Extraction of Essential Oil from the Bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo)

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Abstract

In this research work, one Myanmar indigenous medicinal plant *Cinnamomum zeylanicum* Blume. was selected for chemical analysis. The preliminary detection of phytochemical compounds present in the bark of *Cinnamomum zeylanicum* Blume. were carried out by standard procedures. The antimicrobial activity of sample in various solvent systems was determined by Agar well diffusion method on six selected organisms. The antimicrobial activity of ethanol extract of bark of *Cinnamomum zeylanicum* Blume. was determined by using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) assay. The elemental analysis of sample were measured by Energy Dispersive X-rays Fluorescence (EDXRF) method. The essential oil was extracted from the bark of *Cinnamomum zeylanicum* Blume. by steam distillation method. The functional groups of extracted oil from the bark of *Cinnamomum zeylanicum* Blume. were assigned by Fourier Transform Infrared (FT-IR) spectral data. Moreover, the extracted essential oil was analyzed by Gas Chromatography Mass (GCMS) Spectrometry.

Keyword: *Cinnamomum zeylanicum* Blume., phytochemical, antimicrobial, antioxidant, EDXRF, FT-IR, GC-MS

Introduction

Medicinal herbal plants are cheaper, more accessible to the most of the population in the world. Thus, there is need to encourage the use of medicinal plants as potential sources of new drugs. There has been as highly increased interest for herbal remedial several parts of the world. *Cinnamomum zeylanicum* Blume. is an evergreen tropical tree, belonging to the Lauraceae family. (Das S, 1999)

In Myanmar, the plant is usually referred to Thit-kya-bo. The barks and leaves of *Cinnamomum zeylanicum* Blume. are widely used as spice and flavouring agent in foods and for various applications in medicine. The barks and leaves of *Cinnamomum zeylanicum* Blume.commonly used as spices in home kitchens and their distilled essential oils are used as flavouring agent in the food. *Cinnamomum zeylanicum* Blume. contains antioxidants and other active ingredients which are found in the water soluble portions. (Kiruba, 2011)

The bark of the tree is dried and used for spice in the United States. It has been established that the oils and extracts *Cinnamomum zeylanicum* Blume. possess a distinct antioxidant activity. Steam distillation is the simplest method to extract the essential oil from the bark of *Cinnamomum zeylanicum* Blume. The process is cheaper than other extraction methods. It does not require any solvent and is safer than other methods. The advantage of steam distillation is that it is relatively cheap to operate at basic level, and the properties of the

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oils produced by this method are not altered. Medicinal herbal plants are widely used in treating various diseases and illnesses. (Priyagrace, 2009)

Botanical Description

Family	-	Lauraceae
English name	-	Cinnamon
Botanical name	-	*Cinnamomum zeylanicum* Blume.
Myanmar name	-	Thit-kya-bo
Part used	-	Bark

Figure (1) The barks of Thit-kya-bo

Materials and Methods

Sample collection

The bark of *Cinnamomum zeylanicum* Blume. were collected from Pyin Oo Lwin Township, Mandalay Region. They were chopped into small pieces and used throughout the experiment.

Preliminary Phytochemical Constituents of Bark of *Cinnamomum zeylanicum* Blume.

The phytochemical tests were carried out to detect the presence or absence of organic constituents in the bark of *Cinnamomum zeylanicum* Blume. (Harborne, 1993)

Antimicrobial Activities of Crude Extracts of the Bark of *Cinnamomum zeylanicum* Blume.

The antimicrobial activities of crude extract sample of the bark of useful plant were examined by using Agar well diffusion method at Central Research and Development Centre (CRDC), Insein, Yangon. (Magaldi, 2004) (Valgas, 2007)

Antioxidant Activity of Bark of *Cinnamomum zeylanicum* Blume.

The antioxidant activity of the bark of sensitive plants was done by using DPPH assay method at Department of Chemistry, University of Mandalay. In this experiment, the antioxidant activity of the bark of sensitive plants was done by using DPPH assay method at Department of Chemistry, University of Mandalay. In this experiment, 1, 1-diphenyl-2-picryl hydrazyl (DPPH) powder was used as stable free radical. Ascorbic acid was used as standard antioxidant. Ethanol (Analar grade) was also used as solvent. The absorbance was determined at 517 nm wavelength. (Stjepan and Bozidar, 2006) (Lovo, 2010)

Elemental Analysis of the bark of *Cinnamomum zeylanicum* Blume.

