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Phytochemical Screening, Determination of Antimicrobial Activities of Three Myanmar Indigenous Medicinal Plants and Identification of Functional Groups from *Vitex limonifolia* Wall (Kyun-khauk-nwe)

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Abstract

Three Myanmar indigenous medicinal plants were collected and tested for their phytochemical constituents. Moreover, the antibacterial activities of these plants were performed by agar well diffusion method and minimum inhibitory concentration (MIC) method. A pure bioactive organic compound could be isolated from the barks of *Vitex limonifolia* Wall (Kyun-khauk-nwe) by using separation methods, such as Thin Layer and Column Chromatography. In addition, the bioactivities of this pure compound was re-examined by using MIC (Minimum Inhibitory Concentration) method and the prominent functional groups could be assigned by using FT-IR spectrum.

Introduction

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimation suggests that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs.

Few plant species that provide medicinal herbs have been scientifically evaluated for their possible medical application. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and developing countries.

Medicinal plants are of great economic importance. They are used as raw materials for the extraction of active constituents in pure form, as precursors for synthetic vitamins and steroids and as preparations for herbal and indigenous medicines. Plants are not only the major source of energy rich foods in most societies, but are also an indispensable source of vitamins and other substances promoting healthy growth. In the last 30 years there has been a resurgence of interest in the use of plants as medicines. It is important to distinguish between three different types of medicine traditional, herbal and pharmaceutical.

Nowadays, plant materials are used throughout the industrialized and developing countries as home remedies, over the counter drugs and raw materials for the pharmaceutical industry. In general all medicines, whether they are of synthetic or plant origin, should fulfil the basic requirements of being efficacious and safe. In Myanmar, there are many medicinal plants. Most of them have not been identified yet. Therefore, the study of indigenous medicinal plants and their usage in therapy plays a very important role.

In the present work, three medicinal plants were selected for phytochemical tests, antibacterial activities tests and the prominent functional groups determination of compound in this filled. They are *Vitex limonifolia* Wall (Kyun-khauk-nwe), *Rhus cotinus* Linn (Byi-sin), *Aerua javanfa* (Ohn-phwe). In this research work, Kyun-khauk-nwe was selected only to correlate traditional thrapeutive uses and to develop the Myanmar traditional medicine for local uses.

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Aim

The aim of the paper is to determine phytochemical screening, antibacterial activities and the prominent functional groups from selected medicinal plants.

Objectives

- To study phytochemicals, antimicrobial activities and the prominent functional groups of compound from three medicinal plants
- To correlate traditional therapeutic uses and to develop the Myanmar traditional medicine for local uses.

Botanical Description

Family : Verbenaceae
Scientific name : *Vitex limonifolia* Wall
Myanmar name : Kyun-khauk-nwe
Part used : Bark

Outstanding Features

Perennial, deciduous trees, up to 15 m high; have with wolly quadrangular young branches, palmately trifoliate-compound. Leaves with broadly winged petioles, terminal panicles of fascicled cymes with many violet flowers, and small black drupes bearing dark purple juice, subtended by persistent bracts and calyx, smooth. Seeds oblong or obovate. It is frequently found in the forests and flowering and fruiting period is from June to September.

Medicinal Uses

The barks, leaves, flowers, fruits or roots of various *Vitex* species are used as a general tonic, anthelmintic and in the treatment of gestro-intestinal disorders.

Materials and Methods

In this paper, one Myanmar indigenous medicinal plant, namely *Vitex limonifolia* Wall, was selected for detailed chemical analysis.

Sample Collection

The bark of *Vitex limonifolia* Wall (Kyun-khauk-nwe) was collected at Mya-Kha-Naut Hill in Patheingyi Township, Mandalay Region. Then it was chopped into small pieces, dried in the air and stored in stoppered bottle and used throughout the experiment.

Phytochemical Screening of Three Selected Myanmar Indigenous Medicinal Plants

The preliminary phytochemical tests were done on all selected plants and the results obtained are tabulated in Table (1).

