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Extraction and Isolation of Pure Bioactive Organic Compound from Root of *Croton oblongifolius* Roxb. (Thetyin-gyi)

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Abstract: In this research, one Myanmar indigenous medicinal plant, *Croton oblongifolius* Roxb. (Thetyin-gyi) collected from Loikaw University campus, in Kayah State, Myanmar, was selected for the chemical analysis. The aim of the present research is to be aware of the local people of the effective use of root and stem bark of Thetyin-gyi amply grown in Myanmar. Preliminary phytochemical constituents of root and stem bark of Thetyin-gyi were determined by standard methods. Some phytochemical constituents such as alkaloid, flavonoid, steroid, terpene, glycoside, phenolic compound, reducing sugar and saponin were observed in the samples. The elemental composition of the samples was also determined by using Energy Dispersive X-ray Fluorescence (EDXRF) spectrophotometer. The percentage of calcium and potassium were found the highest amount in the samples.

The percentage of manganese, iron, copper, rubidium and strontium were found in the range of 6.054 to 0.131%. The antimicrobial activities of the samples were tested by agar well diffusion method on six types of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican and Escherichia coli*. Furthermore, a pure compound was isolated from the root of Thetyin-gyi as white crystal (0.02 g) by using thin layer and column chromatography.

The yield percent was found to be (4.54 %) based upon the ethyl acetate crude extract. Moreover, antimicrobial activities of this pure compound were rechecked by using agar well diffusion method. The isolated pure compound responded to medium activities on three tested organisms such as *Bacillus pumilus, Candida albican* and *Escherichia coli*. Finally, the functional groups of isolated pure compound could be determined by FT-IR spectroscopic method. Present investigations indicate that locally available Thetyin-gyi contains active components of great medicinal values.

Keywords: *Croton oblongifolius*, antimicrobial activities, thin layer and column chromatography, FT-IR.

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Introduction

The plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered. Large numbers of plants are constantly being screened for their possible pharmacological value (Singh, 2011). Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Grover *et al.*, 2002). Medicinal Plants have been contributing for the management of cancer since time immemorial. Plants are sources of natural antioxidants, and some of the compounds have significant antioxidative properties and health benefits (Exarchou *et al.*, 1998).

The cancer chemopreventive activities of naturally occurring phytocompounds are of great interest (Shahidi *et al.*, 1992). Historically, nature has been provided a source of medicinal products which lead for the treatment of human diseases. Natural products provide us a source of structurally novel and highly bioactive agents; some of them are in clinical use since a long time. Due to the rapid development of new diseases and resistance microorganisms, the research in natural compounds are of enormous interest and forcing to explore new compounds coming from different sources (Gordaliza, 2010 and 2012; Menna *et al.*, 2013).

Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda, a rather encyclopedia in Myanmar. Extraction of the bioactive plant constituents has always been a challenging task for the researchers.

The *Croton oblongifolius* genus belongs to the Euphorbiaceae family. It is a middle-sized tree and grows in India and Thailand (Ah Shin Nagathein, 1976). It grows wild, more common in Magway, Mandalay and Sagaing District, Myanmar. It is a very useful plant and it plays a great part in the field of Myanmar indigenous medicine. The root, stem bark, leaf and seed of Thetyin-gyi are used medicinally (Shaikh, 2006). The root possesses much medicinal value.

The leaves and flowers are used as salad and soup. The roots are used to treat dysentery. The leaves are used as tonic, the flowers against flatworms, the fruits to treat dysmenorrhoea. The bark is also used to treat chronic enlargement of the liver and remittent fever. It is applied externally to the hepatic region in chronic hepatitis (Salatino *et al.*, 2007).

Decoction of the root bark with black pepper is given in diarrhoea and dysentery. The seeds and fruits are purgative. Various parts of the plants are used in spleen troubles, madness, epilepsy, convulsion, scabies, venereal sores, syphilis, ulcers, hydrocele, cholera, neuralgia and pneumonia (Tun & Than, 2006).

In this research, determination of phytochemical constituents, mineral content and antimicrobial activities of root and stem bark of Thetyin-gyi collected from Loikaw University campus were carried out. Furthermore, extraction and isolation of pure bioactive organic compound from root of Thetyin-gyi were carried out.

Materials and Methods

Sample Collection

The root and stem bark of *Croton oblongifolius* Roxb. (Thetyin-gyi) were collected from Loikaw University campus in Kayah State, Myanmar. These were cleaned from dust, washed with water, sliced and dried at room temperature for one month. The dried materials were powdered by using grinding machine, and stored in airtight glass bottles until they were used.

