

## **INVESTIGATION OF SOME BIOACTIVITIES, PHYTOCHEMICAL SCREENING AND NUTRITIONAL VALUES OF *Byttneria pilosa* Roxb. (Sat-le-pyat)**

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### **Abstract**

The present work deals with the investigation of phytochemical screening, nutritional values and some biological activities of *Byttneria pilosa* Roxb. (Sat-le-pyat roots, leaves and stems). The plant was collected from Kyauk Taw Township, Rakhine State, Myanmar. The root of this plant is chewed or juice is tropically applied as antidote in case of poisoning. Paste prepared from tender stem with leaves is tied to around limbs for the treatment of fractured bones by the Khumi community. According to the phytochemical investigation, sat-le-pyat roots, leaves and stems revealed the presence of alkaloids, starch, phenolic compounds, flavonoids, carbohydrates,  $\alpha$ -amino acids, glycosides, tannins, saponins, terpenoids and steroids and the absence of reducing sugars and cyanogenic glucosides. The nutritional values of Sat-le-pyat plants showed that carbohydrate (66.18 %) and fiber (10.35 %) in roots, carbohydrate (52.55 %) and protein (17.39 %) in leaves and carbohydrate (46.11 %) and fiber (30.44 %) in stems were present as major nutrient than others. In the present chemical investigation,  $\beta$ -sitosterol was isolated from ethyl acetate extract of Sat-le-pyat roots by applying column and thin layer chromatography. The isolated compound was identified by FT IR and physicochemical properties. The antimicrobial activities of PE,  $\text{CHCl}_3$ , EtOAc, 95 % EtOH, MeOH and  $\text{H}_2\text{O}$  extracts of roots, leaves and stems were tested against six different microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* by agar well diffusion method. The result of antimicrobial activity revealed that EtOAc extracts gave significant activity against all species of microorganisms (12~ 30 mm) but  $\text{H}_2\text{O}$  extract exhibited low activity against all species of microorganisms (11~12 mm) except *Bacillus subtilis*, *Bacillus pumilus* and *Candida albicans*. The antioxidant activity of ethanol and aqueous extracts was determined by free radical scavenging DPPH assay method. From the results, the antioxidant property of aqueous extract ( $\text{IC}_{50}$ =12.20  $\mu\text{g}/\text{mL}$ ) was higher than ethanol extract ( $\text{IC}_{50}$ =48.61  $\mu\text{g}/\text{mL}$ ) in roots. The antioxidant power ( $\text{IC}_{50}$ ) of aqueous extract and ethanol extract of leaves was found to be 15.09  $\mu\text{g}/\text{mL}$  and 19.59  $\mu\text{g}/\text{mL}$ . And also,

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the antioxidant property of ethanol extract ( $IC_{50}=11.79 \mu\text{g/mL}$ ) showed higher activity than aqueous extract ( $IC_{50}=29.34 \mu\text{g/mL}$ ) in stems.

**Keywords:** *Byttneria pilosa* Roxb., antioxidant activity, DPPH, antimicrobial activity

## Introduction

*Byttneria pilosa* Roxb. (Locally known as Harijora, Tribal Name, Salam Vra (Morma) and belongs to the family of Sterculiaceae. *B. pilosa* is a large woody climber with grooved, strigose, branchlets. The plant is very popular in tribal community of Bangladesh like Chakma, Marma, Khumi etc. for its medicinal benefit. Leaves are suborbicular, palmately 3-lobed, pilose on both surfaces. Flowers are minute campanulate, in a lax much branched inflorescence. Capsule is globose, size of a large chery, studded with subulate barbed prickles. The plant is used in the treatment of fractured bones.

The crushed stems are applied to affected areas for the treatment of boils, dandruff lice infestation, rheumatagia, snake bite and syphilis. An infusion of the leaves used in baths for the treatment of scabies by the Chakma people. Paste prepared from tender stem with leaves is tied to around limbs for the treatment of fractured bones by the Khumi community. Roots are used to prepare a paste, which is applied to affected areas for the treatment of elephantiasis in Tripura community. The root of this plant is chewed or juice is tropically applied as antidote in case of poisoning. Roots are pounded and the paste is applied for washing hair. It is reported to produce good foam but irritate the eyes (Zaman *et al.*, 2015). It is found in Forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Sylhet, Srimongal, Gajni (Sherpur) and Habiganj of Bangladesh.

