PHYTOCHEMICAL CONSTITUENTS, NUTRITIONAL VALUES AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *SARACA INDICA* L. (THAWKA)

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Abstract

Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs. *Saracaindica* L. (Thawka) is an important indigenous plant with lots of traditional importance and has been claimed to possess various activities such as antioxidant, antimicrobial, anticancer, anti-diabetic etc. Therefore the leaf and bark of the plant has been chosen for the investigation of some biological activities. The samples were collected and identified. By phytochemical tests, carbohydrates, glycosides, organic acid, phenolic compounds, saponins, steroids and terpenoids were detected in the leaf; and alkaloids, carbohydrates, flavonoids, organic acids, saponins, starch, steroids, tannins and terpenoids, in the bark. Proximate analysis of the leaf showed ash (6.8 %), moisture (11.25 %), protein (13.24 %), fat (3.84 %), fiber (38.21 %), carbohydrates (26.66 %) and energy value (194 kcal/100 g); and for the bark, ash (12.96 %), moisture (15.96 %), protein (5.55 %), fat (4.15 %, fiber (25.71 %), carbohydrates (35.67 %) and energy value (202 kcal/100 g). Antimicrobial activity was screened by agar well diffusion method on six strains of bacteria. The highest inhibition zones were observed on *Pseudomonas aeruginosa* (32 mm) and *Bacilliuspumilus* (30 mm)for EtOAc extract of the bark, followed by *Bacilliuspumilus*and *Candida albicans*(16 mm each) for EtOAc extract of the leaf. No activity was found in the PE and watery extracts of both of the bark and leaf. According to the spectrophotometric DPPH assay, the antioxidant activity (IC_{50}) decreases in the order, ethanol extract of leaf (2.86 µg/mL), ethanol extract of bark (6.70 µg/mL), watery extract of bark (8.35 µg/mL) and watery extract of leaf (12.98 µg/mL), the reference used being vitamin C (1.17 µg/mL). Two compounds namely **A** (β-sitosteryl acetate), **B**(β-sitosterol) were isolated by SiO_2 column chromatography and identified by FT IR.

***Keywords*:** *Saracaindica* L., phytochemical constituents, antimicrobial activity, antioxidant activity

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Introduction

Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs. *Saracaindica* L. is an important indigenous plant with lots of traditional importance belonging to the family Caesalpinaceae. This plant possesses various activities such as analgesic, antipyretic, fungitoxic, anthelmintic, anti-diabetic, antimicrobial, antiulcer, anti-inflammatory activities etc. It is distributed in evergreen forests of India up to an elevation of about 750 m. It is found throughout Myanmar, India, especially in Himalaya, Kerala, Bengal and whole south region. The plant is perennial and woody throughout. The stems are erect or ascending, greater than 2 m tall, solid, glabrous or sparsely glabrate. Petals are separate which are orange-yellow in colour. Fruit is legume type, unilocular, freely dehiscent, ablong. Both leaf and bark contain epicatechin, catechin, gallic acid, kaempferol, leucopelargonidin, palmitic acid, stearic acid, oleic acid and β-sitosterol. The plant has therefore been chosen for the present work to validate some of its acclaimed bioactivities (Anshu*et al*, 2014).

Botanical Aspects of *Saracaindica* L.

Botanical name	:	*Saracaindica* L.
Myanmar name	:	Thawka
Family	:	Caesalpinaceae
Genus	:	*Saraca*
Species	:	*indica*
Parts used	:	leaf and bark

(a) Flowers (b) Bark (c)Plant

Figure 1: Thawka (a) flowers, (b) bark and (c) plant

Materials and Methods

Collection and Preparation of Plant Samples

The leaf and bark of Thawka were collected from University of Yangon Campus. The collected sample was identified by authorized botanist at the Department of Botany, University of Yangon.

The leaf and bark were dried for two weeks then the dried samples were made into powder by using electric grinder. The dried powdered samples were separately stored in air-tight containers to prevent moisture changes and contamination.

