Investigation of Antimicrobial Activity and Identification of Chemical Constituents in Essential Oil Extracted from the Bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo)

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Abstract

Cinnamomum zeylanicum Blume. (Thit-kya-bo), popularly known as cinnamon, is widely used in food flavours, cosmetics and pharmaceuticals. The essential oil of cinnamon has been used to have pharmacological effects in the treatment of type II diabetes, toothache, anti-inflammatory, anti-ulcer, anti-microbial, hypolipidemic potential. Investigations on the compositions of these oils revealed that they are rich in monoterpenoids and phenyl propanoids. (E)-cinnamaldehyde is the main component of cinnamon bark oil. The purpose of this research is to study the investigation of antimicrobial activity and extraction of essential oil from the bark of *Cinnamonum zeylanicum* Blume. (Thit-kya-bo). The sample was collected from U Byit quarter, Myitkyina Township, Kachin State. The elemental analysis were determined by using EDXRF spectrometry. Antimicrobial activity of crude extracts: pet - ether, ethyl acetate, ethanol and water of Thit-kya-bo were subjected for screening antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Bacillus pumilus*, *Escherichia coli* and *Candida albicans* by agar well diffusion method. Essential oils from Cinnamon bark was extracted by water distillation method. The chemical constituents in essential oil of Thit-kya-bo bark were detected by GC-MS method.

Keywords **:** *Cinnamonum zeylanicum* blume. (Thit-kya-bo), EDXRF, antimincrobial activity, essential oil

Introduction

Cinnamomum zeylanicum (Thit-kya-bo) belongs to family lauraceae. It is a medium sized, bushy, evergreen tree growing up to height of 6-8m. The leaves are 10-15 cm long and shining green when mature. The flowers, which are arranged in panicles have a green colour and have a distinct odour. The ripened fruit is dark-purple and oblong. Cinnamon of trade is the dried inner bark of many Cinnamomum species. The quantity and appearance of Cinnamomum bark depends on the place of production and the care taken in its preparation. It prefers a sheltered place, constant rain, heat and equal temperature. It flavor is due to an aromatic essential oil which makes up 0.5 to 1 % of its composition. *Cinnamomum zeylanicum* is a native of Sri Lanka and tropical Asia. It is grown mostly in Sri Lanka, India. In India it is mostly cultivated in the states of Karnataka and Kerala. It is also grown Australia and Pacific Islands. (The Wealth of India, 1992).

In medicine it acts like other voltile oils and once has a repulation as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system. The essential oil of Cinnamon also has antimicrobial properties, which aid in the preservation of certain foods. (Matan. N., 2006)

Cinnamon contains essential oils such as trans- cinnamaldehyde, caryophyllene oxide, L-borneol, L-bornyl acetate, eugenol, resinous compounds, cinnamic acid, cinnamaldehyde and cinnamate. Some other constituents are Terpinolene, α-Terpineol, α-Cubebene, and α-Thujene. (Vaibhavi, 2010). The present work focused on investigation of animicrobial activity and identification of chemical constituents in essential oil extracted from the bark of *Cinnamomun zeylanicum* Blume. (Thit-kya-bo). The phytograph of Thit-kya-bo plant, leaves and barks are shown in Figure 1.

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(a) **(b)**
(c)

Figure 1. Photographs of Thyt-kya-bo (a) Plant, (b) Leaves (c) Barks

Materials and Methods

The bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo) was selected for investigation of antimicrobial activity and for extraction of essential oils. It was identified at the Department of Botany, University of Myitkyina. Thit-kya-bo bark was collected from U Byit quarter, Myitkyina Township, Kachin State. The collected dried sample was ground into powder by grinder. The dried powdered sample was stored in the air-tight containers to prevent the moisture and other contaminations.

Material and Method

Sample Preparation

The bark of *Cinnamonum zeylanicum* Blume. (Thit-kya-bo) was collected form U Byit quarter, Myitkyina Township, Kachin State. The collected dried sample was ground into powder by grinder. The dried powdered sample was stored in the air- tight containers to prevent the moisture and other contamination.