Possible anti-obesity, thrombolytic and cytotoxic activities were previously investigated in Bangladesh but the antioxidant and antimicrobial activities of this plant has not been tested to date. (Ibrahim *et al.*, 2015) In Myanmar, *B. pilosa* Roxb. is widely grown in Rakhine State. The root of *B. pilosa* has been used as traditional medicine for the treatment of fractured bones in Rakhine State, Myanmar. In Myanmar, no one has done research on this plant. Thus, to fulfill the scientific evidence in application, *B. pilosa* plant has been chosen to study its chemical constituents and their biological activities such as antimicrobial and antioxidant activity.



**Figure1:** Photographs of (a) fruits, (b) flowers, (c) roots and (d) leaves of *Byttneria pilosa* Roxb.

## Materials and Methods

### Plant Materials

*Byttneria pilosa* Roxb. (Sterculiaceae) roots, leaves and stems were collected in November 2017 at Kyauk-Taw Township, Rakhine State, Myanmar. The species was identified by the authorized botanist at Botany Department, Sittway University, Myanmar.

### Preliminary Phytochemical Tests of Sat-le-pyat

Preliminary detection of phytochemical compounds present in Sat-le-pyat powder sample was carried out according to the phytochemical methods (Evans *et al*, 2003; Marini-Bettolo *et al.*, 1981; M-Tin Wa, 1972; Robinson, 1983; Shriner *et al.*, 1980; Trease *et al.*, 1978; Harborne, 1984; Harborne, 1993; Vogel, 1956).

### Determination of Nutritional Values

The determination of moisture content, ash content, protein content, fat content, fiber content and carbohydrate contents were carried out at Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigation, Yangon. The procedures were performed by the standard methods (AOAC, 2000; Raghuramula *et al*, 1983).

### **Extraction and Isolation of Organic Constituent from Ethylacetate Extract of Sat-le-pyat Roots by Column Chromatography**

Dried powdered root of *Byttneria pilosa* Roxb. (300 g) was extracted with 1000 cm<sup>3</sup> of 95 % ethanol for about one week by percolation method followed by filtration. This procedure was carried out three times. After evaporation of the solvent, the resulting crude extracts were successively partitioned between organic solvents (PE, EtOAc) and water. Evaporation of solvents from the organic layers and aqueous layer provided 1 g of PE, 3 g of EtOAc and 7.5 g of water extracts respectively.

3 g of the EtOAc extract was chromatographed over silica gel 60 (70-230 mesh, Merck) using PE only, PE: EtOAc (9:1, 8.5:1.5, 8:2, 7:3, 6:4 v/v) solvent system. A total eleven fractions were obtained. One compound was isolated from fraction 9. The isolated compound was purified by washing pet ether and followed by recrystallization from ethyl acetate. The isolated compound was identified by FT IR and physicochemical properties. Some physicochemical properties of isolated compound such as R<sub>f</sub> value, melting point, solubility tests and thin layer chromatography with various visualizing reagents were also investigated.

Melting points were determined in open capillaries by a Gallenkamp melting point apparatus. The isolated compound was visualized by spraying reagent such as 5 % H<sub>2</sub>SO<sub>4</sub>, anisaldehyde-H<sub>2</sub>SO<sub>4</sub>, vanillin-H<sub>2</sub>SO<sub>4</sub>, Libermann Burchard reagent and 1 % FeCl<sub>3</sub> solution on TLC chromatograms and also treated with iodine vapour. The colours observed on the corresponding TLC chromatograms for isolated compounds were examined and recorded. When treating with 5 % H<sub>2</sub>SO<sub>4</sub>, anisaldehyde-H<sub>2</sub>SO<sub>4</sub>, vanillin-H<sub>2</sub>SO<sub>4</sub> and Libermann Burchard reagent, the TLC plates must be heated. The isolated compound was also taken to determine the solubility's in pet-ether, chloroform, ethyl acetate, methanol, ethanol and water.

### **Preparation of Plant Extracts for Biological Activity**

The dried powdered sample (100g) was extracted with 500 mL of 95 % ethanol for about one week by percolation method followed by filtration. This procedure was carried out three times. The total combined filtrate was concentrated by distilling and evaporated to obtain ethanol extract.

Similarly, pet ether, methanol, chloroform and ethyl acetate were also prepared according to the above procedure. Aqueous extract was also prepared by boiling 20 g of sample with 100 mL of distilled water for three hours and filtered. It was repeated three times and the filtrates were combined followed by removal of the water to give aqueous extract.