Figure 1. The tree, root and stem bark of Thetyin-gyi

Preliminary Phytochemical Test

The phytochemical constituents of the samples were determined by standard methods (Harborne, 1984). The results were shown in table 1.

Elemental Analysis of the Crude Samples

The elemental composition of the ground and sieved samples were examined by EDXRF spectrophotometer at Universities' Research Center, University of Yangon. The resulting data of the samples were shown in figure 2 and 3.

Antimicrobial Activities of the Crude Samples

Four solvent extracts of each sample were sent to the Pharmaceutical Research Department, Ministry of Industries, Insein, Yangon (Myanmar) for the measurement of antimicrobial activities. In this experiment, antimicrobial activities of each sample were tested on six organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Escherichia coli* by using agar well diffusion method. The results were described in table 3.

Extraction and Isolation of Pure Compound from the Root of Thetyin-gyi

Air dried sample 200 g was percolated with 95% ethanol (400 ml) for about two months. Percolated solution was filtered and concentrated to yield residue. It was extracted with ethyl acetate and evaporated. The ethyl acetate crude sample (0.44 g) was obtained. It was separated by column chromatography over silica gel (70-230 mesh) eluted by various volume ratios of n-hexane and ethyl acetate from non-polar to polar.

After the column separation, total (88) fractions were obtained. Each fraction was checked by TLC. These fractions were combined according to same R_f value under UV lamp and iodine vapour. Eleven combined fractions were obtained. Finally, the fraction (E) gave one spot on TLC in (R_f = 0.55) with n-hexane : ethyl acetate (3:2 v/v) and UV active.

The pure compound white crystal (0.02 g), was obtained. The yield percent was found to be (4.54%) based upon the ethyl acetate crude extract.

Antimicrobial Activities of Pure Compound

Antimicrobial activities of pure compound were rechecked by using agar well diffusion method with six organisms. The results were shown in figure 4.

Spectroscopic Study of Pure Compound

Pure compound was subjected to analysis by FT-IR spectrophotometer at the Department of Chemistry, Monywa University. The results were described in table 4.

Results and Discussion

The results obtained from phytochemical screening of root and stem bark of Thetyin-gyi were tabulated in table 1.

Table 1. Results of Phytochemical Tests of the Samples

No.	Test	Solvent Extract	Reagent	Observation	Root	Stem bark
1	Alkaloid	1% HCl	Wagner's reagent	Brown ppt	+	+
			Mayer's reagent	White ppt	+	+
			Dragendroff's reagent	Orange ppt	+	+
2	Flavonoid	95% EtOH	EtOH, Mg ribbon, conc:HCl	Pink colour	+	+
3	Steroid	petroleum ether	$(CH_3CO)_2O$, conc:H_2SO_4	Blue to green	+	+
4	Terpene	chloroform	$(CH_3CO)_2O$, conc:H_2SO_4	Red or pink	+	+
5	Glycoside	Distilled water	glacial acetic acid, 5% $FeCl_3$, conc:H_2SO_4	Bluish green colour	–	+
6	Phenolic compound	Distilled water	5% $FeCl_3$ solution	Brown colour	–	+
7	Reducing sugar	Distilled water	Benedict's solution	Brick red ppt	+	+
8	Saponin	Distilled water	Distilled water	Formation of frothing	+	+
9	Tannin	Distilled water	1% gelatin solution	White ppt	–	+
(+) Presence of constituent , (–) Absence of constituent						

According to this table, the root and stem bark of Thetyin-gyi contain alkaloid, flavonoid, steroid, terpene, reducing sugar and saponin respectively. Moreover, the stem bark of Thetyin-gyi consists of glycoside, phenolic compound and tannin.

The results of the root and stem bark of Thetyin-gyi by EDXRF spectrophotometer were shown in figure 2 and 3. These results were compared in table 2.