Preliminary Phytochemical Investigation

A few grams of dried powdered sample was subjected to the tests of alkaloids, amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, tannins, starch, steroids and terpenoids (Harborne, J, 1984 ; M- Tin Wa, 1972).

Proximate Analysis

The analysis of the dried powdered sample were made such as moisture content by oven drying method, protein content by micro Kjeldahl distillation method, fat content by Soxhlet extraction, fiber content by fiber cap method, ash content by ashing in furnace method and carbohydrates content by calculation method (AOAC, 2000).

Investigation of Antimicrobial Activity

The antimicrobial activity of the extracts of the dried bark and leaf samples in solvents of different polarities, namely ethanol, ethyl acetate, petroleum ether (PE) and water was studiedon 6 strains of microorganisms, namely *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candidaalbicans* by agar well diffusion method at the Central Research Development Center, Ministry of Industry (1), lower Myanmar, Yangon Region (Karthiheyan, 2012).

Investigation of Antioxidant Activity

DPPH (2,2-diphenyl-1-1-picryl hydrazyl) free radical scavenging assay was chosen to assess the antioxidant activity of the plant materials. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system.

In the present work, the antioxidant activity of ethanol and watery extracts of both of the leaf and bark of Thawka was studied by spectrometric DPPH free radical scavenging assay (Marinova and Batchvarov, 2011).

Isolation of Compounds A and B from bark of Thawka

The compounds **A** and **B** were isolated form the EtOAc extract of the bark by using column chromatographic method by gradient elution using PE, EtOAc and MeOH. Six fractions (F_1 to F_6) were obtained as mixtures. Successive fractions obtained were combined on the basis of their behaviors on TLC. The fraction F_1 was washed with EtOH and compound **A** was obtained as white crystals. The fraction F_2 was washed with EtOH and compound **B** was obtained as white needles.

Results and Discussion

Phytochemical Investigation of Thawka

Preliminary phytochemical investigation was carried out in order to know the types of phytochemical constituents present in leaf and bark of *Saracaindica* L.(Thawka). Carbohydrates, glycosides, organic acids, phenolic compounds, saponins, steroids and terpenoids were present in the leaf, whereas alkaloids, carbohydrates, flavonoids, organic acid, saponins, starch, steroids, tannins and terpenoids were present in the bark.

Nutritional Values of Thawka

Nutritional values of both of the leaf and bark were determined by standard AOAC methods, and the results are summarized in Table 1. The corresponding energy values calculated for the leaf and the bark, respectively, are 194and 202 kcal/100 g.

Table 1:Nutritional Values of Thawka (Leaf and Bark)

No	Parameters	Content (%)	
		Bark	Leaf
1	Ash	12.96	6.8
2	Moisture	15.96	11.25
3	Protein	5.55	13.24
4	Fat	4.15	3.84
5	Fiber	25.71	38.21
6	Carbohydrate	35.67	26.66

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

In the screening of antimicrobial activity by agar well diffusion method, the measurable clear zone diameter, including the well diameter, shows the degree of antimicrobial activity. The well diameter is 10 mm in the present study. The larger the zone diameter observed for an extract, the more active it is on the test organism.

The results of antimicrobial activity are shown in Table 2 and Figures 2, 3, 4 and 5. It was found that PE and watery extracts of both samples showed no activity on all tested microorganisms. The highest inhibition zones observed were 32 mm on *Pseudomonas aeruginosa* for barkand 16mm on *Bacillus pumilus* and *Candida albicans* for leaf, both for EtOAc extracts.

Table 2: Antimicrobial Activity Screening on Thawka (Leaf and Bark)

Types of Extracts	Types of Microorganisms and Diameter of inhibition zone (mm)					
	B.subtilis	*B.pumilus*	*S.aureus*	*P. aeruginosa*	*E.coli*	*C.albicans*
(Leaf)						
PE	-	-	-	-	-	-
EtOAc	14(+)	16(+)	13(+)	-	15(+)	16(+)
EtOH	-	11(+)	11(+)	-	11(+)	13(+)
H_2O	-	-	-	-	-	-
(Bark)						
PE	-	-	-	-	-	-
EtOAc	27(+++)	30(+++)	28(+++)	32(+++)	26(+++)	28(+++)
EtOH	14(+)	13(+)	14(+)	-	15(+)	14(+)
H_2O	-	-	-	-	-	-