Screening of Phytochemical Constituents on the Bark of Thit-kya-bo

The bark extracts were tested for the presence of phytochemical constituents by using standard procedures.

Preparation of Various Crude Extracts from Thit-kya-bo

25 g of dried powdered sample was percolated with 75 cm^3 of pet-ether for 6 hours and filtered. The filtrate was placed in a weighted porcelain basin and then evaporated to dryness on a water-bath until it was completely dried. Pet-ether extract was obtained. Ethyl acetate extract , ethanolic extract and watery extract from the bark of Thit-kya-bo were extracted in similar manner mentioned.

Some Elemental Analysis of the Sample by Energy Dispersive X-Ray Fluorescence (ED-XRF) Spectrometry

For this measurement, pellets of the sample were first made x-ray spectrometer permits simultaneous analysis of light element to heavy element. Energy dispersive X-ray fluorescence

spectrometer (Shimadzu EDX-720) can analyze the elements from Na to U under vacuum condition. X-ray fluorescence uses X-rays to excite an unknown sample. The individual elements in each sample are detected by using semiconductor (Si - Li) that permits multi-elements, simultaneous analysis. In this way, EDX-720 spectrometer determines elements that are presents in the sample. Analysis of some elements in the bark of Thit-kya-bo were measured by EDXRF method using EDX-720 instrument at the Universities' Research Centre (URC), Yangon. The results sbtained are shown in Table 1.

Screening of Antimicrobial Activity of Various Crude Extracts by Agar Well Diffusion Method

The antimicrobial activities of different crude extracts such as pet-ether, ethyl acetate, 95 % ethanol and watery extracts from the bark of Thit-kya-bo were determined against six strains of microorganisms such as *Bacillus subtilis, Bacillus pumalis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli* by employing agar well diffusion method at Fermentation Department, Development Center of Pharmaceutical Technology, Ministry of Industry I, Yangon, Myanmar.

Extraction of Essential Oil From the Bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo) by Water Distillation Method.

The dried powdered sample of Thit-kya-bo (25 g) and distilled water (25 ml) were placed in the 500 ml round-bottomed flask. The flask was fitted to Clevenger's apparatus which has regulated to maintain a rate of condensate of 1-3 hour. The volume of essential oil was collected in the receiver flask. After the water distillation process, the product was collected and separated two layers of immiscible liquids such as water and oil by using separatory funnel. The oil was then partitioned with pet-ether in a separating funnel. The pet-ether extract was dried anhydrous sodium sulphate, filtered and evaporated to get the essential oil which has then weight until to the constant and kept in air tight bottle. Yield percent of essential oil was calculated according to the following equation.

$$\text{yield percent \%} = \frac{\text{volume of essential oil (ml) X 100\%}}{\text{weight of sample in (g)}}$$

Figure 2. Photographs Showing Extraction of Essential Oil from the Bark of *Cinnamonum zeylanicum* Blume. (Thit-kya-bo) with Clevenger's apparatus

Identification of Chemical Constituents in Essential Oil Extracted from the Bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo) by GC-MS methods.

For identification of chemical constituents in essential oil GC-MS spectrometer was used to examine the molecular weight and formula of organic compound. The organic compounds in essential oil from *Cinnamomum zeylanicum* Blume. (Thit-kya-bo) were detected by GC-MS method.

Results and Discussion

Screening of phytochemical Constituents on the Bark of Thit-kya-bo

The phytochemical tests revealed the presence of flavonoids, glycosides, phenolic compounds, tannins, carbohydrate, organic acids, saponin glycosides, steroids and terpenoids. But alkaloids, ∝-amino acid, reducing sugars, and starch were not detected in this sample.