### ***In vitro* Screening of Antimicrobial Activity by Agar Well Diffusion Method**

The antimicrobial activity of different crude extracts such as 95 % EtOH, PE, MeOH, EtOAc, CHCl<sub>3</sub> and water extracts were tested with six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* species by using agar well diffusion method at Pharmaceutical Research Department, Ministry of Industry, Yangon (Finegold *et al.*, 1978; Cruickshank, 1975; Mar Mar Nyein *et al.*, 1991; Jain *et al.*, 1974).

### **Screening of Antioxidant Activity of Crude Extracts by DPPH assay**

The ability of aqueous extract and ethanol extract to scavenge free DPPH radicals was determined by using the DPPH assay. DPPH radical scavenging activity was determined by UV spectrophotometric method. The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95 % ethanol using shaker. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 µM DPPH solutions and 1.5 mL of test sample solution. Both extracts were dissolved in ethanol and a series of different concentrations was prepared. The sample solution was allowed to stand at room temperature for 30 min. After 30 min, the absorbance values of these solutions were measured at 517 nm using UV- visible spectrophotometer and calculated IC<sub>50</sub> values. In this study, six different concentrations (6.25, 12.5, 25, 50, 100, 200, 400 µg/mL) of each extract were prepared by serial dilution. Ascorbic acid was used as standard. The experiment was done in triplicate. The percent oxidative inhibition values of crude extracts was measured at different concentrations. The antioxidant activity is expressed as % radical scavenging activity (% RSA) and 50 % inhibition concentration (IC<sub>50</sub>). When the concentrations of the samples were increased, the absorbance value decreased i.e. % inhibition or radical scavenging activities were also

increased. From the average value of % inhibition, 50 % inhibition concentration ( $IC_{50}$ ) were calculated by linear regressive excel program.

## **Results and Discussion**

The phytochemical screening of Sat-le-pyat (roots, leaves and stems) revealed the presence of terpenoids, alkaloids, flavonoids, carbohydrates, phenolic compounds, saponins, tannins, steroids, terpenoids, glycosides, starch and  $\alpha$ - amino acids but reducing sugars and cyanogenic glycosides were absent. The results are shown in Table 1.

The quantitative analyses of total ash and moisture contents have been done according to the methods described in AOAC (2000). The total ash in the sample is the inorganic residue remaining after the organic matter has been burnt away. Moisture content sometimes reflects the half-life of material. The larger the moisture content, the shorter the half-life. The determination of nutrient values showed that carbohydrate (66.18 %) and fiber (10.35 %) are present as major nutrient than others such as protein (3.84 %), crude fat (1.63 %), moisture (12.00 %) and ash (6.00 %) in roots, carbohydrate (52.55 %), protein (17.39 %), fat (11.09 %), fiber (6.97 %), moisture (4.00 %) and ash (8.00 %) in leaves and carbohydrate (46.11 %), protein (5.15 %), fat (4.30 %), fiber (30.44 %), moisture (4.00 %) and ash (10.00 %) in stems respectively. The results are described in Table 2.

The isolated compound (0.3 g, 0.06 %) was obtained as white needle crystal from fraction 9 of the ethylacetate extract of root by column chromatography on silica gel using PE: EtOAc (9:1 and 8.5:1.5 v/v ) solvent system. Isolated compound is soluble in pet-ether, chloroform, ethyl acetate, methanol and ethanol but insoluble in water. Therefore, the isolated compound may be organic compounds possessing moderate polarity. The compound is unactive under UV light.

Isolated compound: (melting point 140 °C,  $R_f$  =0.28 in PE: EtOAc (9:1 v/v) solvent system). Isolated compound provides positive tests for steroids because of its physicochemical properties. In addition, Co-TLC was also done with authentic  $\beta$ -sitosterol. The  $R_f$  value of compound was found to be identical with that of  $\beta$ -sitosterol in any solvent system and they also gave

the same behavior on TLC. Therefore it can be inferred that the isolated compound was  $\beta$ -sitosterol. On the basis of these colour tests, the isolated compound was classified and the some physicochemical properties of isolated compound was shown in Table 3 and Figure 4.