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

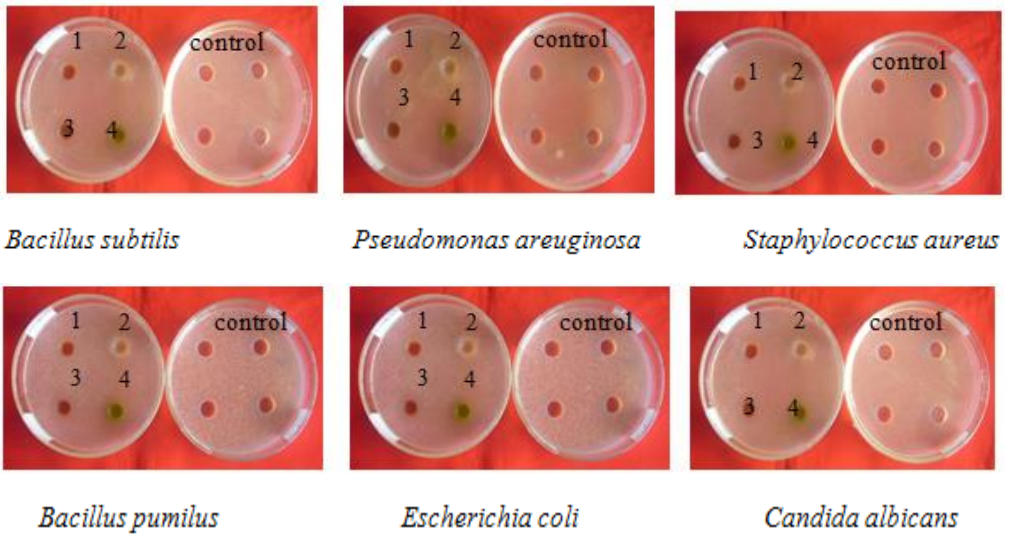


Figure 2: Screening of antimicrobial activities of different extracts of the leaf of Thawka

1 =PE extract 2 =H_2O extract
3 =EtOAc extract 4 =EtOH extract

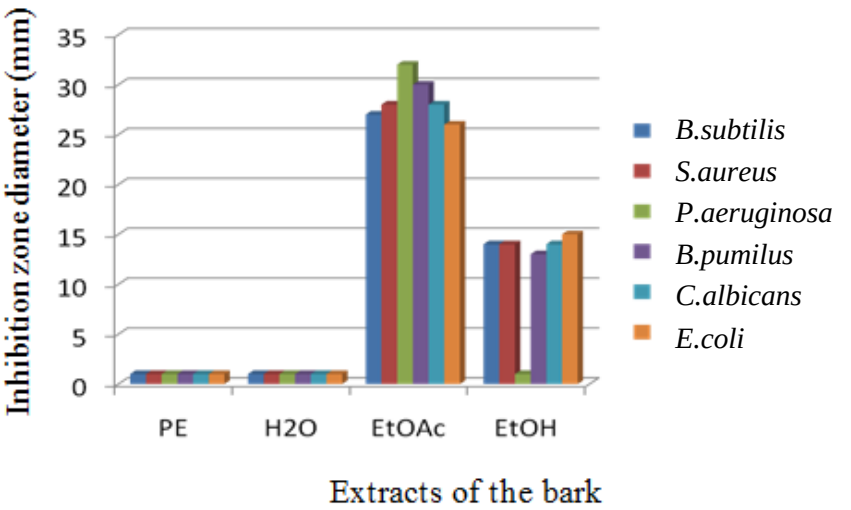
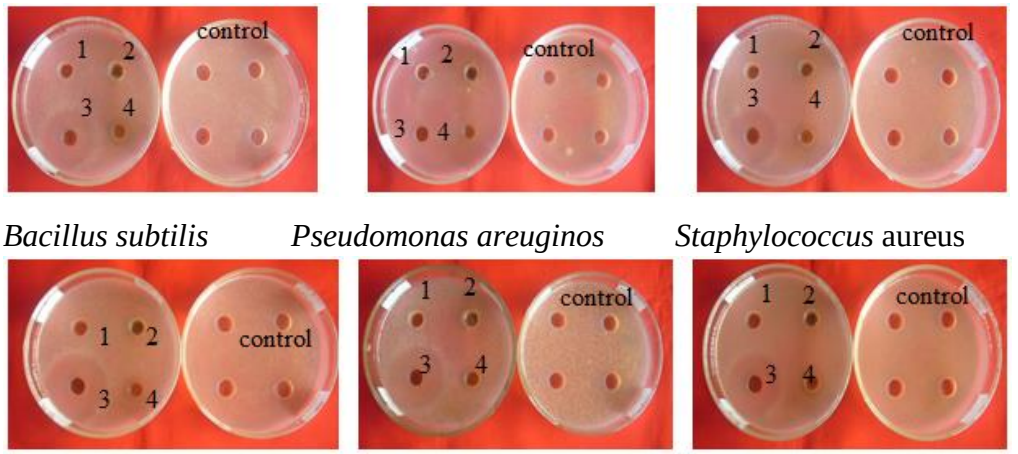


