Antimicrobial Activity And Phytochemical Constituents Of *Terminalia Chebula* Retz. (Phan-Kha)

Ei Ei Sann[1], San San Aye[2]

Abstract

Developing countries, where dysentery and diarrhea are endemic, depend strongly on traditional medicine as a source of inexpensive treatments because it is based on plants that are abundantly available in our countries. Consequently, Myanmar medicinal plants (Phan-Kha) which are used for the treatment of dysentery and diarrhea in Myanmar were selected to study in order to find the scientific basis for such use. Polar and non-polar extracts of the Phan-Kha fruits were screened on six microorganisms by agar well diffusion method. Phytochemical examination for types of organic constituents present in Phan-Kha fruits was carried out. The preliminary phytochemical tests indicated that various types of compounds, such as phenolic compounds, glycosides, carbohydrates, amino acids, tannins, steroids and terpenoids were present in the selected fruits samples, except for starch, alkaloids and flavonoids. The polar extracts of Phan-Kha showed antimicrobial activities (17 mm - 40 mm) against all the six microorganisms tested. β-sitosterol (0.36 %) was isolated from Phan-Kha. β-sitosterol isolated from Phan-Kha was confirmed by authentic β-sitosterol and also compared with melting point determination. Moreover, the functional group present in this compound was also confirmed by FT IR determination.

Keywords*:* antimicrobial activities, phytochemical, β-sitosterol, Phan-Kha fruits

Introduction

Terminalia chebula Retz. (Phan-Kha)

Terminalia chebula Retz. of the family Combretceae is an important tree of pharmaceutical and trade value. It is distributed in the forests of Northern India, Eastern India, and the Southern Peninsula. The fruit of the plant is rich in tannin and commonly known as myrobalan or chebulic myrobalan. In Indian pharmacopeia, the fruit of *T. chebula* is extensively used as adjuvants to other medicines in almost all diseases e.g jaundice, plexopathy, hiccough, cephalalgia, epilepsy, leprosy, and astringent, anti-inflammatory, digestive, cardiotonic (Phetkate *et al.*, 2012). The effect of tannin from *T. chebula* on the infectivity of potato virus X was also reported.

The present research work deals with the antimicrobial screening of polar and non-polar extracts and the column chromatographic isolation of some organic constituents of *T. chebula* fruits.

Materials and Methods

Plant materials

The fruits of Phan-Kha were collected from the local market in Yangon Region. These samples were ground to get a fine powder by grinding machine. The drug powders were then stored in an air-tight container.

[1] Lecturer, Dr, Department of Chemistry, Bago University
[2] Professor, Dr, Department of Chemistry, University of Yangon

Preparation of crude extracts

100 g of each dried powdered sample was percolated in 500 mL of petroleum ether (PE, 60-80 °C) for one week and filtered. This procedure was repeated three times. Then the filtrate was concentrated by a vacuum rotatory evaporator to get the respective PE extract. Similarly, ethyl acetate, acetone, and 80 % EtOH extracts of each dried powdered sample were prepared according to the above procedure. In the preparation of watery extract; 100 g of each dried powdered sample was soaked in 500 mL of distilled water into the conical flask. These flasks were boiled in the water bath for 6 h and filtered. This process was carried out three times. The combined filtrates were dried over a water bath at 100 °C to get the corresponding watery extract.

Preliminary Phytochemical Investigation

Phytochemical tests for the fruits of *T. Chebula* were carried out according to the reported methods to investigate the presence and absence of phytoconstituents such as alkaloids, amino acid, carbohydrates, glycosides, flavonoids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoids (M-Tin Wa, 1972 and Harbone, 1984).

Antimicrobial screening

The antimicrobial activity of the prepared crude extracts was determined by the agar well diffusion method. Four small holes of 10 mm diameter each were cut out in the inoculated agar to place samples to be tested. The volume of each sample placed in each well was 0.1 mL. The Petri dish was then incubated at 37 ºC for 48 h, and the diameters of clear inhibition zones around the wells, if appeared, were measured (Finegold and Martin, 1982).