Some Elemental Analysis of the Sample by Energy Dispersive X-Ray Fluorescence (EDXRF) Spectrometry

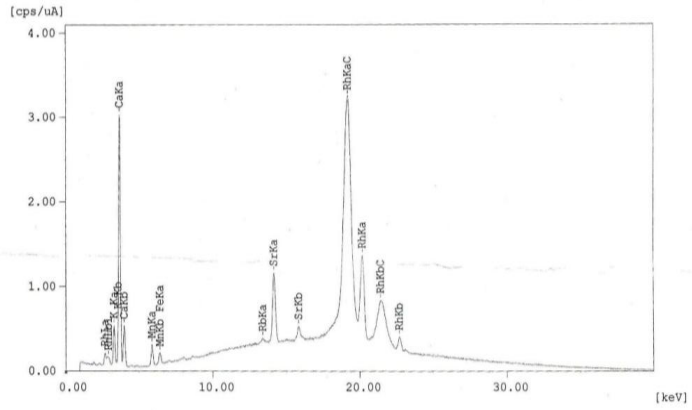
In this work, relative abundance of elements present in Thit-kya-bo was determined by EDXRF spectrometer. The EDXRF spectrum of the sample was shown in Figure (3) and data was listed in Table (1). In addition, the relative abundance of calcium (Ca) and potassium (K) were observed to be highest. A few amount of Manganese (Mn), Strontium (Sr), iron (Fe) and rubidium (Rb) were observed in this sample. Calcium is the most abundance mineral in the human body. It helps numerous functions, such as building strong bones, tramsmitting nerve impulses, making hormones and maintaining a regular heartbeat (Website 1). Potassium is essential for the body's growth and maintenance. It is also essential role in the response of nerves to stimulation and in the contraction of muscles.

Sample : Ma Yaw Sant
Operator: NTD
Comment : Pallet
Group : Solid Air
Date : 2017-12-20 14:39:21

Measurement Condition

Instrument: EDX-720 Atmosphere: Air Collimator: 10(mm)

Analyte	TG kV	uA	FI	Acq.(keV)	Anal.(keV)	Time(sec)	DT(%)
Si-U	Rh 50	36-Auto	----	0 - 40	0.02-39.98	Live- 100	41



Quantitative Result

Analyte	Result	[3-sigma]	Proc.-Calc.	Line	Int.(cps/uA)
Ca	81.015 %	[0.872]	Quan-FP	CaKa	20.7432
K	11.837 %	[0.288]	Quan-FP	K Ka	3.1728
Mn	3.602 %	[0.104]	Quan-FP	MnKa	2.0030
Sr	2.093 %	[0.048]	Quan-FP	SrKa	9.5511
Fe	1.286 %	[0.118]	Quan-FP	FeKa	0.9700
Rb	0.167 %	[0.040]	Quan-FP	RbKa	0.6787

Figure 3. EDXRF Spectrum of Bark of Thit-kya-bo

Table 1. Relative Abundance of Some Elements in the Bark of Thit-kya-bo by EDXRF method

No.	Elements	Relative Abundance%
1.	Calcium (Ca)	81.015 %
2.	Potassium (K)	11.837 %
3.	Manganese (Mn)	3. 602 %
4.	Strontium (Sr)	2.093 %
5.	Iron (Fe)	1.286 %
6.	Rubidium (Rb)	0.167 %

Antimicrobial Activity of Bark of Thit-kya-bo by Agar Well Diffusion Method

Screening of antimicrobial activity of various crude extracts such as pet-ether, ethyl acetate, ethanol and watery extracts from bark of Thit-kya-bo were investigated by employing agar well diffusion method. The samples were tested on six species of microorganism such as *Bacillus substilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* species. The inhibition zone diameter shows the degree of the antimicrobial activity. The larger the inhibition zone diameters, the higher the antimicrobial activity. The inhibition zones of crude extracts against six microorganisms tested are shown in Figure (4). It is found that pet-ether extract and ethyl acetate extract of bark of Thit-kya-bo showed a moderate antimicrobial activity against six microorganisms with inhibition zone diameter ranged in 11 mm to 14 mm. In addition, ethanol extract of Thit-kya-bo exhibited antimicrobial activity against all test microorganisms except *Staphylococcus aureus*. Water extract of Thit-kya-bo was observed antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* except *Bacillus pumilus, Candida albicans* and *E-coli.*