The elemental composition of bark of *Cinnamomum zeylanicum* Blume. were examined by the Energy Dispersive X-ray Fluorescence (EDXRF) spectrophotometer at Department of Chemistry, Monywa University. (SPECTRO XEPOS EDXRF Spectrometer, Germany)

Extraction of Essential Oil by Steam Distillation

The extraction of essential oil was done by steam distillation method at Department of Chemistry, University of Mandalay. The yield percent of extraction oil was determined. (Pratt, 2013) The apparatus was used, 2 L of distilled water was poured into the still body and perforated cone was set over it, 300 g of the sample was placed on the perforated cone of the still. It was heated carefully without decomposition of oil. The time taken was five hours per day.

After heating for five hours, a mixture of volatile oil and stream was came out passed into the condenser. The oil collecting on the surface of the water was separated by using petroleum ether and separating funnel .And then petroleum ether in essential oil was allowed to evaporate. The filtrate was stored for the use of next functional group determination. The dehydrated oil obtained by passing through the anhydrous sodium sulphate preserves for best quality. The above experiment was carried out for three times, each time using 300 g of sample.

Figure (2) Steam distillation apparatus

Figure (3) The extracted oil by using separating funnel

Study on FT-IR spectrum of Extracted Oil

The Fourier Transform Infrared spectrum of extracted oil was measured at Department of Chemistry, University of Mandalay. The FT-IR spectrum informs the prominent functional groups containing in the compound. The FT-IR spectrum of extracted oil was measured at the Department of Chemistry, University of Mandalay. (Silverstein, 2005) The infrared spectrum of extracted oil was described in Figure.

Determination of Chemical compositions by GC-MS

The bark of *Cinnamomum zeylanicum* Blume. was extracted with steam distillation and analyzed by GC-MS using methanol as solvent for identification of different compounds, at the Department of Chemistry, University Research Center (URC), Mandalay.

Results and Discussion

Preliminary Phytochemical Screening of the Bark of *Cinnamomum zeylanicum* Blume.

Table (1) Results of Phytochemical Screening of the Bark of *Cinnamomum zeylanicum* Blume.

No.	Test	Reagent	Observation	Bark
1.	Alkaloid	Wagner's solution	Orange ppt	+
2.	Flavonoid	Conc: HCl, Mg turning	Yellow color solution	+
3.	Glycoside	10 % lead acetate	Yellow ppt	+
4.	Lipophenol	0.5 KOH, 4 drops of NaOH	Yellow color solution	+
5.	Phenolic	10 % $FeCl_3$	Greenish blue color solution	+
6.	Polyphenol	1 % $FeCl_3$ and 1 % K_3 $[Fe(CN)_6]$	Greenish blue color solution	+
7.	Reducing Sugar	Benedict's solution	Red ppt	+
8.	Saponin	$NaHCO_3$	Forth	+
9.	Steroid	Pet ether, $CHCl_3$,, acetic anhydride, Conc: H_2SO_4	Reddish brown ppt	+
10.	Terpene	Acetic anhydride, $CHCl_3$, conc: H_2SO_4	Brown ppt	+

(+) = presence (-) = absence ppt = precipitate

According to above table, the sample contained alkaloid, flavonoid, glycoside, lipophenol, phenolic, polyphenol, reducing sugar, saponin, steroid and terpene.

Determination of Antimicrobial Activities of Crude Extracts of the Bark of *C. zeylanicum* Blume.

Table (2) The Results of Antimicrobial Activities of Crude Extract Sample of the Bark of *Cinnamomum zeylanicum* Blume.