Figure 3: Bar graph of inhibition zone diameters of different leaf extracts on each type of microorganisms



Bacillus subtilis *Pseudomonas areuginos* *Staphylococcus* aureus

Bacillus pumilus *Escherichia coli* *Candida albicans*

Figure 4: Screening of antimicrobial activities of different extracts of the bark of Thawka

1 = PE extract 2 = H_2O extract

3 = EtOAc extract 4 = EtOH extract

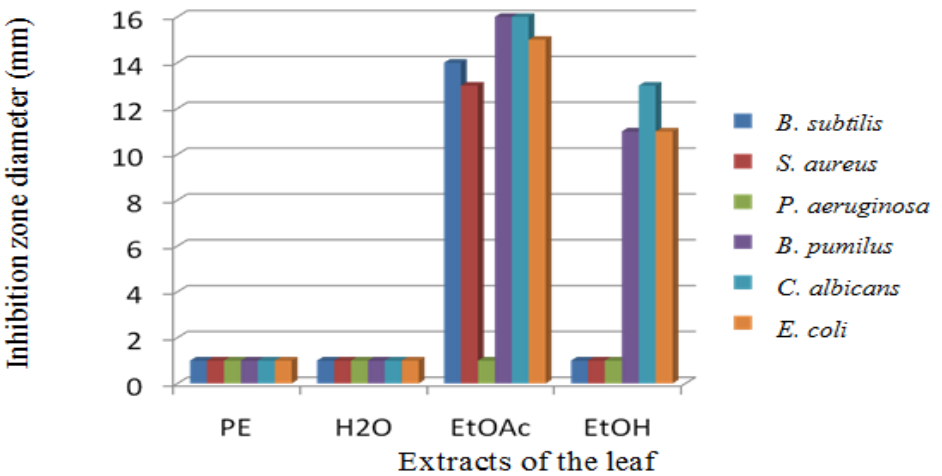


Figure 5: Bar graph of inhibition zone diameters of different bark extracts on each type of microorganisms

Investigation of Antioxidant Activity of Crude Extracts by DPPH Assay Method

The antioxidant activity of the EtOH and watery extracts of leaf and bark of Thawka was investigated by DPPH radical scavenging assay. There were different concentrations of each extract and vitamin C was used as standard. The extract was mixed with DPPH, decolorized due to hydrogen donating ability. The radical scavenging activity (RSA) of crude extracts was expressed in terms of % RSA and IC_{50}.The results are shown in Table 2 and Figure 6 and 7.

According to the results, among the extracts, the EtOH extract of leaf (2.86µg/mL) possessed highest antioxidant activity.

