EXTRACTION OF BIOACTIVE COMPOUNDS FROM CURRY LEAVES (MURRAYA KOENIGII) USING SOLVENT EXTRACTION METHOD

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Abstract— *Murraya koenigii* also known as curry leaves are used as a flavour ingredient and has a lot of bioactive components which can be found inside the plant. The amount of people in Malaysia that are still not aware of curry leaves medicinal values due to lack of exposure is quite alarming. This research objective are to extract essential oils from *Murraya koenigii* by using solvent extraction method, to determine the highest oil content in *Murraya koenigii* by using three different solvent which are pure methanol, pure ethanol and distilled water, to identify bioactives content in *Murraya koenigii* using GC-MS to determine chemical structure in *Murraya koenigii* using FTIR. The result shows that Methanol solvent yield the highest oil content. The functional group was also identified where it was classified as O-H, C-H bond, Benzene and Esters group. Bioactive compounds of the essential oil performed by using GC-MS detected the presence of α-Pinene, α-Caryophyllene, α-Terpineol and Estragole. All four has potential medical benefits to human which α-Pinene exhibits anti-inflammatory and anti-microbial properties while α-Caryophyllene has anticarcinogenic properties, α-Terpineol reduces mechanical hypernociception and inflammatory response and Estragole gives local anesthetic activity. Since the bioactives compound are sucessfully found in *Murraya koenigii* leaves, it is hoped that Malaysians will be much aware with the health benefits of *Murraya koenigii* and will start to consume it instead of throwing it away.

Keywords— **murraya koenigii (L) leaves, soxhlet extraction, essential oil, bioactive compounds, antioxidant activity.**

I. INTRODUCTION

Nowadays, a lot of deadly diseases breaking out all over the world are due to unhealthy consumption of food. In Malaysia, curry leaf is widely used as a flavouring ingredient and unlike other herbs such as coriander or mint leaves, people put curry leaves to the dishes just for their aroma and taste. The curry leaf won't be consumed and will be discarded as waste due to its pungent and bitter taste. Thus, it is quite alarming as many people especially in Malaysia are still not aware that curry leaves has many medicinal values due to lack of exposure about curry leaves beneficial health to the human. Instead of throwing the leaves away into the dustbin, the curry leaves can be taken to be investigated on its useful and beneficial component since the information about the secondary metabolite content and the antioxidant and anticancer activity of Malaysian curry leaf is still scarce. Thus, additional research on the bioactive compounds in curry leaves will help to further evaluate the health benefits from this plant. The objectives of the research are to extract essential oils from Murraya koenigii leaves (curry leaves) by using solvent extraction method. Second, to determine the highest oil content in Murraya koenigii leaves by using three different solvents. Third, to identify chemical structure in Murraya koenigii leaves using Fourier Transform Infrared

Spectroscopy (FTIR) and last, to identify bioactives content in Murraya koenigii leaves using Gas Chromatogtaphy– Mass Spectrometry (GC-MS). In this research, the highest oil content in Murraya koenigii will be determine by using three different solvents which are pure methanol, pure ethanol and distilled water. The extracted curry leaves will then be analyzed by using: Gas Chromatogtaphy – Mass Spectrometry (GC-MS) to determine the bioactives content in Murraya koenigii and Fourier Transform Infrared Spectroscopy (FTIR) to identify the chemical structure in the leaves

II. METHODOLOGY

A. Plant Material

Fresh leaves of *Murraya koenigii* was purchased from supermarket and was washed thoroughly under running tap water to remove any impurities on the leaves. The curry leaves are shade-dried until all moisture completely removed. The dried curry leaves was then grind for 10 seconds using spice grinder to get fine particles of powder. The method of preparation of *Murraya koenigii* leaves was followed as according Disegha and Izionworu (2014) with some modification.

B. Soxhlet Extraction

Using 1:60 sample to solvent ratio (Goldsmith et. al., 2014), 3g to 5g of *Murraya koenigii* was placed in the thimble of Soxhlet extractor and 200 ml of solvent was added to round bottom flask attached to Soxhlet. Each extraction was performed for 6 hours. Once extraction has completed, the extract was then filtered and later concentrated using rotary evaporator. The extracted Murraya koenigii leaves was added 1 mL in GC vial before subjected to Gas Chromatography-Mass Spectrometry (GC-MS) and FT-IR analysis.