Inhibition Zone							
Samples	**Solvent**	***B-sub***	***S-aureus***	***P-aeruginosa***	***B - pumlis***	***Candida***	***E-coli***
Cinnamomum zeylanicum Nees	n- hexane	11 mm (+)	–	–	11mm (+)	12mm (+)	–
	EtOAc	25mm (+++)	25mm (+++)	25mm (+++)	25mm (+++)	25mm (+++)	25mm (+++)
	EtoH	19mm (++)	19mm (++)	18mm (++)	18mm (++)	19mm (++)	18mm (++)
Control	n-hexane	–	–	–	–	–	–
	EtOAc	–	–	–	–	–	–
	EtoH	–	–	–	–	–	–

Agar well ~ 10 mm Organism
10 mm ~ 14 mm (+) = low activity
15 mm ~ 19 mm (++) = medium activity
20 mm ~ above (+++) = high activity

I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus*
V = *Candida albicans*
VI = *E. coli*

According to this table, n-hexane extract of useful plants are no activity on all organisms. Ethyl acetate extract of the sample responds high activities on all organisms. Moreover, ethanol extract of sample gives rise to medium activity on six organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus*, *Candida albicans*, and *E. coli*.

Determination of Antioxidant Activity of the Standard Ascorbic Acid

The result of IC_{50} value of the standard ascorbic acid was shown in Table(3).

Table (3) Results of IC_{50} Value of the Standard Ascorbic Acid

Concentration (µg/mL)	Mean Absorbance	Mean % Inhibition	IC_{50} (µg/mL)
50	0.297	68.50	17.99
25	0.350	61.61	
12.5	0.483	48.78	
6.25	0.562	40.41	
3.125	0.608	35.52	

IC_{50} value was calculated by using linear regressive equation.

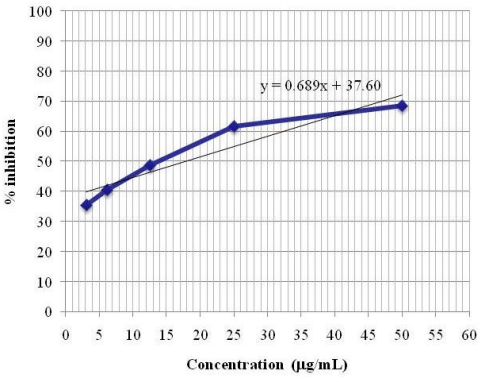

Figure (4) %Plot of Inhibition Vs Different Concentration

Determination of Antioxidant Activity of Bark of *Cinnamomum zeylanicum* Blume. by DPPH Assay

The result of IC_{50} value of the sample was shown in Table (4).

Table (4) Result of IC_{50} Value of the Sample

Concentration (µg/mg)	Mean Absorbance	Mean % Inhibition	IC_{50} (µg/mL)
160	0.312	75.77	12.15
120	0.336	72.83	
80	0.360	69.44	
60	0.386	65.35	
20	0.428	39.50	

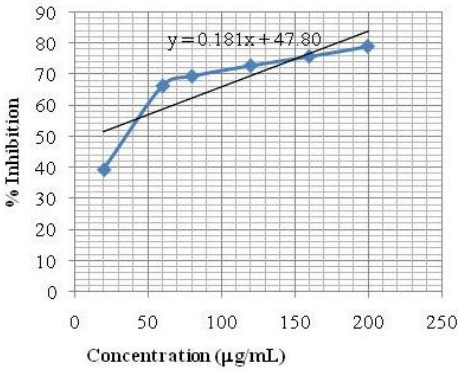

Figure (5) Plot of % Inhibition Vs Concentration of Sample

IC_{50} value was calculated by using linear regressive equation.

According to this table, the antioxidant activity of sample was determined in DPPH free radical scavenging assay. In DPPH screening assay that IC_{50} value of sample was found to be 12.15µg/mL. It was very much higher than that of standard ascorbic acid (IC_{50} 17.99 µg/mL). So, the sample extract has higher antioxidant activity than standard ascorbic acid.

Determination of the Elemental Analysis of the Bark of *Cinnamomum zeylanicum* Blume.

The elemental composition of the bark of *Cinnamomum zeylanicum* Blume. were measured at Department of Physics, University of Mandalay by Energy Dispersive X-ray Fluorescence analysis. The results are shown in Table (5).

Table (5) Mineral Contents of the Bark of *Cinnamomum zeylanicum* Blume.

No.	Symbol	Element	Relative Abundance (%)
1.	Ca	Calcium	1.6300
2.	Si	Silicon	0.0102
3.	K	potassium	0.6021
4.	Cl	Chlorine	1.4890
5.	Al	Aluminum	0.0163
6.	Fe	Iron	0.0148
7.	P	Phosphorous	0.0358
8.	S	Sulphur	0.0241
9.	Ti	Vanadium	0.0017
10.	Ba	Barium	0.0096
11.	Sr	Strontium	0.0029

According to EDXRF results, calcium content of sample is higher than the others. Some minerals are essential for a healthy diet. High mineral contents sometimes cause retardation of the growth of certain microorganisms.