Table 2: % RSA (Radical Scanvenging Activity) and IC_{50} Values of Watery and Ethanol Extracts of Leaf and Bark of Thawka and Vitamin C

Test Samples	%RSA±SD at Different Concentration (µg/mL)						IC50(µg/mL)
	0.625	1.25	2.5	5	10	20	
Watery Leaf	7.89 ± 0.11	9.45 ± 0.33	12.73 ± 2.1	27.27 ± 0.11	43.75 ± 0.66	64.77 ± 7.84	12.98
Watery Bark	5.78 ± 0.44	9.61 ± 0.33	20 ± 1.1	31.48± 3.84	59.14± 0.99	65.86± 4.31	8.35
EtOH Leaf	9.50 ± 0.42	23.6 ± 0.57	46.6 ± 1.41	69.9 ± 0.41	71.4 ± 0.85	72.9 ± 0.56	2.86
EtOH Bark	4.3 ± 1.84	8.4 ± 1.98	22.7 ± 0.98	39.3 ± 0.98	70.8 ± 0.00	72.6 ± 0.57	6.70
*Vitamin C	25.20 ± 1.40	53.58 ± 0.88	65.53 ± 1.13	74.82 ± 0.59	83.32 ± 0.78	91.21 ± 0.48	1.17

SD- Standard Deviation

* Used as Standard

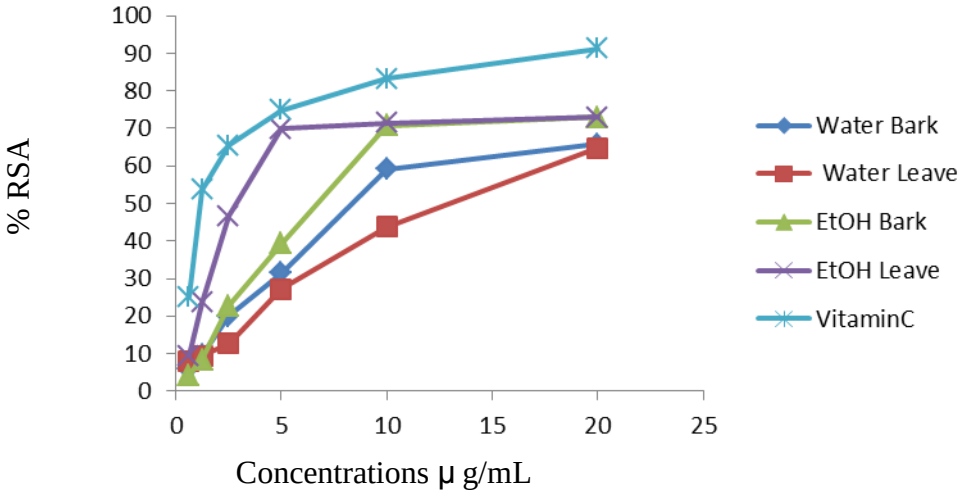


Figure 6: Radical scavenging activity (%RSA) of the EtOH and watery extracts of Leaf and Bark of Thawkavs concentrations