FT IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 3427 ( $\nu_{\text{O-H}}$  of alcohol), 2931, 2865 ( $\nu_{\text{C-H}}$  of  $\text{CH}_3$  &  $\text{CH}_2$ ), 1714 ( $\nu_{\text{C=O}}$ ), 1462, 1381 ( $\delta_{\text{C-H}}$ ), 1052, 1022 ( $\nu_{\text{C-O-C}}$ ). The FT IR spectral data of compound were found to be similar to that of  $\beta$ -sitosterol. (Merck Index, 2001). The FT IR spectrum of compound was shown in Figure 2. The FT IR spectra of the isolated compound (black) and  $\beta$ -sitosterol (red) were shown in Figure 3.

Antimicrobial activity of six crude extracts of Sat-le-pyat (roots, leaves and stems) were tested on six strains of microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli species* by agar well diffusion method. This method is based on the zone diameter in millimeter (mm) of agar well. The measurable zone diameter, including the well diameter, shows the degree of antibacterial activity. The larger zone diameter shows the higher activity on the test bacteria.

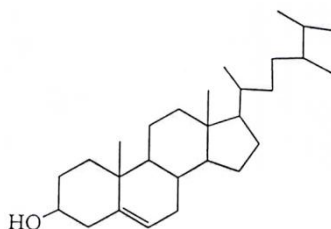
The results of antimicrobial activity revealed that EtOAc extracts gave significant activity against all species of microorganisms (12~30mm), however  $\text{H}_2\text{O}$  extract exhibited low activity against all species of microorganisms (11~12 mm) except *Bacillus subtilis*, *Bacillus pumilus* and *Candida albicans*. The results of antimicrobial activity are shown in Table 4.

The antioxidant activity of 95 % ethanol and aqueous extracts from Sat-le-pyat (roots, leaves and stems) was studied by DPPH free radical scavenging assay method. The results are summarized in Table 5 and Figures 5, 6, 7.

From the results, the antioxidant properties were observed in the increasing order of aqueous extract ( $\text{IC}_{50}=12.20 \mu\text{g/mL}$ ) > ethanol extract ( $\text{IC}_{50}=48.61 \mu\text{g/mL}$ ) in roots, aqueous extract ( $\text{IC}_{50}=15.09 \mu\text{g/mL}$ ) > ethanol extract ( $\text{IC}_{50}=19.59 \mu\text{g/mL}$ ) in leaves, ethanol extract ( $\text{IC}_{50}=11.79 \mu\text{g/mL}$ ) > aqueous extract ( $\text{IC}_{50}=29.34 \mu\text{g/mL}$ ) in stems. The extracts were compared with standard ascorbic acid ( $\text{IC}_{50}=1.90 \mu\text{g/mL}$ ). Among these extracts, the

ethanol extract of stems showed higher antioxidant activity than other extracts of roots and leaves. However, it was also found that both extracts have the lower antioxidant activity than standard ascorbic acid ( $IC_{50} = 1.90 \mu\text{g/mL}$ ). The larger the % RSA showed the higher the antioxidant activity. In contrast, the lower the  $IC_{50}$  value, the more effective antioxidant activity.

In brief, all above scientific data may contribute to the utilization of the roots, leaves and stems of Sat-le-pyat plant in Myanmar traditional medicine for the treatment of skin diseases, fractured bones, wound infection, food poisoning and diarrhea.



Compound ( $\beta$ -sitosterol)  $C_{29}H_{50}O$ , mol.wt.414, m.pt.141°C

**Table 1: Results of Phytochemical Investigation of *Byttneria pilosa* Roxb. (Sat-le-pyat Roots, Leaves and Stems) by Test Tubes Method**

No.	Tests	Extract	Test reagents	Observation	Remark		
					A	B	C
1	Alkaloids	1 %HCl	Mayer reagent	White ppt	+	+	+
			Dragendorff's reagent	Orange ppt	+	+	+
			Wagner's reagent	Reddish brown ppt	+	+	+
			sodium picrate	Yellow ppt	+	+	+
2	$\alpha$ -amino acids	$H_2O$	Ninhydrin	pink spot	+	+	+
3	Carbohydrates	$H_2O$	10 % $\alpha$ -naphthol & Conc. $H_2SO_4$	Red ring	+	+	+
4	Glycosides	$H_2O$	10 % Lead acetate	White ppt	+	+	+
5	Phenolic compounds	$H_2O$	10 % $FeCl_3$	Deep blue colour	+	+	+
6	Starch	$H_2O$	$I_2$ solution	Deep blue colour	+	+	+
7	Saponins	$H_2O$	Distilled water	Frothing	+	+	+