Extraction of Essential Oil by Steam Distillation Method

Table (6) The Yield (%) of Essential Oil by Steam Distillation Method

No. of Experiment	Weight of Sample (g)	Weight of extracted essential oil	% of essential oil
1	300	0.0049	0.00163
2	298	0.0048	0.00160
3	299	0.0047	0.00156

According to this table, the average yield percent of essential oil is 0.0016% based on the weight of barks of Thit-kya- bo.

Determination FT-IR Assignment of Essential Oil

FT-IR spectrum of Essential Oil was measured at the Department of Chemistry, University of Mandalay. According to FT-IR spectrums, the assignments were shown in Table (5). (Silverstein, 2005)

The FT-IR spectrum of essential oil was measured at the Department of Chemistry, University of Mandalay was shown in Figure.

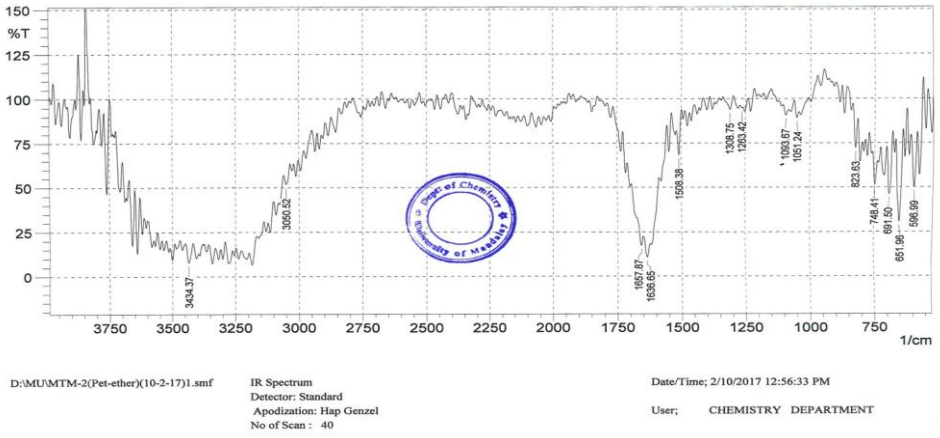

Figure (6) FT-IR spectrum of isolated Thitkya-bo essential oil

Table (7) Characteristic Absorption Peaks of FT-IR Spectrum Assignment for Essential Oil

Absorption Peak (cm^{-1})	Assignment (Functional group)
3434.37	O-H stretching vibration of of alcohol group
3050.52	C-H stretching vibration of sp2 hydrocarbon
1657.87	C=O stretching vibration of carbonyl group
1636.65, 1508.38	*C*–––*C* ring skeletal vibration of aromatic ring
1308.75	O-H bending vibration of alcohol group
1093.67, 1051.24	C-C-O stretching vibration of ether group
823.63	C-H out of plane bending vibration of trans or E alkene group
748.41	C-H out of plane bending vibration of cis or Z alkene

According to FT-IR assignment, the essential oil consists of O-H functional group, carbonyl group, aromatic benzene ring, C-H hydrocarbon group, C-C-O stretching vibration of alcohol group, Trans or E and cis or Z alkenic group respectively.

Identification of Components by GCMS

Identification of the extracted oil components was based using a National Institute of Standards and Technology (NIST) mass spectral library according to the reference mass spectra from published sources, and retention indices (RI). The essential oil from the bark of *Cinnamomum zeylanicum* Blume. by GCMS analysis showed the presence of some compounds, shows in Table.