Isolation of compound from *T. chebula* (Phan-Kha)

The dried fruit powder (50 g) was extracted with petroleum ether (60-80 ºC) for about one week by percolation method followed by filtration. PE extract of Phan-Kha was fractionated by column chromatography using PE: ethyl acetate (9:1, 5:1) as eluent. Finally, colorless crystals of β-sitosterol (0.36 %) were isolated. β-sitosterol is one of several phytosterols with a chemical structure similar to that of cholesterol. It is a white, waxy with a characteristic odour. The isolated compound was subjected to Co-TLC analysis with a standard β-sitosterol sample. The chromatogram was developed in the chosen solvent system for the compound isolated. After the plate has dried, the R_f value of the isolated sample was measured. Localization of the spot was made by viewing directly under UV 254 nm and 365 nm light. And then, it was treated with 5 % H_2SO_4, anisaldehyde-H_2SO_4, and ceric sulphate followed by heating, respectively.

Results and Discussion

Phytochemical Constituents in the fruits of *T.Chebula*

The phytochemical constituents present in the leaves of *T.Chebula* were investigated by the test tube method. The phytochemical tests revealed that amino acid, carbohydrates, glycosides, tannins, phenolic compounds, steroids, and terpenoids were present in the sample. However, alkaloids, flavonoids and starch were not detected in the fruits of *T.Chebula*.

Table 1. Result of Preliminary Phytochemical examination of *T.Chebula* (Phan- Kha) fruit

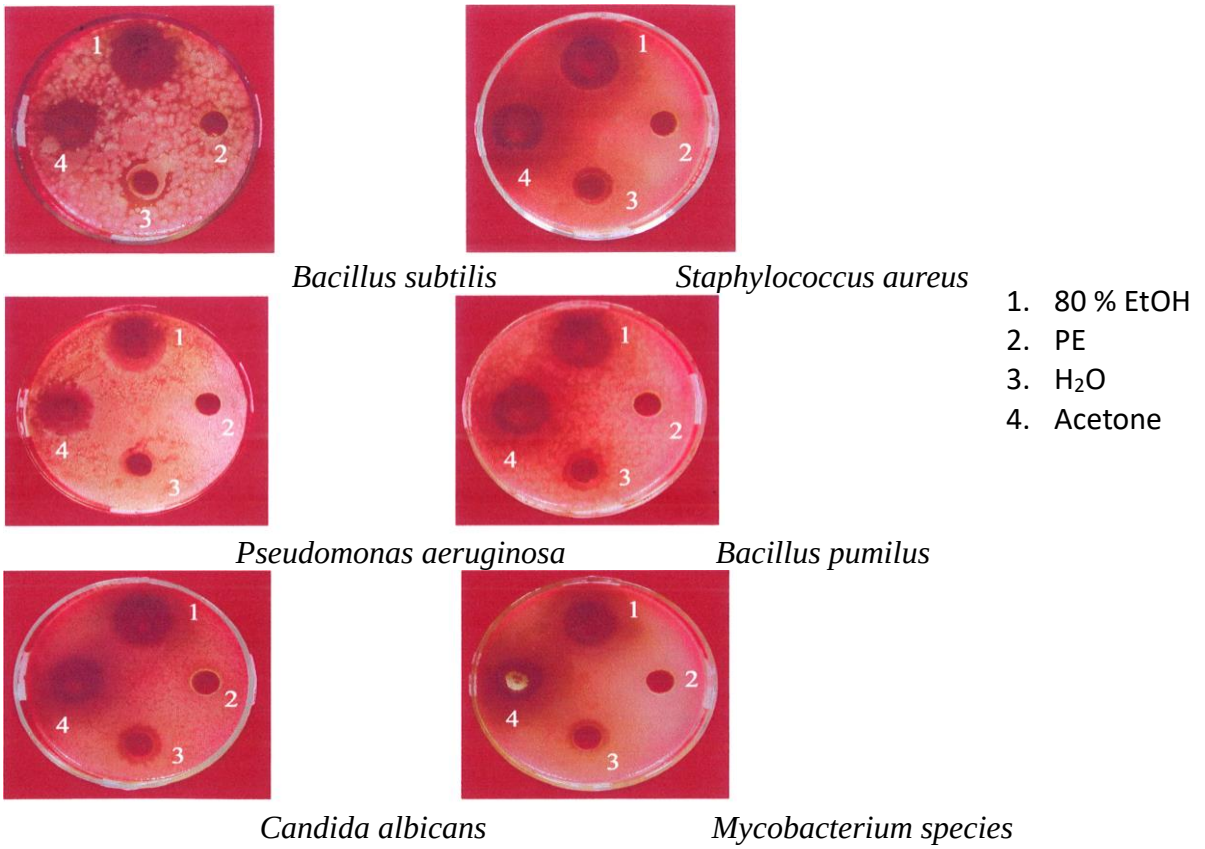
No.	Test	Extract	Test reagent	Observation	Result
1	Alkaloids	1% HCl	Dragendroff's, Mayer's, Wagner's reagent.	no orange ppt. no white ppt. no reddish brown ppt	_
2	Amino acids	H_2O	Ninhydrin reagent	Pink color spot	+
3	Carbohydrates	H_2O	10 % α-naphthol, Conc: H_2SO_4	Red ring	+
4	Phenolic compound	EtOH	1% $FeCl_3$ solution,1%$K_3Fe(CN)_6$	Dark blue color	+
5	Glycosides	H_2O	10 % Lead acetate solution	White ppt.	+
6	Steroids& Terpenoids	PE	Acetic anhydride, Conc: H_2SO_4	Greenish Blue	+
7	Tannin	H_2O	1 % Gelatin	White ppt.	+
8	Starch	H_2O	1 % Iodine solution	no bluish-black ppt.	_
9	Flavonoids	EtOH	Mg turning, Conc: HCl	no pink color	_