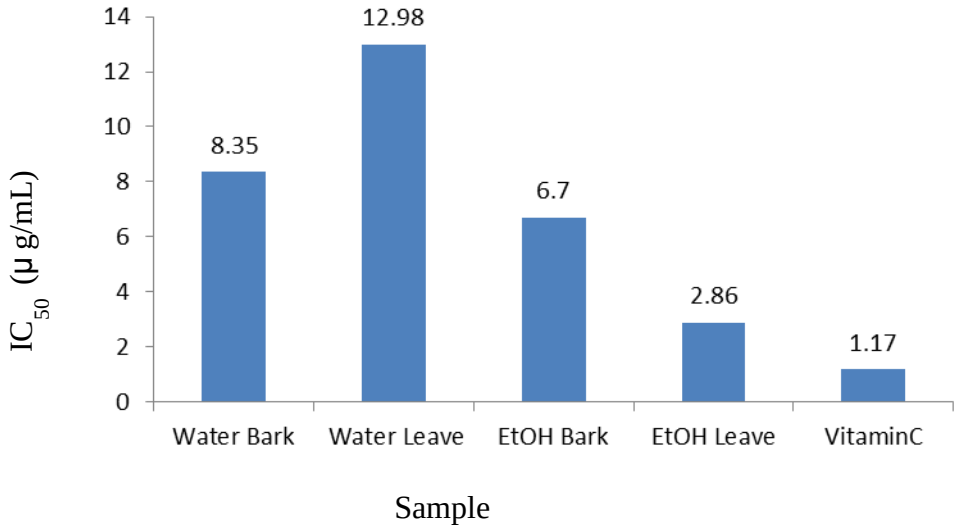


Figure 7: A bar graph of IC_{50} (µ g/mL) of the EtOH and watery extracts of Leaf and Bark of Thawka

Identification of isolated compounds

Compounds **A** and **B** were isolated from EtOAc extract of the bark by column chromatographic method as white crystals as UV inactive compounds. The isolated compounds **A**and **B** have very similar FT IR spectra (Figures 8 and 9), the main difference being the two additional ester bands at 1730 (C=O stretching) and 1272 (C-O stretching) cm^{-1} in **A**; whereas for **B**, it is the stronger band at 3478 cm^{-1} (O-H stretching).Other characteristic bands representing the olefinic group around 800cm^{-1} is present in both compounds. In fact the IR spectrum of **A** is almost superimposable with that of β-sitosteryl acetate (Figure 10), whereas the IR spectrum of **B** (Figure 11) is almost superimposable with that of β-sitosterol (Figure 12). Therefore **A** and **B** are β-sitosteryl acetate and β-sitosterol. The assignment of the IR bands of the two compounds is summarized in Table 3.