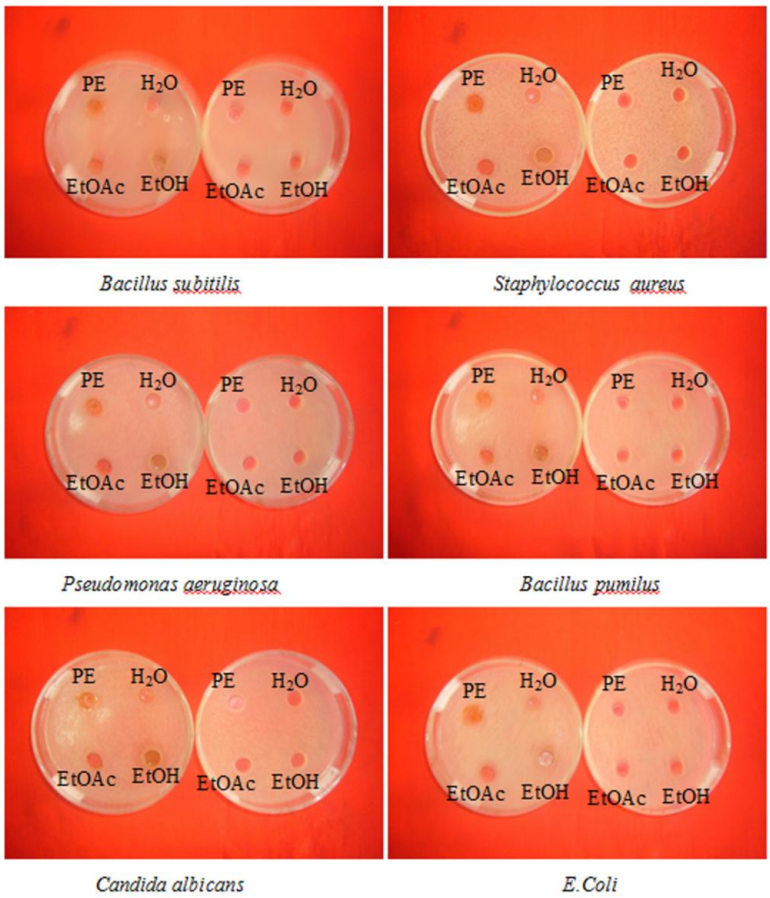


Figure 4. The Images of Inhibition Zones of Various Crude Extracts Against Six Microorganisms

Extraction of Essential Oil from the bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo) by Water Distillation Method.

Extraction of essential oil from the dried powdered sample of Thit-kya-bo was carried out by water distillation method using Clevenger's apparatus and yield percentage was determined to be 0.2348 %.

Identification of Chemical Constituents in Essential Oil from the bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo) by GC-MS Method.

The GC-MS chromatogram or essential oil extracted from the bark of Thit-kya-bo is shown in Figure (5). According to GC-MS chromatogram, the peak appears at the retention time 7.21 min with 100 % relative abundance. At this retention time 7.21 min, the GC-MS spectrum (Figure 6) shows the molecular ion peak at m/z 132, indicating the molecular weight of compound A is (E)-Cinnamaldehyde at the retention time 10.21 min, the GC-MS spectrum (Figure 7) shows the molecular ion peak at m/z 190 which indicates the molecular formula $C_{11}H_{10}O_3$. Thus it can be observed that compound B is 7-methoxy– 4-methyl coumarin. In addition, at the retention time 11.13 min, represents the molecular ion peak at m/z 222 with the formula $C_{13}H_{26}O$ (Figure 8)

which is indicates molecular weight of compound C to be 222 and so compound C is (2E, 6E)-3,7,11–trimethyl 2,6,10–dodecatrien–1-ol (Farnesol isomer).