C. Analysis of Essential Oil

- GC-MS

  For the determination of the chemical constituents contained in the essential oil of Murraya koenigii leaves, GCMS machine was used. This machine model : Varian 450-GC, Varian 240-MS. This GC-MS use Helium injector temperature is 250°C ; Ion-source temperature is at 280 °C. The oven temperature is programmed from 50 °C (isothermal for 4min.), with an increase of 3°C/min, to 280 °C and held for 10 minutes. While isothermal is at 280 °C and the total running time is 90.67 minutes

- FT-IR

  The identification of chemical structure of Murraya koenigii is achieved by using FT-IR analysis. FTIR model: Perkin Elmer, Spectrum One FT-IR Spectrometer. Scanning the spectrum in the range 450–4000 cm^{-1} at a resolution of 4 cm^{-1} was carried out (Bonde et. al., 2012).

II. RESULTS AND DISCUSSION

A. Oil content

Out of these three solvents, methanol give the highest essential oil yield from Murraya koenigii (16.292% (w/w)), followed by ethanol extract (11.161% (w/w)) and the lowest was water (4.110% (w/w)).

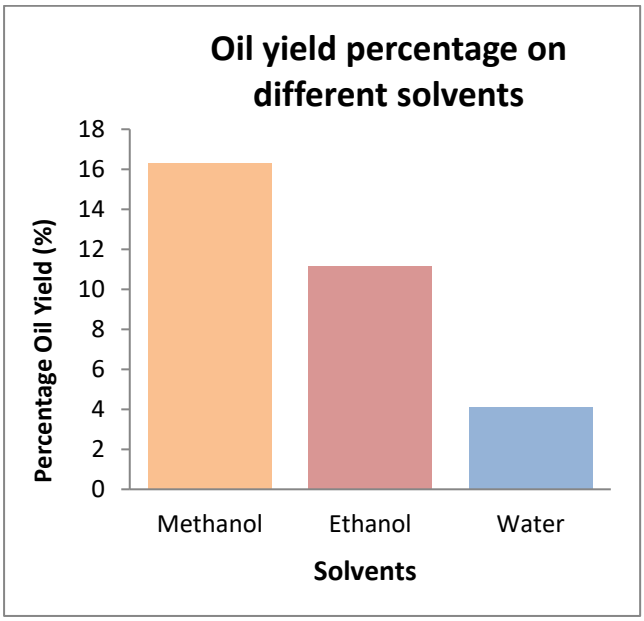

Figure 1: *Comparison oil yield between three solvents.*

Alcohol which is a polar solvent act very good as solvent for extraction. Out of three solvent, Methanol gives the highest oil yield compared to Ethanol. This is due to the reason that methanol has higher polarity than ethanol. Methanol which also has higher dieletric constant than ethanol enable it to extract more polar compounds (Bischoff, 2013). This shows that methanol is the best solvent for the extraction of essential oil of Murraya koenigii leaves.

B. FT-IR of Murraya koenigii

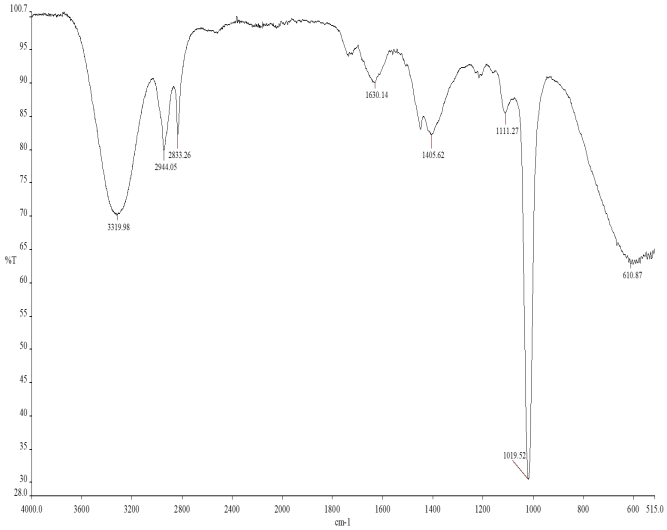

Figure 2: *IR spectrum of Murraya koenigii leaves from methanol solvent*

The functional group in Murraya koenigii from Methanol solvent was successfully identified by using Fourier Transform Infrared Spectroscopy (FTIR) as the functional group of Murraya koenigii leaves was identified and classified as O-H, C-H bond, Benzene and Esters group as seen in Table 1.