Antimicrobial activity

In the present work, the samples were tested on six strains of microorganisms which include *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans,* and *Mycobacterium* species. The measurable zone diameter, including the agar well diameter, shows the degree of antimicrobial activity. It was found that the polar extracts of *T. chebula* showed antimicrobial activities against all the six microorganisms tested (Figure 1 and Table 2). It was also observed that the PE extracts of *T. chebula* did not show any antimicrobial activity. The larger the inhibition zone diameter, the greater is the antimicrobial activity.

Identification of isolated compound

(β-sitosterol): The isolated compound was UV inactive. It was identified by some colour tests on TLC, 5 % H_2SO_4, ceric sulphate solutions, and anisaldehyde H_2SO_4 were employed as spraying agents followed by heating. It was also identified by comparison study (Co-TLC) with standard β-sitosterol (Figure 2). The same R_f values (0.27) were observed for the isolated compound and standard β-sitosterol with PE: EtOAc (5:1). The coloration of the isolated compound was found to be identical to that of standard β-sitosterol with each spraying reagent. It gave violet, brown and purple-bluish colors when it was treated with 5 % H_2SO_4, anisaldehyde-H_2SO_4, and ceric sulphate followed by heating, respectively.β-sitosterol: Colorless needle (0.36 % yield, mp. 138-140 ºC) (Merck index, 2001), FT IR ν_{max}^{kBr} cm^{-1}: 3400(ν O-H), 2939-2877 (ν C-H), 1643 (ν C=C), 1049 (ν C-O) (Figure 3).