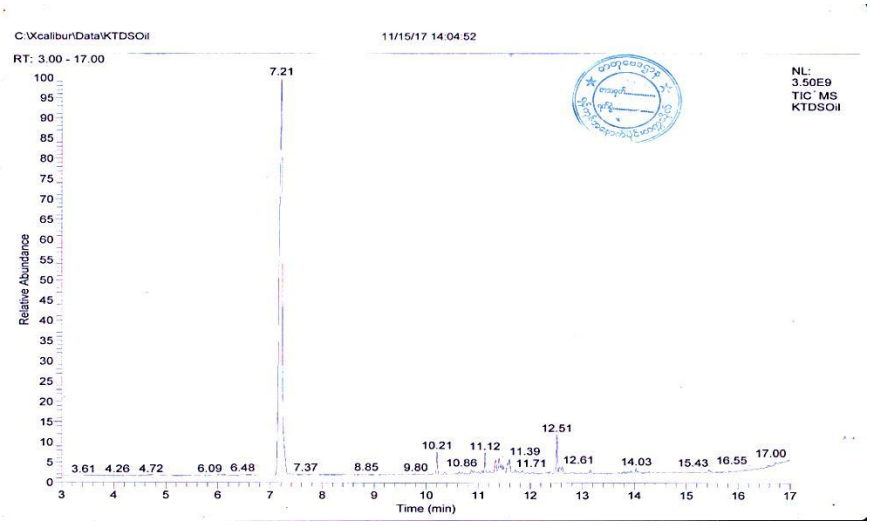


Figure 5. GC-MS Chromatogram of Essential Oil from the Bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo)

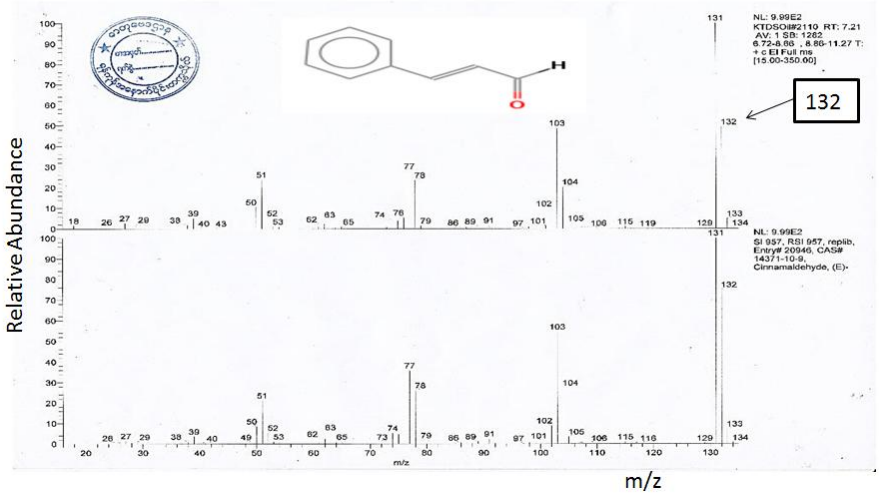


Figure 6. Mass Spectrum of Compound A at Retention Time 7.21 min and (E)-Cinnamaldehyde

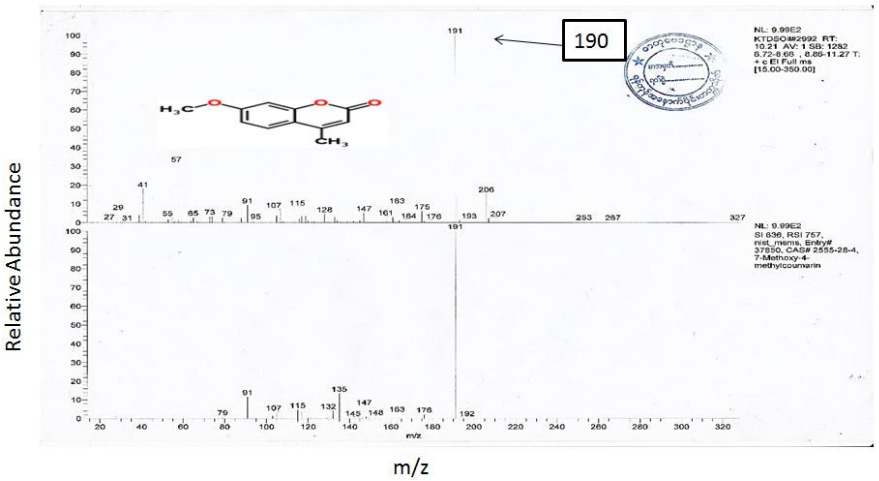


Figure 7. Mass Spectrum of Compound B at Retention Time 10.21 min and 7-methoxy-4-methylcoumarin