Table 1. FTIR Analysis of Murraya koenigii

Wavelength (cm^{-1})	Absorption Intensity	Functional Group
3319.98	Medium	O-H (carboxylic acids)
2944.05	Medium	C-H bond
2833.26	Medium	C-H bond
1630.14	Strong	Benzene
1405.62	-	Finger print region
1111.27	C-O	Esters
1019.52	C-N	Esters
610.87	-	Finger print region

C. GC-MS of Murraya koenigii

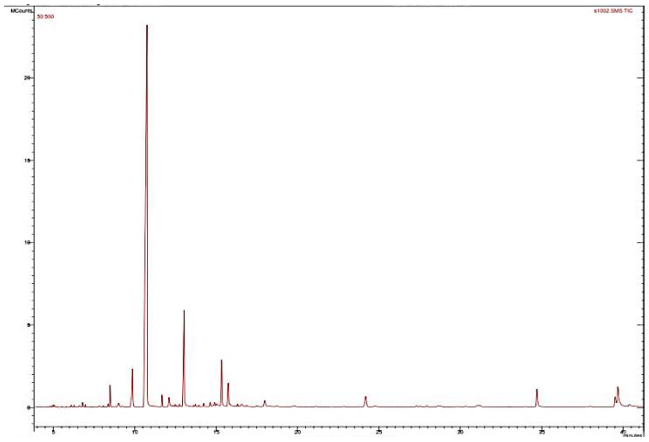

Figure 3: *GC-MS Chromatogram of Murraya koenigii in Methanol solvent*

The outcomes from GC-MS of Murraya koenigii from methanol solvent showed several bioactive compounds which were; Estragole (51.669%, 0.423% and 1.288%), Azulene (0.228%, 23.475%, 1.168), Limonene (0.994%, 3.922%, 0.992%), as well as α-Pinene (0.048%, 10.727%, 0.540%), α-Terpineol (0.363%, 0.010%, 0.318%) and α-Phellandrene (0.048%, 0.437%, 0.223)

Table 2. Bioactive component of Murraya koenigii from first run of Methanol solvent

Methanol		
Retention Time(min)	**Chemical Constituents**	**Composition**
4.372	á-Pinene	0.048
5.012	Cis-á-Terpineol	0.363
10.699	Estragole	51.669
12.997	2-Propanone	8.102
13.721	Caryophyllene	2.600
22.834	Gurjunene	0.188

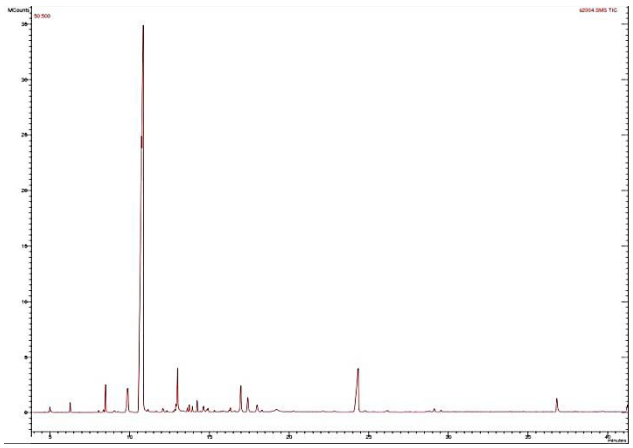

Figure 4: *GC-MS Chromatogram of Murraya koenigii in Ethanol solvent*

As for GC-MS analysis of Murraya koenigii from ethanol extracts bioactive compounds such as Aristolene (55.318%, 3.931%, 0.065%), followed by Gurjunene (8.522%, 0.154%,

0.333%), α-Panasinsense (6.508%, 0.114%, 5.689%), Naphthalene (2.908%, 3.615%, 0.142%), as well as Caryophyllene (0.427%, 0.381%, 0.465%), Mequinol (0.211%, 0.284%, 0.233%), α-Pinene (0.052%, 0.264%, 0.142%) and α-Terpineol (0.393%, 0.120%, 0.055%).