Yadanabon University Research Journal, 2019, Vol-10, No.1

Table 1. Results of Phytochemical Tests on Plant Extract

No	Tests	Reagent used	Sample		
			I	II	III
1	Alkaloid	Dragendroff's reagent	+	+	+
2	Flavonoid	dil HCl, Mg turnings	+	+	–
3	Terpene	$CHCl_3$, conc: H_2SO_4, acetic anhydride	+	–	+
4	Steroid	$CHCl_3$, conc: H_2SO_4, acetic anhydride	–	+	–
5	Saponin	EtOH, conc: H_2SO_4	–	+	–
6	Polyphenol	10 % $FeCl_3$ solution	+	–	+
7	Glycoside	10 % lead acetate	+	+	+

– = absence

+ = presence I = Kyun-khauk-nwe, II = Byi-sin, III = Ohn-phwe

According to this table, these plants extracts consist of alkaloid, steroid, flavonoid, terpene, saponin, polyphenol and glycoside respectively.

Antimicrobial Activities of Three Myanmar Indigenous Medicinal Plants

Four solvent extracts of Kyun-khauk-nwe, Byi-sin and Ohn-phwe were sent to DCPT, Insein, Yangon, for the measurement of antibacterial activities applying agar well diffusion method on six organisms. These results are tabulated in Table (2).

Table 2. Antimicrobial Activities of Selected Myanmar Indigenous Medicinal Plants

Sample	Solvents	Organisms					
		I	II	III	IV	V	VI
Kyun-khauk-nwe	n-hexane	+	–	–	–	+	++
	$CHCl_3$	+++	–	+	+	+	++
	EtOAc	++	++	++	+++	++	++
	EtOH	+	++	++	++	++	++
Byi-sin	n-hexane	–	–	–	–	–	–
	$CHCl_3$	–	–	–	++	–	–
	EtOAc	+	++	++	++	++	++
	EtOH	+	+	++	++	++	++
Ohn-phwe	n-hexane	+	+	–	+	–	–
	$CHCl_3$	–	+	+	–	–	–
	EtOAc	+++	+++	+++	+++	+++	+++
	EtOH	++	++	++	+	–	++

Agar well – 10 mm

10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++)

20 mm above (+++)

Organisms

I = *Bacillus subtilis*

II = *Staphylococcus aureus*

III = *Pseudomonas aeruginosa*

IV = *Bacillus pumalis*

V = *Candida albican*

VI = *Mycobacterium* species

According to these results from Table (2), the ethyl acetate and ethanol of Kyun-khauk-nwe have the higher activities on organisms.

Yadanabon University Research Journal, 2019, Vol-10, No.1

Extraction and Isolation of Pure Compound from *Vitex limonifolia* Wall (Kyun-khauk-nwe)

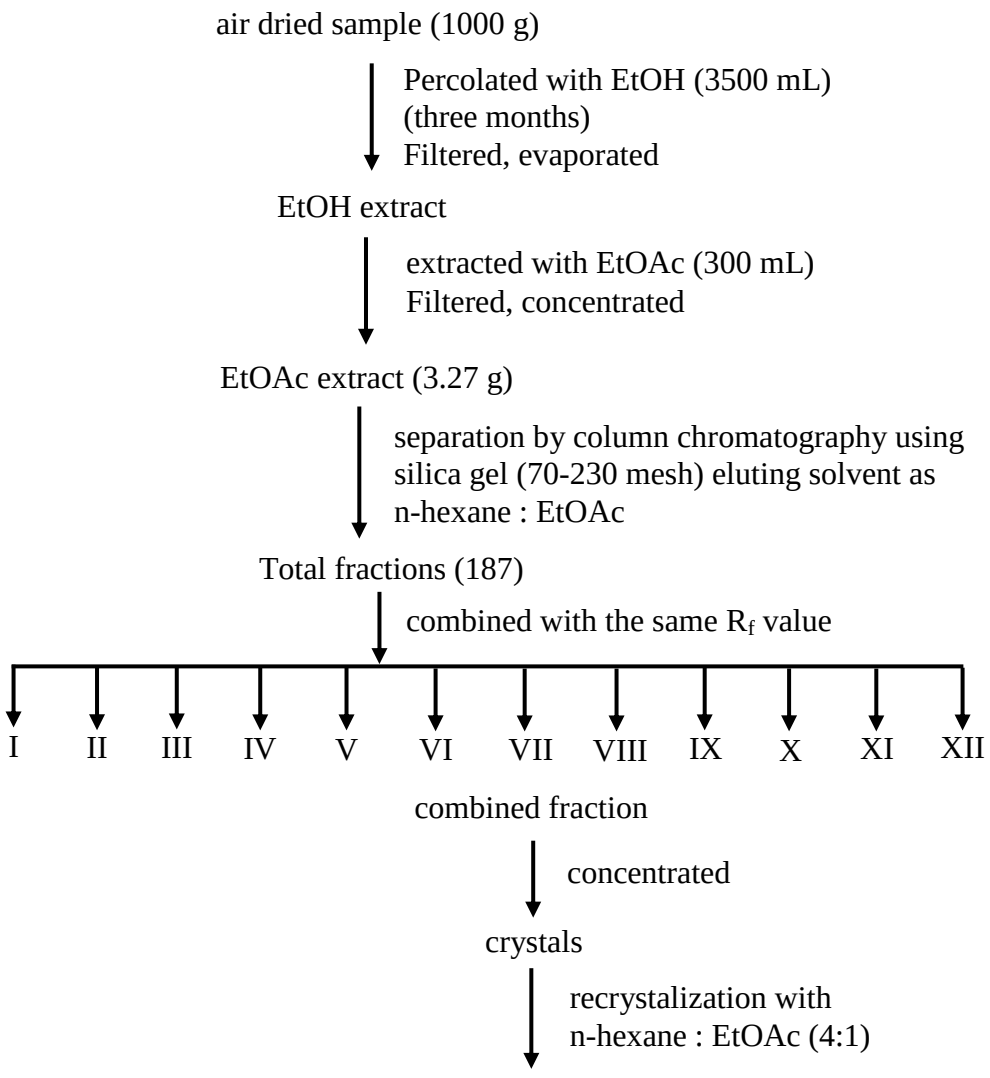
The air dried barks of Kyun-khauk-nwe (1000 g) were extracted with 95 % ethanol (3500 mL) for three months. The extract solution was filtered and the filtrate was evaporated in vacuum. The residue obtained was reextracted with ethyl acetate (300 mL). This ethyl acetate extract was evaporated and concentrated.