No.	Tests	Extract	Test reagents	Observation	Remark		
					A	B	C
8	Tannins	H <sub>2</sub> O	2 % NaCl,1% gelatin	White ppt	+	+	+
9	Flavonoids	EtOH	Conc. HCl and Mg turning	Pink colour	+	+	+
10	Steroids	P.E	Acetic anhydride and conH <sub>2</sub> SO <sub>4</sub>	greenish blue	+	+	+
11	Terpenoids	CH <sub>2</sub> Cl <sub>2</sub>	Acetic anhydride and conH <sub>2</sub> SO <sub>4</sub>	Colour change	+	+	+
12	Cyanogenic Glycosides	H <sub>2</sub> O	Sodium picrate paper	No colour change	-	-	-
13	Reducing sugar	H <sub>2</sub> SO <sub>4</sub>	Benedict's solution	No colour change	-	-	-

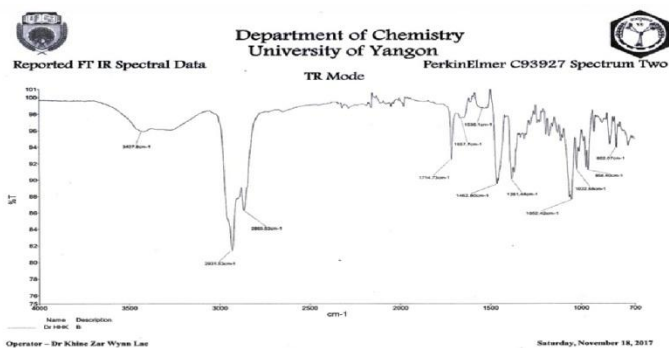
( + ) Present ( - ) Absent ( A = roots, B = leaves, C = stems)

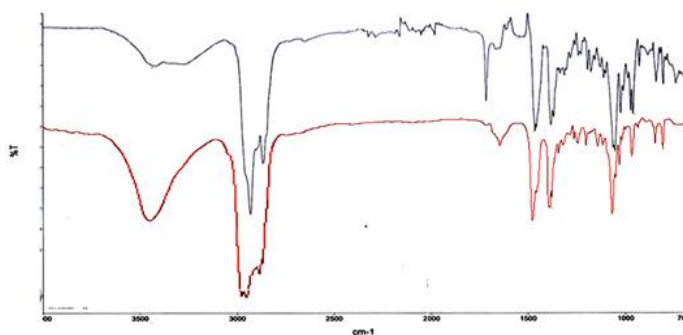
**Table 2 : Nutritional Values of the Sat-le-pyat Roots, Leaves and Stems**

Sr. No.	Type of nutrient	Contents (%)		
		roots	leaves	stems
1	Moisture	12.00	4.00	4.00
2	Ash	6.00	8.00	10.00
3	Protein	3.84	17.39	5.15
4	Crude fiber	10.35	6.97	30.44
5	Crude fat	1.63	11.09	4.30
6	Carbohydrate	66.18	52.55	46.11
7	Energy value (kcal/100 g)	294.75	379.57	243.74

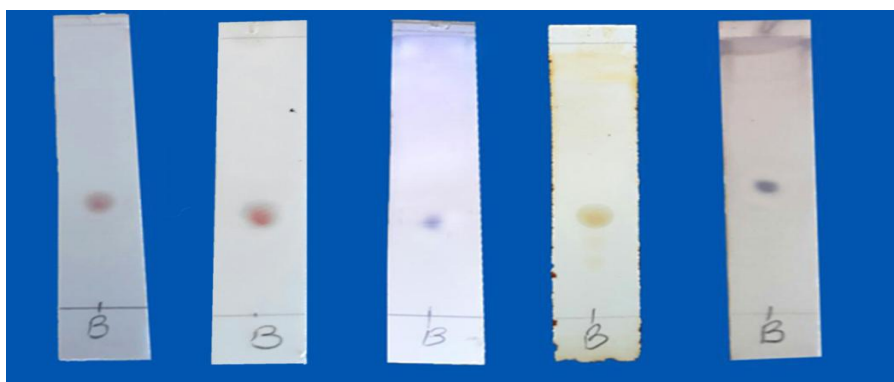
**Table 3: Some Physicochemical Properties of Isolated Compound**