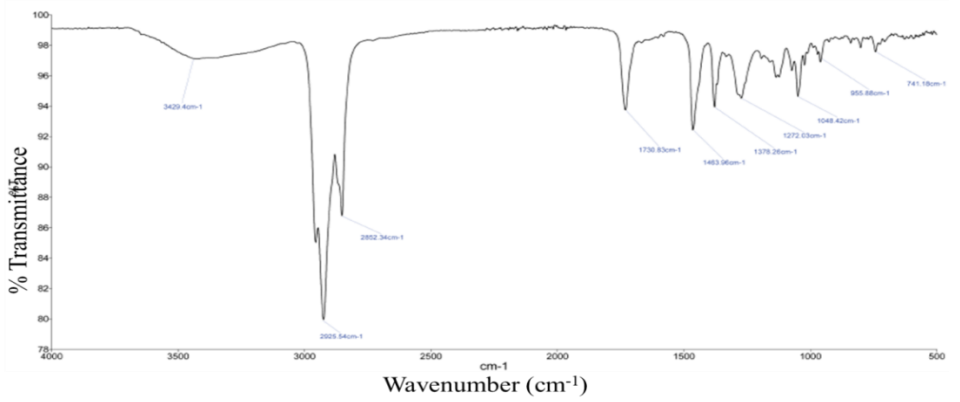


Figure 8: FT IR spectrum of isolated compound **A**

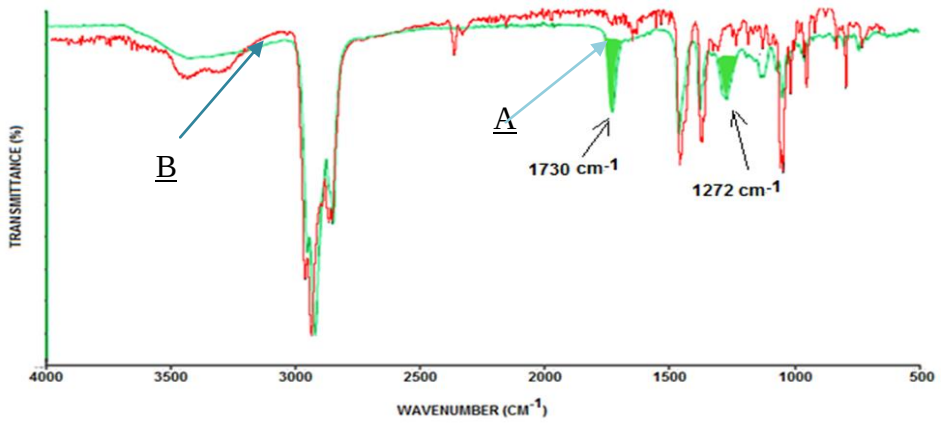


Figure 9: Comparison of the FT IR spectra of isolated compounds **A** and **B**

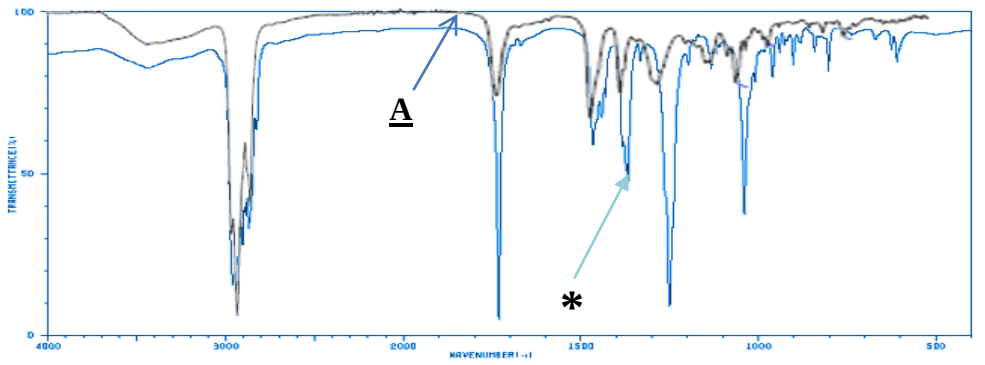


Figure 10: Comparison of FT IR spectrum of isolated compound **A** with the reference spectrum* of β sitosteryl acetate