Table 3. Bioactive component of Murraya koenigii from first run of Ethanol solvent

Ethanol		
Retention Time(min)	**Chemical Constituents**	**Composition**
4.997	α -Pinene	0.264
5.180	α -Terpineol	0.120
9.850	Naphthalene	2.908
10.722	Estragole	64.362
10.801	Aristolene	55.318
22.840	Gurjunene	0.210

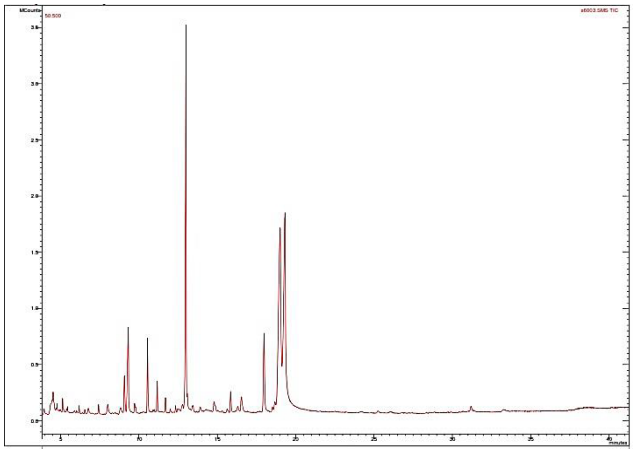

Figure 5: *GC-MS Chromatogram of Murraya koenigii in Water solvent*

GC-MS analysis of Murraya koenigii from water extract showed bioactive compounds such as Estragole (4.321%, 2.466%, 2.705%), Limonene (1.420%,0.279%, 0.435%), α-Terpineol (1.662%, 1.021%, 0.299%) and Caryophyllene (0.649%, 16.086%, 0.266%)

Table 4. Bioactive component of Murraya koenigii from first run of Water solvent

Water		
Retention Time(min)	**Chemical Constituents**	**Composition**
3.552	Ecgonine	0.404
7.991	cis-á-Terpineol	1.021
8.452	á-Pinene	0.417
9.054	Mequinol	1.448
10.529	Estragole	2.466
19.283	à-Caryophyllene	16.086

The main bioactive components of *Murraya koenigii* leaves are essential oil such as α-Pinene, α-Caryophyllene, α-Terpineol and Estragole which have potential medicinal value that bring benefits to human.

α-Pinene which was obtained from all three solvents exhibits anti-inflammatory and anti-microbial properties. This was proven by a study conducted by Wang et. al., (2012) which proved that α-Pinene exhibits anti-inflammatory effect in human Chondrocytes. While a study by Rufino et. al., (2014) proved that α-Pinene has anti-microbial properties.

Next is α-Caryophyllene which belongs to Terpenes family. α-Caryophyllene can be found in spices and herb. The biological activites of Caryophyllenes are it is anti-inflammatory, antibiotic, antioxidant and anticarcinogenic (Legault and Pichette, 2007). It can be confirmed that Caryophyllene has anticarcinogenic properties by the study conducted by Legault and Pichette (2007), Caryophyllene facilitates the passage of paclitaxel through the membrane which potentiates its anti-cancer activity and thus confirms the anti-cancer properties of Caryophyllene.

Another major component in *Murraya koenigii* leaves is α-Terpineol which also belongs to group Terpenes. It is one of the monoterpene alcohol isomers and naturally occurring in plants. α-Terpineol is one of the major components of essential oil of varius plant species. According to Oliveira et. al., (2012), α-Terpineol reduces mechanical hypernociception and inflammatory response. Lastly, Estragole which is a relatively nontoxic terpenoid ether, is an important constituent of many essential oils with extensive applications in folk medicine and aromatherapy. It is also known to have potent local anesthetic activity (Leal-Cardoso, 2004). Thus, it is proven that *Murraya koenigii* leaves has many medical benefits and brings great benefits for those who consume it.

III. CONCLUSION

The essential oil of Murraya koenigii leaves was successful extracted by using solvent extraction method. The second objective which is to determine the highest oil yield from Murraya koenigii leaves in three different solvent was also a success as it was found that methanol

yield the highest oil content compared to other two. Next, the functional group in Murraya koenigii was successfully identified by using Fourier Transform Infrared Spectroscopy (FTIR) as the functional group identified and classified as O-H, C-H bond, Benzene and Esters group. Lastly, the bioactive component was identified by using Gas Chromatogtaphy– Mass Spectrometry GC-MS and bioactive compound found was found to be α-Pinene, α-Caryophyllene, α-Terpineol and Estragol. All four has potential medical benefits to human which α-Pinene exhibits anti-inflammatory and anti-microbial properties while α-Caryophyllene has anticarcinogenic properties. While α-Terpineol reduces mechanical hypernociception and inflammatory response and Estragole gives local anesthetic activity. Since the bioactives compound are sucessfully found in *Murraya koenigii* leaves, thus the last objective was successfully achieved.

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