Experiment	Observation	Remark
Physical state	White needle crystal	-
Melting point(°C)	140 °C	-
R <sub>f</sub> value	0.28 (PE : EtOAc =9: 1v/v)	-
UV(254-365nm)	inactive	No Conjugated double bond
2,4-DNP solution test	No yellow ppt	C=O absent
1 % FeCl <sub>3</sub> solution test	No colour change	Phenolic OH absent
10 % Lead acetate solution	No white ppt	Glycoside absent
Liebermann Burchard test	green	steroid
5 % H <sub>2</sub> SO <sub>4</sub> , Δ	Cherry red to brown	steroid
Vanillin-H <sub>2</sub> SO <sub>4</sub> , Δ	blue	steroid
Anisaldehyde-H <sub>2</sub> SO <sub>4</sub> , Δ	blue	steroid
I <sub>2</sub> vapour	yellow	steroid

**Figure 2: FT IR spectrum of isolated compound**



**Figure 3:** FT IR spectra of the (a) isolated compound (black) and (b)  $\beta$ -sitosterol (red)\*  
\*SDBS spectral database



5%  $H_2SO_4$ ,  $\Delta$     Libermann-Burchard,  $\Delta$     Vinillin,  $\Delta$      $I_2$     Anisaldehyde- $H_2SO_4$ ,  $\Delta$

**Figure 4:** Thin layer chromatograms of isolated compound

**Table 4: Antimicrobial Activities of Crude Extracts of Sat-le-pyat (Roots, Leaves and Stems) on Six Species of Microorganisms**

Sample	Extracts	Inhibition zone diameters (mm) of different extracts against microorganism tested					
		I	II	III	IV	V	VI
Roots	PE	12 (+)	12 (+)	13 (+)	12 (+)	13 (+)	15 (++)
	CHCl <sub>3</sub>	13 (+)	13 (+)	14 (+)	13 (+)	14 (+)	14 (+)
	MeOH	13 (+)	13 (+)	15 (++)	13 (+)	13 (+)	14 (+)
	EtOAc	13 (+)	17 (++)	15 (++)	17 (++)	14 (+)	15 (++)
	EtOH	12 (+)	13 (+)	18 (++)	18 (++)	15 (++)	15 (++)
	H <sub>2</sub> O	-	11 (+)	12 (+)	-	-	11 (+)
	Leaves	PE	12 (+)	12 (+)	12 (+)	17 (++)	15 (++)
CHCl <sub>3</sub>	13 (+)	13 (+)	12 (+)	12 (+)	13 (+)	12 (+)	
MeOH	15 (++)	14 (+)	13 (+)	13 (+)	13 (+)	13 (+)	
EtOAc	18 (++)	15 (++)	15 (++)	13 (+)	17 (++)	12 (+)	
EtOH	13 (+)	13 (+)	13 (+)	14 (+)	13 (+)	13 (+)	
H <sub>2</sub> O	12 (+)	11 (+)	11 (+)	12 (+)	12 (+)	11 (+)	
Stems	PE	13 (+)	11 (+)	12 (+)	11 (+)	12 (+)	12 (+)
	CHCl <sub>3</sub>	13 (+)	12 (+)	13 (+)	12 (+)	13 (+)	13 (+)
	MeOH	13 (+)	14 (+)	15 (++)	13 (+)	14 (+)	13 (+)
	EtOAc	25 (+++)	25 (+++)	30 (+++)	20 (+++)	29 (+++)	28 (+++)
	EtOH	17 (++)	15 (++)	12 (++)	14 (+)	17 (++)	15 (+)
	H <sub>2</sub> O	11 (+)	11 (+)	11 (+)	-	11 (+)	12 (+)