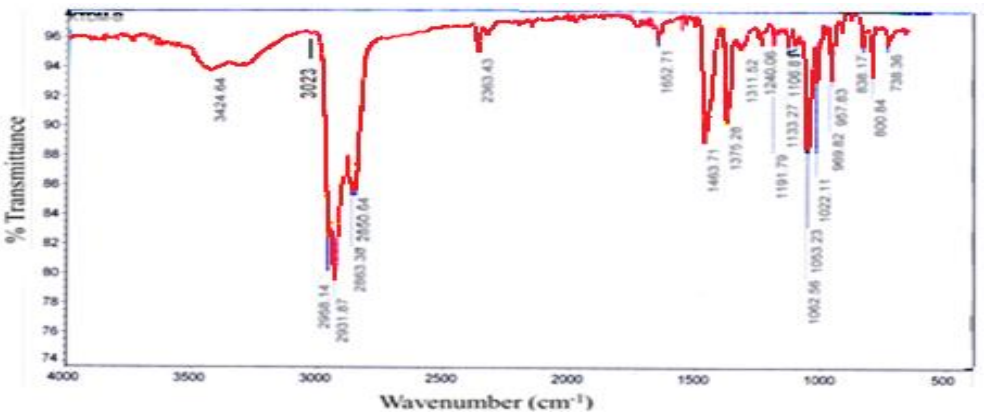


Figure 11: FT IR spectrum of isolated compound **B**

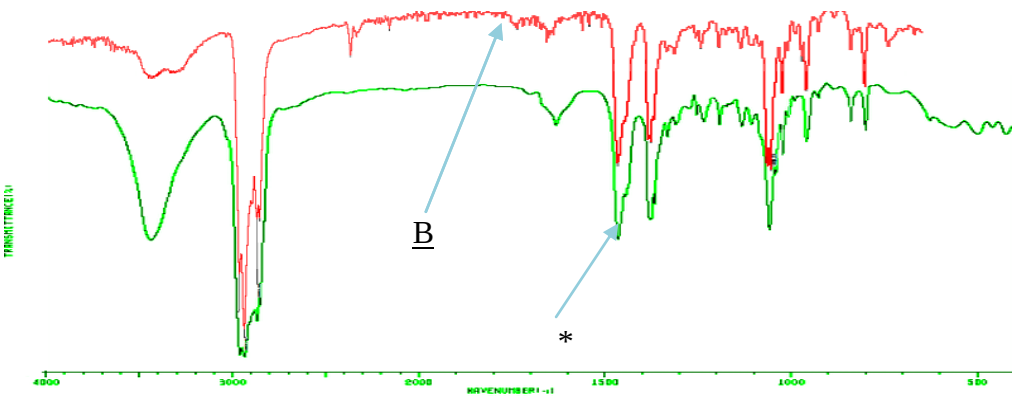


Figure 12: Comparison of FT IR spectra of the isolated compound **B** with the reference spectrum* of βsitosterol

*Spectral Database for Organic Compounds (SDBS), https://sdbs.db.aist.go.jp

Table 3 : Assignment of FT IR Spectral Data of Compound A and B

No	Wave number(cm^{-1})		Vibrational Mode	Band Assignment*
	Compound A	Compound B		
1	-	3424	ν O-H	OH
2	-	3023	ν =C-H	olefinic C-H
3	2953	2958	$\nu_{as}CH_3$	CH_3
4	2925	2931	$\nu_{as}CH_2$	CH_2
5	2866	2863	$\nu_{sy}CH_3$	CH_3
6	2852	2850	$\nu_{sy}CH_2$	CH_2
7	1730	-	$\nu_{C=O}$	carbonyl group of ester
8	1463	1463	δ CH_2, $\delta_{as}CH_3$	CH_3, CH_2
9	1378	1375	$\delta_{sy}CH_3$	$_{sy}CH_3$
10	1272,1043	1062,1053	ν C-O	C-O
11	787	800	δ_{oop}C-H	olefinic C-H out of plane bending

* Silverstein *et al.*, 2005; Pretsch *et al.*, 1989

Conclusion

In concluding the present work, the following inteferences can be deduced. Results of phytochemical investigations revealed that carbohydrates, glycosides, organic acids, phenolic compounds, flavonoids, saponins, steroids and terpenoids were present in the leaf;alkaloids, carbohydrates, flavonoids, glycosides, organic acids, saponins, starch, steroids, tannins and terpenoids were present in the bark.

Nutritional values were, ash(6.80%), moisture(11.25 %), protein (13.24 %), fat (3.84 %), fiber (38.21 %), carbohydrate(26.66 %) and energy value (194 kal/100g) in leaf and ash (2.96 %), moisture(15.96 %), protein (5.55 %), fat (4.15 %),fiber (25.71 %), carbohydrate(35.67 %) and energy value(202 kcal/100g) in bark.

From the screening of antimicrobial activity by agar well diffusion method on 6 strains of bacteria, the largest inhibition zone diameter of 32 mm was observed on *Pseudomonas aeruginos a* for the EtOAc extract of the bark; only half of this, *i.e.,* 16mm inhibition zone diameter was observed as the

highest activity for the EtOAc extract of the leaf on *Bacillus subtilis* and *Candida albicans*.

Antioxidant activity by spectrometric DPPH assay method indicated IC_{50} (µg/mL) values of standard, Vitamin C (1.17 µg/mL), ethanol extract of leaf (2.86 µg/mL), ethanol extract of bark (6.70 µg/mL), watery extract of bark (8.35 µg/mL) and watery extract of leaf (12.98 µg/mL).By the silica gel column chromatographic separation, five compounds were isolated from EtOAc extract of the bark and flower of Thawka.

Compound **A**and **B** were also isolated from the EtOAc extract of bark by using solvent system (PE:EtOAc; 3:7 v/v) as white needles crystals. Compound **A** was identified to be β-sitosteryl acetate by analysis of the FT IR spectral data and also by comparison with reference spectrum of β-sitosteryl acetate. Compound **B** was identified to be β-sitosterol by analysis of the FT IR and also by comparison with the reference FT IR spectral data of β-sitosterol.

Thus the ethyl acetate extract of the bark has good prospects for the formulation of antibacterial drugs; and for uses where antioxidant activity is desired, the ethanol extract of the leaf is more promising.

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