The ethyl acetate extract (3.27 g) was chromatographed on a silica gel column, eluting with n-hexane and ethyl acetate with various ratios from non-polar to polar, to produce (174) fractions. Each fraction was checked by TLC with suitable solvent system. The fractions with the same R_f value were combined to give (12) combined fractions.

The major one is combined fraction (VII), because of a single spot on TLC under UV detector, and the highest yield. So, it was evaporated in vacuum yielding 50.7 mg of needle shape crystal compound. Then it was washed with n-hexane and ethyl acetate with various volume ratios giving the pure white needle shape compound (37.9 mg) with the yield of 1.16 % based upon the crude EtOAc extract.

The FT-IR spectrum of an isolated pure compound was measured at the Department of Chemistry, University of Mandalay.

Flow Sheet for the Isolation of Pure Compound from Kyun-khauk-nwe



Yadanabon University Research Journal, 2019, Vol-10, No.1

The Antimicrobial Activities of Pure Compound by Microplate Dilution Method

The antimicrobial activities of pure compound were rechecked with MIC (minimum inhibitory concentration) by micro plate dilution method applying on eight selected organisms in DMR (Department of Medical Research), Yangon. These results are shown in Table (3).

Table 3. Results Antimicrobial Activities of Pure Compound

Organisms	Downstream Serial Dilution (mg/mL)										
	I 2	II 1	III 0.5	IV 0.25	V 0.125	VI 0.62	VII 0.31	VIII 0.015	IX 0.007	X 0.0035	XI 0.0017
A	–	–	+	+	+	+	+	+	+	+	+
B	–	+	+	+	+	+	+	+	+	+	+
C	–	–	–	–	–	3	7	23	+	+	+
D	–	–	+	+	+	+	+	+	+	+	+
E	–	–	3	7	+	+	+	+	+	+	+
F	–	+	+	+	+	+	+	+	+	+	+
G	–	–	+	+	+	+	+	+	+	+	+
H	–	–	–	–	–	–	–	–	–	3	7

(–) = Absence, (+) = uncountable colony, (No:) Number of colony

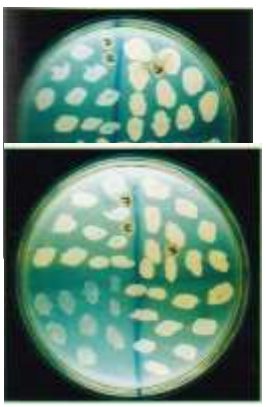
Organisms

A = *Proteus morganii*
B = *Satmonella typhi*
C = *Staphylococcus aureus*
D = *E. coli* ATU
E = *Vibrio parahaemlytius*
F = *Shigella fluxnei*
G = *Bacillus subtilis*
H = *Vibrio cholerae* 0139

According to this table, pure compound responds the highest activities (lowest MIC value) on one selected bacterium (H) *Vibrio cholerae*.