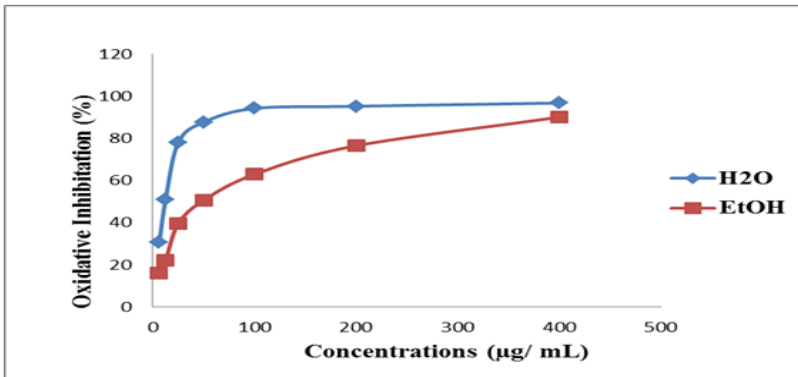
- |                                    |                                    |
|------------------------------------|------------------------------------|
| I. <i>Bacillus subtilis</i>        | I. <i>Bacillus subtilis</i>        |
| II. <i>Staphylococcus aureus</i>   | II. <i>Staphylococcus aureus</i>   |
| III. <i>Pseudomonas aeruginosa</i> | III. <i>Pseudomonas aeruginosa</i> |
| IV. <i>Bacillus pumilus</i>        | IV. <i>Bacillus pumilus</i>        |
| V. <i>Candida albicans</i>         | V. <i>Candida albicans</i>         |
| VI. <i>Escherichia coli</i>        | VI. <i>Escherichia coli</i>        |

**Table 5: Percent Oxidative Inhibition and IC<sub>50</sub> Values of Crude Extracts of *Byttneria pilosa* Roxb.**

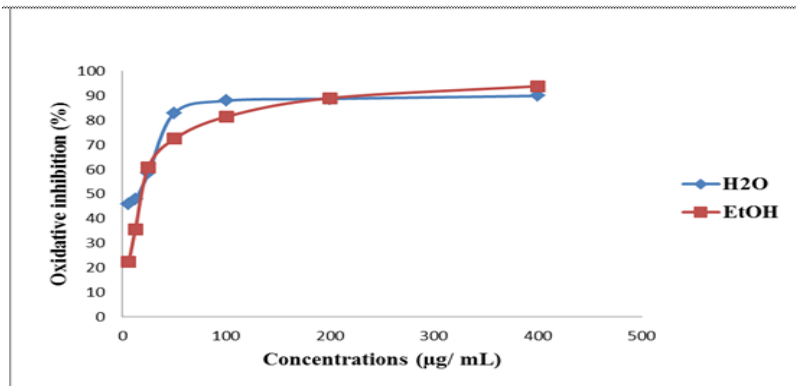
Samples	Extracts	% Inhibition (mean ±SD)							IC <sub>50</sub> (µg/mL)
		In different concentrations (µg/ mL)							
		6.25	12.5	25	50	100	200	400	
Roots	Aqueous	30.49	50.97	78.13	87.72	94.27	95.31	96.86	12.20
		±	±	±	±	±	±	±	
	EtOH	3.88	0.64	1.99	0.58	0.10	0.18	0.53	48.61
		±	±	±	±	±	±	±	
Leaves	Aqueous	2.14	3.91	1.97	4.28	3.70	2.14	2.14	15.09
		±	±	±	±	±	±	±	
	EtOH	45.98	47.79	58.43	82.93	87.95	88.75	89.95	19.59
		±	±	±	±	±	±	±	
Stems	Aqueous	2.85	8.23	2.08	3.42	0.60	0.35	0.92	29.34
		±	±	±	±	±	±	±	
	EtOH	7.12	20.73	46.54	66.46	85.57	86.79	91.66	11.79
		±	±	±	±	±	±	±	
		3.36	2.80	7.75	4.26	2.46	0.93	0.93	
		21	53.72	65.57	73.50	79.85	90.35	93.04	
		±	±	±	±	±	±	±	
		2.60	3.20	0.73	0.42	5.72	1.80	2.40	

**Table 6: Percent Oxidative Inhibition and IC<sub>50</sub> Values of Standard Ascorbic Acid**

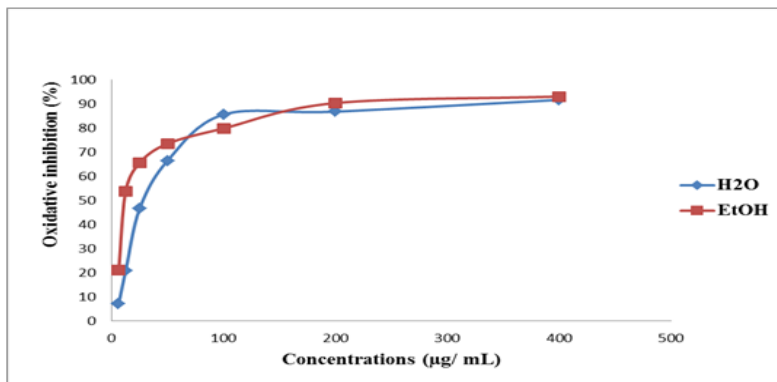
Standard	% Inhibition (mean ±SD)					IC <sub>50</sub> (µg/mL)
	In different concentrations (µg/ mL)					
	0.16	0.8	4.0	20.0	100.0	
Ascorbic acid	16.34±	39.20±	70.52±	88.09±	95.95±	1.90
	2.33	1.41	2.59	1.18	3.70	