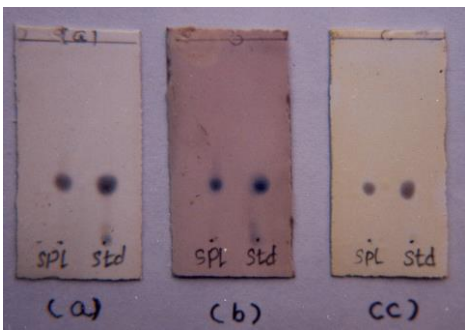
1. 80 % EtOH
2. PE
3. H_2O
4. Acetone

Figure 1. Agar well diffusion tests of *T. chebula* (Phan-Kha) fruit extracts on six microorganisms

Table 2. Results of Antimicrobial Activity of Phan-Kha Fruit

No	Organisms	Inhibition zone diameter (mm)			
		PE	80 % EtOH	Acetone	H_2O
1	*Bacillus subtilis*	–	26	22	20
2	*Staphylococcus aureus*	–	23	20	15
3	*Pseudomonas aeruginosa*	–	22	19	15
4	*Bacillus pumilus*	–	24	25	18
5	*Candida albicans*	–	24	21	17
6	*Mycobacterium species*	–	20	19	17

* Agar well diameter -10 mm, (–) No activity



SPL = Sample
Std = standard β-sitosterol
Spraying agents
(a) = 5 % H_2SO_4
(b) = Anisaldehyde H_2SO_4
(c) = $CeSO_4$
Stationary phase: Silica gel,
Mobile phase : PE: EtOAc (5:1)

Figure 2. Thin layer chromatograms of isolated β–sitosterol from *T. chebula* (Phan-kha)

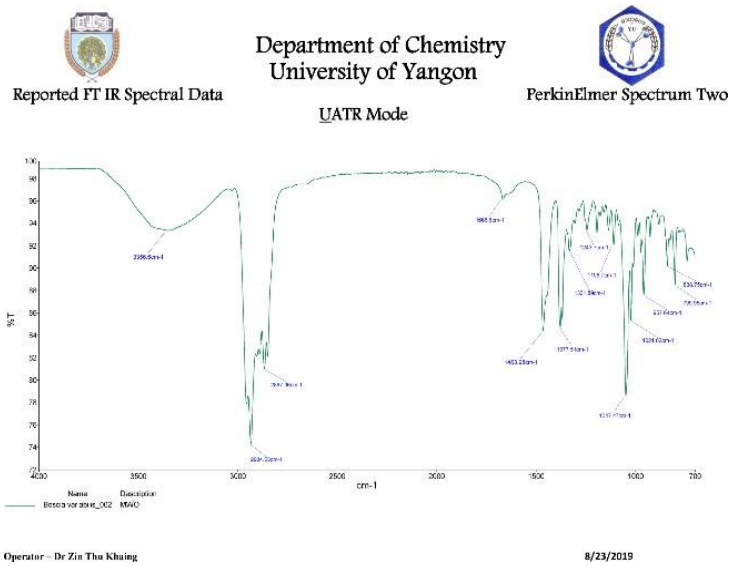


Figure 3 FT IR spectrum data of isolated β-sitosterol from Phan Kha fruits

Table 3. FT IR Spectral Data of Isolated β-sitosterol from Phan Kha fruits

Wave No. (cm $^{-1}$)	Assignment
3356	OH stretching
2934, 2867	CH stretching
1463,1377	CH bending
1047,1021	C-O stretching

Conclusion

From the present research work on "antimicrobial activity and phytochemical constituents of *T. chebula* (Phan-Kha)", the following conclusions can be drawn.

Crude extracts have been prepared from Phan-kha by using non-polar and polar solvents. The antimicrobial activity of the crude extracts was screened by *in vitro* method using agar well diffusion techniques on six microorganisms which include *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus*, *Candida albicans,* and *Mycobacterium* species. Polar extracts of Phan-Kha showed antimicrobial activity against all tested microorganisms (17 mm - 26 mm). However, non-polar extracts of fruits did not show antimicrobial activity. Pet-ether extract of Phan-Kha was separated by column chromatographic method to give β-sitosterol (0.36 %). Therefore, β-sitosterol containing Phan-Kha fruit may be used as a natural antioxidant and antimicrobial agent.

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