The Antibacterial Activities of Pure Compound by Microplate Dilute Method

Various Concentration of Pure Compound with Eight Selected Organisms



Antibacterial Activities of Pure Compound with Eight Selected Organisms

Results and Discussion

The FT-IR spectrum of an isolated pure compound was measured at the Department of Chemistry, University of Mandalay and it was shown in Figure (1).

The band at 3394.5 cm^{-1} indicates the O-H stretching vibration of alcohol group. The band which appear at 3090 cm^{-1} should be C-H stretching vibration of cis or Z alkene. The two intense peaks at 2923.9 cm^{-1} and 2846.7 cm^{-1} imply the C-H stretching vibration of unsymmetrical and symmetrical sp^3 hydrocarbons.

The peak at 1681.8 cm^{-1} indicates the carbonyl group and the peak at 1596.9 and 1512.1 cm^{-1} indicate the C=C stretching vibration of alkenic group. Furthermore, the existence of C-H in plane bending vibration of hydrocarbon could be determined at 1419.5 cm^{-1}, 1288.4 cm^{-1} and 1242.1 cm^{-1}. The peak at 1164.9 and 1103.2 cm^{-1} indicate the mixed C-C-O stretching vibration of alcohol and C-O-C asymmetrical stretching vibration of ether group.

The existence of trans or E and cis or Z alkenes could be detected at 933.5 and 848.6 cm^{-1}. The peak at 617.2 cm^{-1} shows the C-H out of plane bending vibration.

Table 4. The Absorption Peaks and their Assignment of Pure Compound

Absorption peaks (cm^{-1})	Assignments
3394.5	O-H stretching vibration
3090	C-H stretching vibration of alkene group
2923.9, 2846.7	Unsymmetrical and symmetrical stretching of sp^3 hydrocarbons
1681.8	C=O stretching vibration
1596.9, 1512.1	C=C stretching vibration of aromatic group
1419.5, 1288.4	C-H in plane bending of allylic and normal hydrocarbon
1164.9, 1103.2, 1242.1	C-C-O (stretching of alcohol) and C-O-C stretching of ether group
933.5, 848.6	C-H out of plane bending vibration of trans or E and cis or Z alkene
617.2	C-H out of plane bending vibration

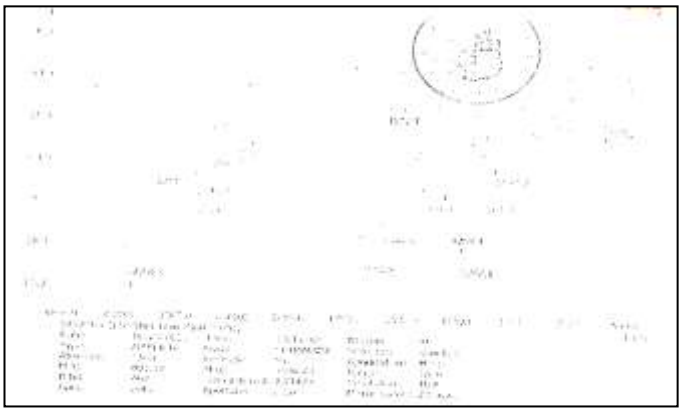


Figure 1. FT-IR Spectrum of Pure Compound

Conclusion

In this paper, firstly, three Myanmar indigenous medicinal plants (Kyun-khauk-nwe, Byi-sin, Ohn-phwe) were selected for phytochemical screening and antibacterial activities. The pure bioactive organic compound from the barks of Kyun-khauk-nwe was isolated by advance chromatographic methods such as Thin Layer and Column Chromatography. The antimicrobial activities of this compound responds the highest activities (lowest MIC value) on one selected bacterium *Vibrio cholerae*. Therefore, this compound may be regarded as effective anti-*Vibrio cholerae*.

The functional groups of this compound were determined by using FT-IR spectrum such as alcohol group, carbonyl group, sp^3 hydrocarbon, ether group, cis or Z alkene and trans or E alkene present in this compound. According to the result, Kyun-khauk-nwe can be applied for traditional therapeutive uses and to develop the Myanmar traditional medicine for local uses.

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