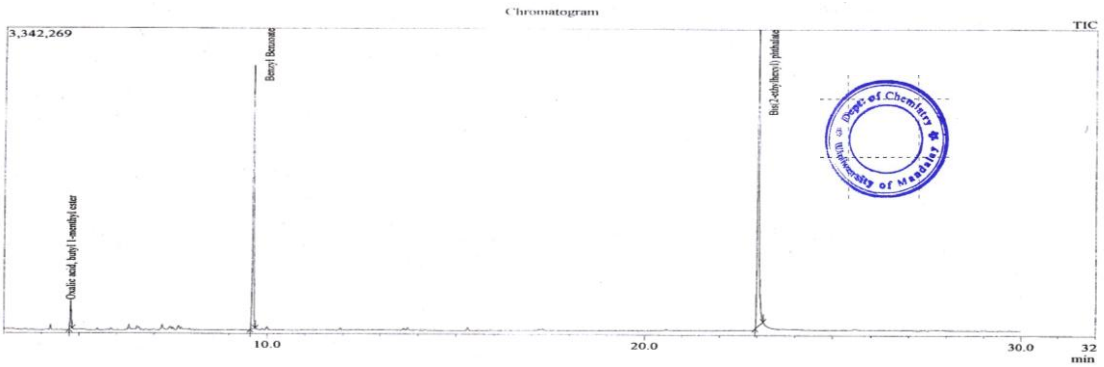

Figure (7) Total ion chromatogram of extracted essential oil

Table (8) Chemical Composition of Extracted Essential Oil

Peak Report TIC

Peak#	R.Time	Area	Area%	Base m/z	Name
1	4.778	622093	3.50	83.05	Oxalic acid, butyl 1-menthyl ester
2	9.622	6917827	38.97	105.05	Benzyl Benzoate
3	23.023	10210094	57.52	149.00	Bis(2-ethylhexyl) phthalate
		17750014	100.00		