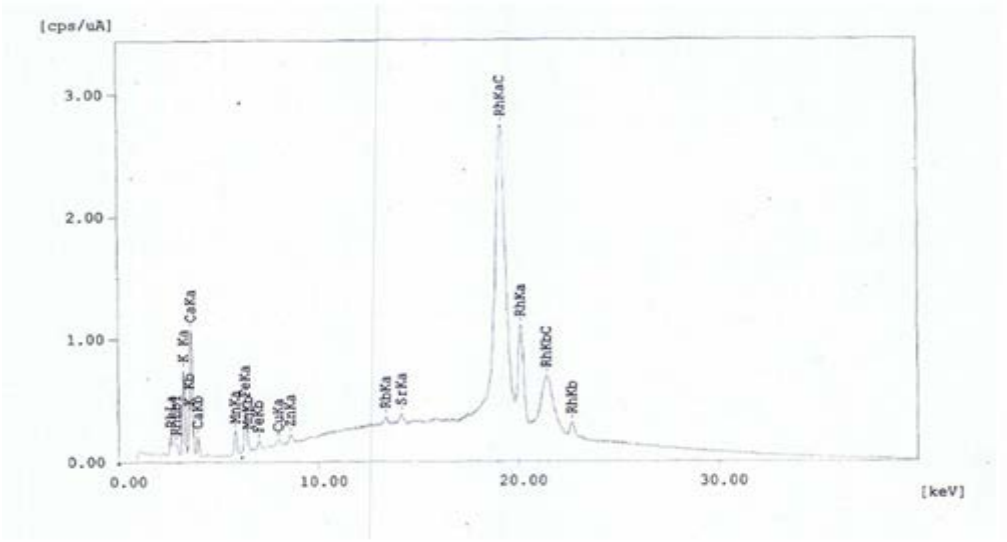


Figure 2. EDXRF spectrum of the root of Thetyin-gyi

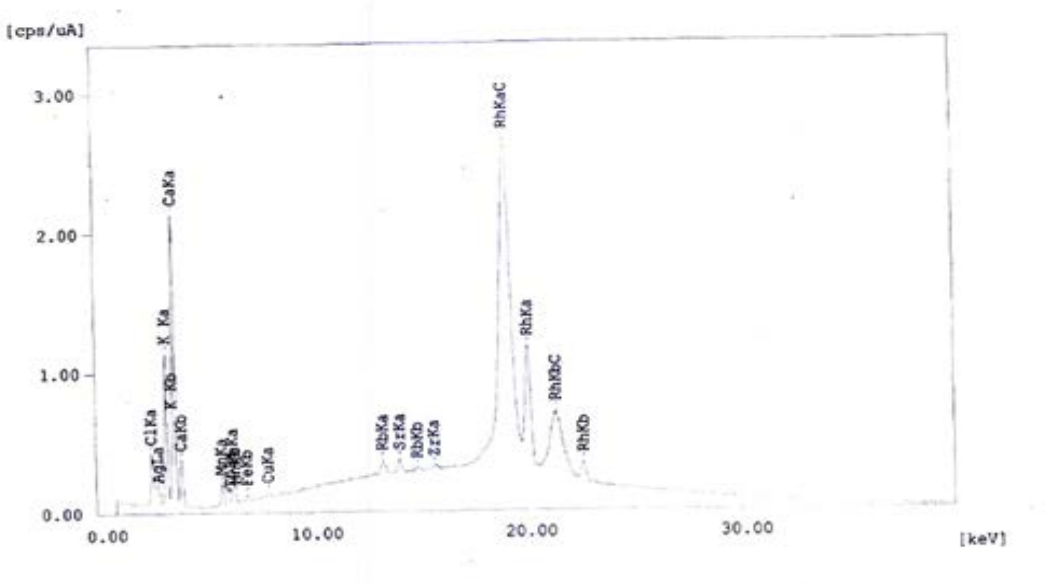


Figure 3. EDXRF spectrum of the stem bark of Thetyin-gyi

Table 2. Results of Mineral Content of the Samples

No.	Element	Root, amount (%)	Stem bark, amount (%)
1.	Chlorine (Cl)	-	13.816
2.	Potassium (K)	28.855	26.624
3.	Calcium (Ca)	59.267	56.078
4.	Manganese (Mn)	3.933	1.382
5.	Iron (Fe)	6.054	1.419
6.	Copper (Cu)	0.602	0.158
7.	Zinc (Zn)	0.705	-
8.	Rubidium (Rb)	0.276	0.179
9.	Strontium (Sr)	0.307	0.132
10.	Zirconium (Zr)	-	0.044
11.	Terbium (Tb)	-	0.170

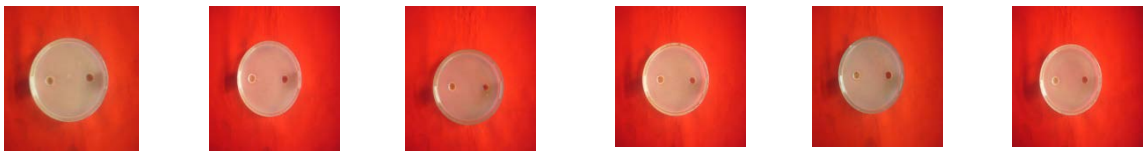
The results of antimicrobial activities of the crude samples and pure compound were compared in table 3.