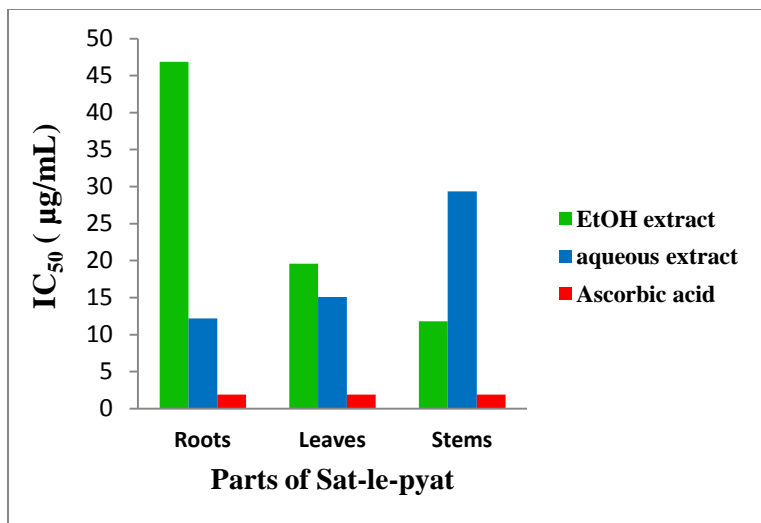
**Figure 5:** Plot of % oxidative inhibition vs concentrations (µg/mL) of aqueous and ethanol extracts of roots



**Figure 6:** Plot of % oxidative inhibition vs concentrations (µg/mL) of aqueous and ethanol extracts of leaves



**Figure 7:** Plot of % oxidative inhibition vs concentrations (µg/mL) of aqueous and ethanol extracts of stems



**Figure 5:** Bar graph of  $IC_{50}$  values of aqueous and 95 % ethanol extracts compared with ascorbic acid

### Conclusion

From the overall assessment of the research, the following inferences could be deduced. Plant extract gave significant antimicrobial and antioxidant activity due to the bioactive constituents present in it. The results demonstrate the presence of bioactive constituents in plant extracts including terpenoids, alkaloids, flavonoids, carbohydrate, phenolic compounds, saponins, tannins, steroids, terpenoids, glycosides, starch and  $\alpha$ - amino acids. From the results of nutritional values determination, Sat-le-pyat (roots, leaves and stems) have higher carbohydrates and fiber content than others nutrients.  $\beta$ -sitosterol was isolated from ethylacetate extract of plant roots.  $\beta$ -sitosterol is one of many sterols that come from plants (phytosterols) and it can reduce cholesterol levels and the risk of some cancers.  $\beta$ -sitosterol has always found in many plants. No one has not been isolated chemical constituents from *Byttneria pilosa* Roxb. roots in the previous investigation. The results of antimicrobial activity revealed that EtOAc extracts gave significant activity against all species of microorganisms (12~ 30 mm) but H<sub>2</sub>O extract exhibited low activity against all species of microorganisms (11~12 mm). Therefore, EtOAc extracts of Sat-le-pyat may be most effective for the treatment of the diseases infected by the tested microorganism such as food poisoning, urinary tract infection, eye infections, skin infection such as cellulitis, boils, impetigo, infection of wounds and burns. From the results of the antioxidant activity of different parts of Sat-le-pyat (roots,

leaves and stems), it was found that ethanol extract of stems ( $IC_{50}=11.79 \mu\text{g/mL}$ ) showed the highest activity followed by aqueous extract of roots ( $IC_{50}=12.20 \mu\text{g/mL}$ ), then aqueous extract of leaves ( $IC_{50}=15.09 \mu\text{g/mL}$ ) respectively. According to these observations, it can be concluded that the Sat-le-pyat plant possessed the antibacterial and antioxidant activities.

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