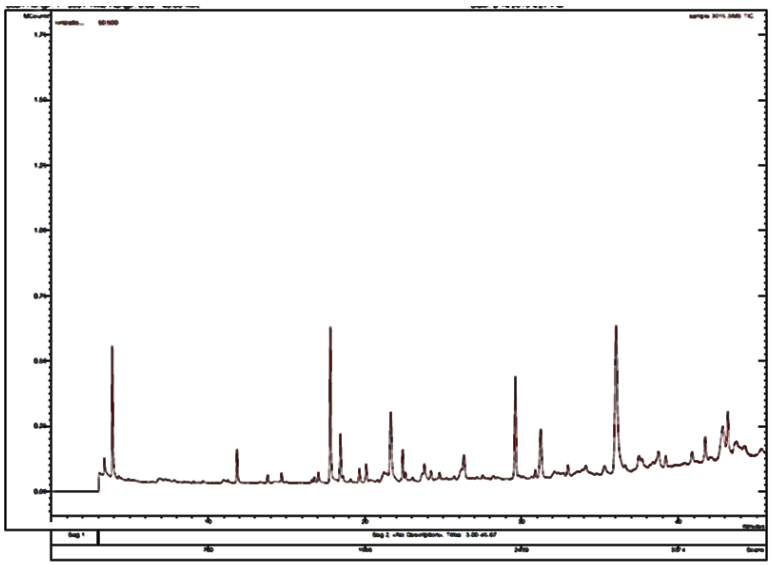

Figure 1 GC-MS Chromatogram of P.odorata leaves extract

Table 2 Compounds indentified by GC-MS and their bioactivity

No.	RT (min)	Peak area (%)	Compound Name	Molecular formula	Molecular weight (g/mol)	Bioactivity
1.	36.025	9.936	Curlone	$C_{15}H_{22}O$	218.3346	Anti-trypanosomal [12]
2.	7.477	0.9	1-Eicosene	$C_{20}H_{40}$	280.5316	Anti-microbial, cytotoxic, Antifungal, Antioxidant [13]
3.	7.830	0.957	α-Phellandrene	$C_{10}H_{16}$	136.2340	Antifungal [14] Promotes immune response [15]
4.	18.429	1.709	Caryophyllene	$C_{15}H_{24}$	204.357	Anti-Inflammatory [16] Anti-cancer [17]
5.	19.639	0.556	3-Carene	$C_{10}H_{16}$	136.2340	Anti-Fungal [18], Anti-Oxidant, Anti-acetylcholinesterase [19]
6.	24.210	0.532	α-Fernesene	$C_{15}H_{24}$	204.3511	Anti-Oxidant [20]
7.	26.314	1.479	Valencene	$C_{15}H_{24}$	204.3511	Aroma active [21]
8.	29.593	3.522	Limonene oxide	$C_{10}H_{16}O$	152.237	Aroma active [21], Acaricidal [22]
9.	32.950	0.516	Terpineol	$C_{10}H_{18}O$	154.253	Antibacteria, Antioxidant [23]
10.	35.743	0.107	Spathulenol	$C_{15}H_{24}O$	220.356	Antiproliferative, Antimycobacterial[24], Anti-microbial [25]
11.	36.025	9.936	Trumerone	$C_{15}H_{24}O$	218.3346	Anti-depressant [26] Microglia activation [27]
12.	36.1027	9.936	Squalene	$C_{30}H_{50}$	410.7180	Emollient, Antioxidant [28] Anti-cancer, Detoxifier [29]
13.	42.831	3.110	Mequinol	C_7H_8O	124.1372	Pharmaceutical Intermediates [30] Antifungal [31]

Different types of solvent have a different capability to extract the various chemical compositions from each plant extract. Dark green color in plants was due to the presence of chlorophyll. These suggest that acetone and ethanol can extract the chlorophyll from plant more efficiently than hexane [32].

Although hexane extraction gave the highest percentage of oil yield extract, acetone was the most effective solvent because it can extract more bioactive compounds compared to other solvent.

Acetone was able to extract 13 bioactive compounds as tabulated in Table 2, followed by ethanol (5 bioactive compounds) and hexane (5bioactive compounds). The major bioactive compounds present in P.odorata leaves extract are curlone (11.074%), trumerone (9.94%), squalene (9.94%) and limonene oxide (3.52%).

Based on Beaufay et. al, curlone is the most promising anti-trypanosomal agent that can be used to treat Human African trypanosomiasis (HAT), known as sleeping sickness caused by *Trypanosoma brucei*, that threatens 61 million sub-Saharan African [12].

It has been suggested that aromatic turmerone inhibits microglia activation, a property that may be useful in treating neurodegenerative disease. The effect ar-turmerone has on neural stem cell (NCS) made it a strong candidate to support regeneration in neurologic disease [27]. Turmerone also poses an anti-depressant like effect by increasing the monoamines level thus decreasing monoamines oxidase-A and stress level of mice [26].

Experimental studies have shown that squalene can effectively inhibit chemically induced skin, colon, and lung tumorigenesis in rodents and act as protective agent during carcinogen treatment[28]. Squalene supplementation is suggested to be accounted to inhibit tumor growth and prevention of normal cells to turn into tumor cells under oxidative stress[29].

Limonen oxide works as acaricidal agents or pesticides as it have been shown to display activity against both larvae and eggs of the tick of *Rhipicephalus (Boophilus) microplus* [22].

C. FTIR Analysis

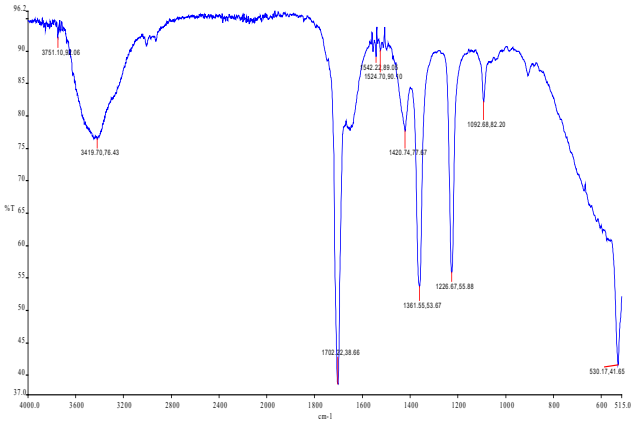

Figure 2 FTIR Spectra on *P.odorata* leaves extract

Table 3 Peak value and Functional groups

Wavenumber (cm^{-1})	Functional group
3419.70	O-H stretch (Alcohols and phenols)
1702.22	C=O stretch (Aldehydes)
1542.22	NO_2 stretch (Nitro compounds)
1420.74	Sp^3 C-H bend
1361.55	C-O (Phenols)
1226.55	C-O-C stretching (cellulose)

The data of peak value and functional group is presented in Table 3. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis confirmed the presence of alcohols, phenols, aldehydes, nitro compounds and cellulose.

III. CONCLUSION

Different types of solvent in solvent extraction methods exhibited different type of bioactive compounds. From quantitative and qualitative analysis that has been done on the extracted oil, acetone was the most effective solvent as it can extract the most bioactive compounds. The major bioactive compounds present in *P.odorata* leave extract are curlone, limonene oxide, turmerone, squalene and mequinol. These compounds have great potential to provide alternatives to conventional drugs since the bioactivity of the compounds was proven in various research despite lacks of human trials.

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