Table 3. Antimicrobial Activities of the Crude Samples and Pure Compound

Samples	Solvent	Organisms					
		I	II	III	IV	V	VI
Thetyin-gyi (root)	n-hexane	12mm (+)	12mm (+)	12mm (+)	12mm (+)	12mm (+)	14mm (+)
	EtOAc	13mm (+)	11mm (+)	12mm (+)	15mm (++)	15mm (++)	16mm (++)
	EtOH	12mm (+)	11mm (+)	12mm (+)	12mm (+)	13mm (+)	12mm (+)
	H_2O	-	-	-	-	-	-
Thetyin-gyi (stem bark)	n-hexane	11mm (+)	11mm (+)	11mm (+)	11mm (+)	12mm (+)	22mm (+++)
	EtOAc	12mm (+)	12mm (+)	12mm (+)	13mm (+)	13mm (+)	17mm (++)
	EtOH	13mm (+)	13mm (+)	14mm (+)	14mm (+)	14mm (+)	20mm (+++)
	H_2O	-	-	-	-	-	-
Pure Compoud	EtOH	15mm (++)	13mm (+)	14mm (+)	16mm (++)	16mm (++)	15mm (++)

Organisms
I - *Bacillus subtilis* (N.C.T.C-8236)
II - *Staphylococcus aureus* (N.C.P.C-6371)
III - *Pseudomonas aeruginosa* (6749)
IV - *Bacillus pumilus* (N.C.I.B-8982)
V - *Candida albicans*
VI - *Escherichia coli* (N.C.I.B-8134)

Agar well ~ 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)



(I) *Bacillus stubtilis* (II) *Staphylococcus aureus* (III) *Pseudomonas aeruginosa* (IV) *Bacillus pumilus* (V) *Candida albicans* (VI) *Escherichia coli*

Figure 4. Antimicrobial activities of the pure compound

According to these data, ethanol and n-hexane crude extracts of stem bark of Thetyin-gyi give high potent activity and ethyl acetate extract shows medium activity on E-coli. Ethyl acetate extract of root of Thetyin-gyi shows medium activity on *Bacillus pumilus*, *Candida albicans* and *E-coli.*

Moreover, the pure compound responds to medium activity on *Bacillus subtilis*, *Bacillus pumilus*, *Candida albicans* and *E-coli.* The results of antimicrobial activities of the pure Compound were shown in figure 4. FT-IR spectrum of pure compound was shown in figure 5.