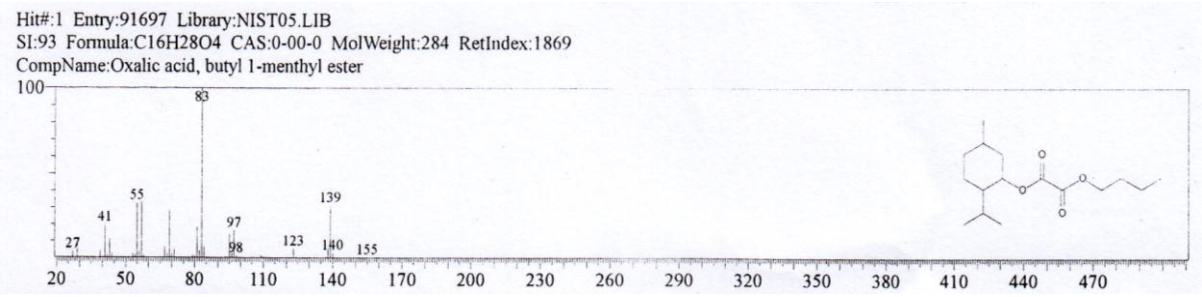

Figure (8) EI mass spectrum of butyl 1-menthyl ester

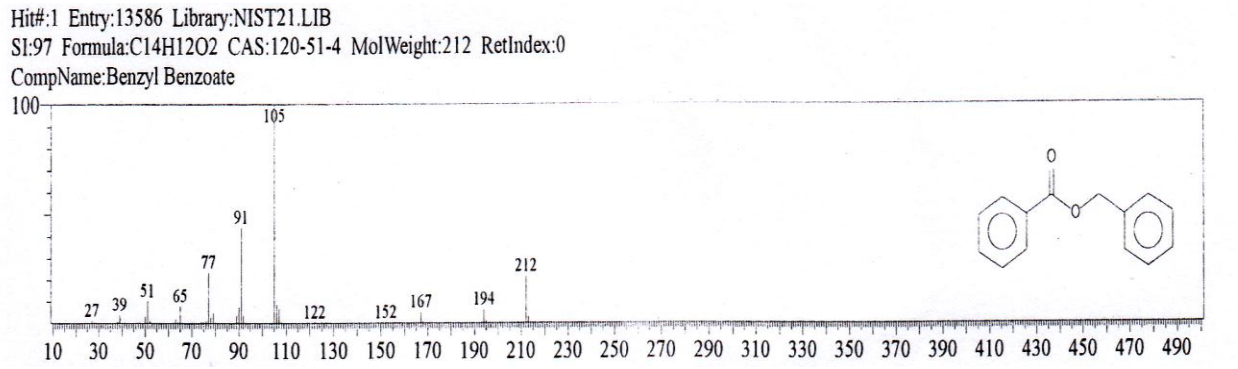

Figure (9) EI mass spectrum of benzyl benzoate

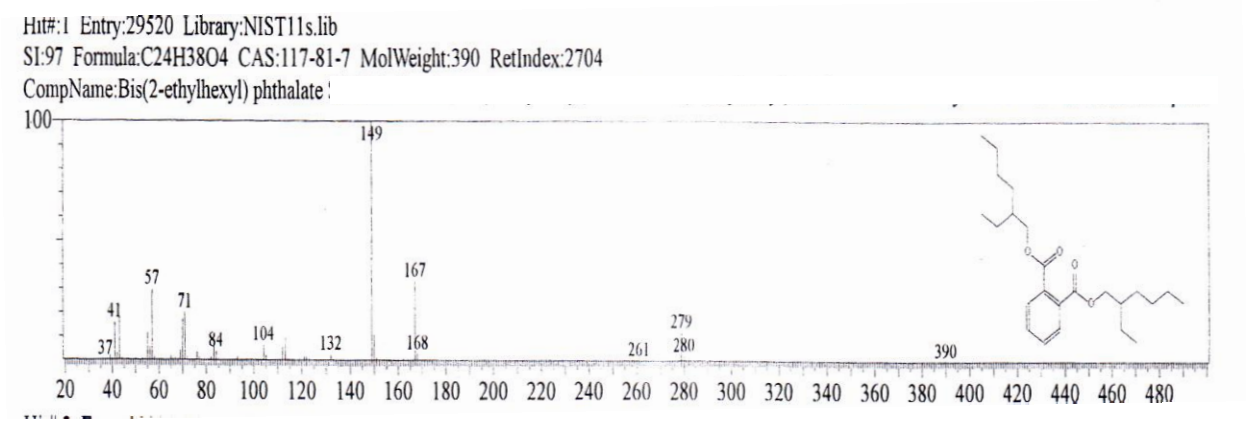

Figure (10) EI mass spectrum of Bis (2-ethylhexyl) phthalate

This is the total ion chromatogram for essential oil extract of the bark of *Cinnamomum zeylanicum* Blume. by GCMS using steam distillation. In this spectrum, X-axis represents time (min) and Y-axis represents percent(%).

According to this experimental data, the extracted essential oil comprised the high level of Bis (2-ethylhexyl) phthalate is comparison with other.

Conclusion

In this research work, the bark of *Cinnamomum zeylanicum* Blume. was collected from Pyin Oo Lwin Township in Mandalay Region. According to phytochemical screening which gave positive tests for alkaloids, flavonoids, glycosides, lipophenol, phenolic, polyphenol, reducing sugars, saponins, steroids and terpenes compound respectively. The antimicrobial activities of various solvent systems were tested by Agar well diffusion method on six selected organisms. The n-hexane extract of useful plants are no activity on all organisms. Ethyl acetate extract of sample responds high activities on all organisms. Moreover, ethanol extract of sample gives rise to medium activity on six organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E.coli.* Percent inhibitions of standard ascorbic acid and ethanol extract of useful plant are

12.15µg/mL. It was very much higher than that of standard ascorbic acid (IC_{50} = 17.99 µg/mL). So, the sample extract has higher antioxidant activity than standard ascorbic acid. The mineral contents of bark of sensitive plant the highest amount of calcium and silicon were found. Calcium is important for optimal bone health throughout the life. The health benefits of silicon also play a vital role in theprevention of atherosclerosis, insomnia, skin disorders and tuberculosis.

In addition, the essential oil was isolated by steam distillation method. The yield percent of essential oil was found to be 0.0016% based in crude sample. According to FT-IR spectral data of essential oil consists of OH functional group, sp^2 hydrocarbon, sp^3hydrocarbon, carbonyl group, aromatic benzene ring, O – H bending vibration of alcohol group, C – C – O stretching vibration, ether group, trans or E and cis or Z alkene group. The results obtained in this study showed that *Cinnamomum zeylanicum* Blume. possess essential oil in the bark of useful plant and that their oil compositions were quantitatively different. Bis(2-ethylhexyl) phthalate was found to be the highest constituent 57.52% in bark extract. It can be used for the purpose of medicinal and beneficial to man-kind.

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