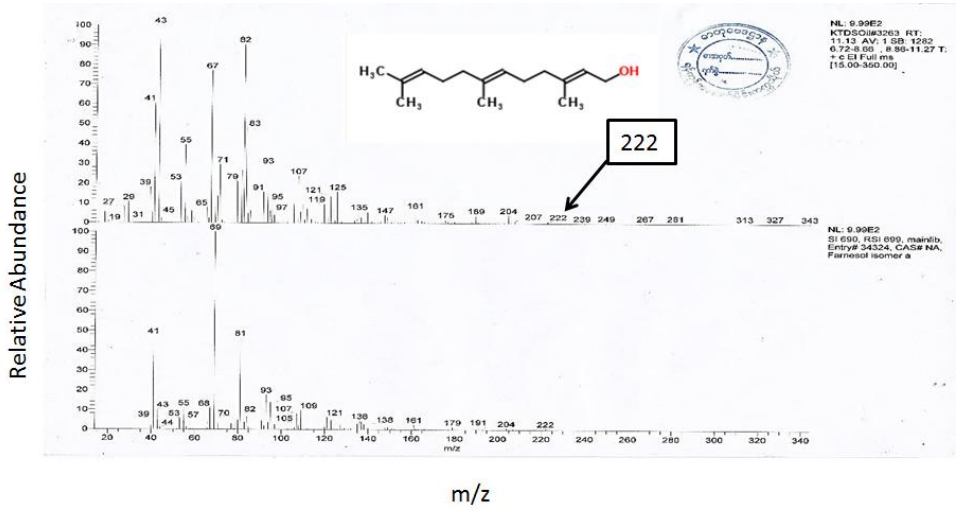


Figure 8. Mass spectrum of Compound C at Retention Time 11.13 min and Farnesol isomer

Conclusion

From overall assessment of the present work concerning with the investigation of antimicrobial activity and identification of essential oil extracted from the bark of *Cinnamomum zeylanicum* Blume. (Thit-kya-bo), the following inferences could be deduced. From elemental analysis, calcium and potassium are the highest content and followed by manganese(Mn), strontium(Sr), iron(Fe) and robidium(Rb) in the bark of Thit-kya-bo determined by EDXRF spectrometer. Moreover, from the results of antiminobial activity by agar well diffusion method, pet-ether extract and ethyl acetate extract of bark of Thit-kya-bo showed a moderate antimicrobial activity against six microorganisms with inhibition zone diameter ranged in 11 mm to 14 mm. In addition, ethanol extract of Thit-kya-bo exhibited antimicrobial activity against all test microorganisms except *Staphylococcus aureus*. Water extract of Thit-kya-bo was observed antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* except *Bacillus pumilus, Candida albicans* and *E-coli.* In water distillation, the solvent was pure without being affected by the solvent. Essential oil (0.2348 %) was extracted

from the bark of Thit-kya-bo and the major component was identified to be (E)-Cinnamaldehyde (m/z 132, R_t 7.21 min, 100 % Relative abundance) 7-Methoxy-4- methyl coumarin (m/z 190, R_t 10.21 min, 50 % Relative abundance) and (2E, 6E)-3,7,11-trimethyl -2,6,10-dodecatrien-1ol (Farnesol isomer), (m/z 222, R_t 11.13 min, 1 % Relative abundance). Consequently, it is widely used in food flavours, cosmetics and pharmaceuticals.

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Online Materials

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