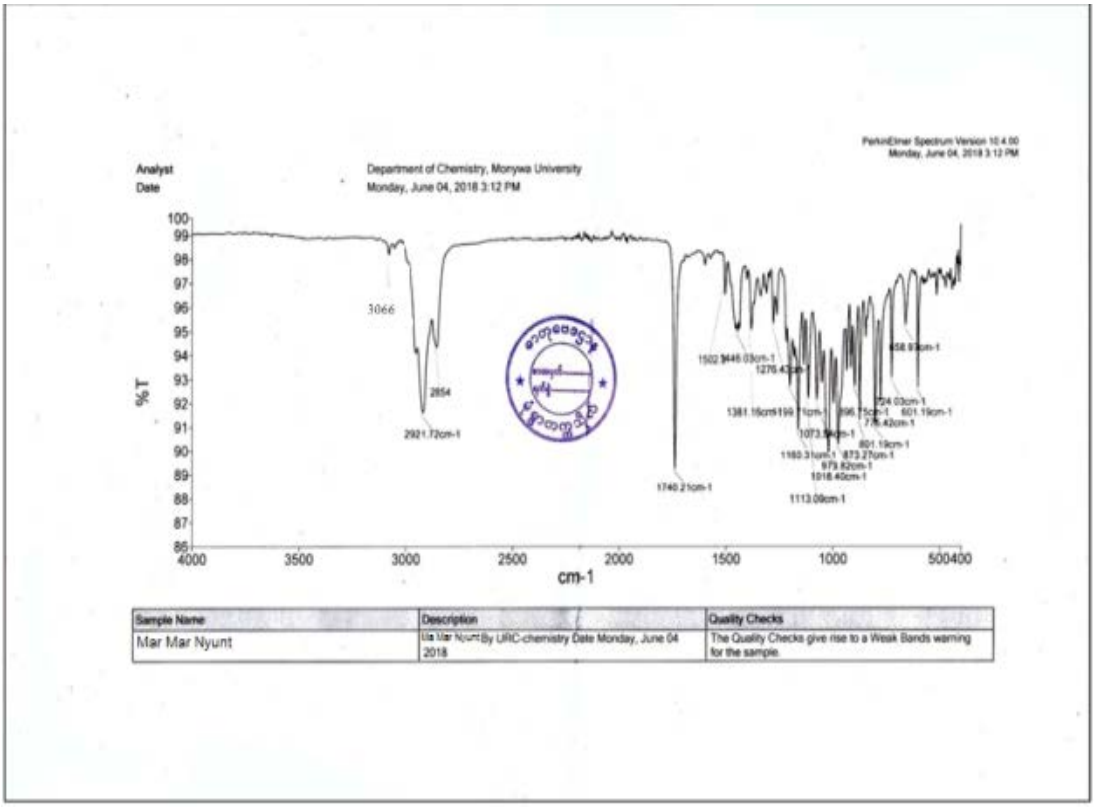


Figure 5. FT-IR spectrum of pure compound

In the spectrum of pure compound, the band which appears at 3066 cm^{-1} indicates C=C - H stretching vibration of sp^2 hydrocarbons. The band which appears at 2921.72 cm^{-1} and 2854 cm^{-1} are due to asymmetrical and symmetrical C-H stretching vibration of sp^3 hydrocarbons. The band at 1740.21 cm^{-1} should be (C=O) stretching frequency of carbonyl group. The band at 1446.03 cm^{-1} is due to C=C stretching vibration of alkenic group. The band at 1381.16 cm^{-1} should be C-H bending vibration of sp^3 hydrocarbons. The band at 1276.4 cm^{-1} is due to (C-CO-O) stretching frequency of ester group. The band at 973.82 cm^{-1}should be C-H out of plane bending vibration of E or trans alkene. The two bands at 776.42 and 724 cm^{-1} are indicated C-H out of plane bending vibration of Z or cis alkene.

The functional groups observed in FT-IR spectrum for the pure compound were tabulated in table 4.

Table 4. Absorption Peak in FT-IR Spectral Data of Pure Compound

No.	Wave Number (cm^{-1})	Functional Groups
1	3066	C=C - H stretching vibration of sp^2 hydrocarbons
2	2921.72 , 2854	asymmetrical and symmetrical C-H stretching vibration of sp^3 hydrocarbons
3	1740.21	C=O stretching frequency of carbonyl group
4	1446.03	C=C stretching vibration of alkenic group
5	1381.16	C-H bending vibration of sp^3 hydrocarbons
6	1276.43	C-CO-O stretching frequency of ester group
7	973.82	C-H out of plane bending vibration of E or trans alkene
8	776.42, 724	C-H out of plane bending vibration of Z or cis alkene

Conclusion

In the present study, Thetyin-gyi selected for chemical analysis was based on its traditional health, local availability and distribution. The root and stem bark of Thetyin-gyi were collected from Loikaw University campus. Qualitative phytochemical screening showed alkaloid, flavonoid, steroid, terpene, reducing sugar and saponin were observed in two samples. In the elemental analysis: potassium, calcium, manganese, iron, copper, rubidium and strontium, were observed in two samples. The percentage of calcium and potassium were found the highest amount in the samples. As a result, these samples help to generate muscles, nerves and skin cells in human body. It also helps bone stay strong. In contrast, trace amount of two heavy metals such as zirconium and terbium were found in stem bark of Thetyin-gyi. As a results, zirconium in stem bark of Thetyin-gyi can cause the formation of granulomas in the lungs if it is inhaled. Terbium in stem bark of Thetyin-gyi is very irritating if they come into contact with the skin and the eyes. Antimicrobial activities of each sample were performed by four solvent extracts by using Agar-well diffusion method. Among them, ethyl acetate extract of stem bark of Thetyin-gyi shows medium activity on *E-coli*. Ethanol, ethyl acetate and n-hexane extracts of root of Thetyin-gyi show low activities on all tested organisms. Ethanol and n-hexane extracts of stem bark of Thetyin-gyi respond to high potent activities on *E-coli*.

Furthermore, pure compound, white crystal (0.02 g, 4.54%) based upon the ethyl acetate crude extract, could be isolated from the ethyl acetate extract of root of Thetyin-gyi by using thin layer and column chromatography. The antimicrobial activity of this pure compound responds to medium activities on three tested organisms such as *Bacillus pumilus, Candida albican* and *E-coli*. In addition, functional groups of pure compound could be determined by using FT-IR spectral data. Present investigations indicate that locally available Thetyin-gyi contains active components of great medicinal values. Therefore Thetyin-gyi could be considered as “a gift of nature”. More research on the study of isolation and identification of pure bioactive organic compounds from the stem bark of Thetyin-gyi can be done.

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