Phytochemical Screening and Some Bioactivities Of *Byttneria pilosa* Roxb. (Sat-le-pyat) Leaves and Stems

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Abstract

This paper is concerned with phytochemical screening, the elemental analysis and assessment of nutritional values and biological activities of *Byttneria pilosa* Roxb. (Sat-le-pyat leaves and stems). Sample collection was carried out in Ponnagyun Township, Rakhine State. The crushed stems are applied to affected areas for the treatment of boils; an infusion of the leaves used in baths for the treatment of scabies (Chakma). Paste prepared from tender stem with leaves is tied to around limbs for the treatment of fractured bones (Khumi). From the phytochemical investigation, Sat-le-pyat leaves and stems revealed the presence of alkaloids, starch, phenolic compounds, flavonoids, carbohydrates, α-amino acids, glycosides, tannins, saponins, terpenoids, and steroids but reducing sugars and cyanogenic glucoside were absent. Nutritional values was determined by A.O.A.C method. The nutritional values of Sat-le-pyat plants showed that 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 other nutrients. Relative abundance of elements in Sat-le-pyat leaves and stems were determined by Energy Dispersive X-rays fluorescence (EDXRF) technique. From this result, it was found that Ca and K were more abundant in Sat-le-pyat leaves and stems. Other elements such as (Sr, Cl, Fe, Mn, Rb, Zn and Cu) were also found to be different compositions (or) amounts in it. The antimicrobial activity of the different crude extracts (MeOH, PE, EtOAc, $CHCl_3$, EtOH, H_2O) of Sat-le-pyat leaves and stems were screened by agar well diffusion method against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* species. Among these extracts, EtOAc extract of stems showed higher activity on the six species of microorganisms (inhibition zone diameter, 20~30 mm) and EtOAc extracts of leaves gave activity against all species of the tested microorganisms (zone of inhibition ranging12~18 mm). H_2O extract of stems did not show activity on *Bacillus pumilus*. All extracts give good activity on all tested microorganisms. The antioxidant activity of ethanol and aqueous extracts was determined by free radical scavenging DPPH assay method. From these results, the antioxidant property of aqueous extract (IC_{50}=15.09 µg/mL) was higher than ethanol extract (IC_{50}=19.59 µg/mL) for the leaves. And also, ethanol extract (IC_{50} = 11.79 µg/mL) showed higher activity than aqueous extract (IC_{50} = 29.34 µg/mL) for the stems.

Keywords: *Byttneria pilosa* Roxb, antimicrobial activity, antioxidant activity, EDXRF, nutritional values, phytochemical test

Introduction

Byttneria pilosa Roxb. (Locally known as Harijora, Tribal Name, Salam Vra (Morma) and is belong 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, rheumatalgia, 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

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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. 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 researched 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 (Ibrahim *et al*, 2015).

(a) (b) (c) (d)

Figure 1 Photographs of (a) fruits, (b) flowers, (c)roots and (d) leaves of *Byttneria pilosa* Roxb**.**

Materials and Methods

Plant Materials

Byttneria pilosa Roxb. (Sterculiaceae) leaves and stems were collected in November 2017 at Ponnagyun Township, Rakhine State, Myanmar. The species was identified by the authorized botanist at Botany Department, Sittway University, Myanmar (Kress, *et al*.,2003 and Heywood, *et al*., 2007).

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; 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).

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 the 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,$CHCl_3$ and water extracts were tested with six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pesudomonas* a*eruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli species* by using agar well diffusion method at Pharmeceautical Research Deperment, 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_{50} 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 were measured at different concentrations. The antioxidant activity is expressed as % radical scavenging activity (% RSA) and 50 % inhibition concentration (IC_{50}). 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}) was calculated by linear regressive excel program.

Results and Discussion

The phytochemical screening of Sat-le-pyat (leaves and stems) revealed the presence of terpenoids, alkaloids, flavonoids, carbohydrates, phenolic compounds, saponins, tannins, steroids, terpenoids, glycosides, starch and α- 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 (52.55 %) and protein (17.39 %) are present as major nutrient than others such as fat (11.09 %), fiber (6.97 %), moisture (4.00 %) and ash (8.00 %) in leaves and carbohydrate (46.11 %) and fiber (30.44 %) are higher content than protein (5.15 %), fat (4.30 %), moisture (4.00 %) and ash (10.00 %) in stems respectively. The results are described in Table 2.

From the results of EDXRF, Sat-le-pyat leaves contained Ca (52.089 %), K (25.706 %), Cl (16.887 %), Mn (3.283 %), Fe (0.966 %), Sr (0.529 %), Zn (0.432 %) and Rb (0.108 %). The relative abundance of elements in Sat-le-pyat stems showed the presence of Ca (57.006 %), K (36.725 %), Mn (3.288 %), Fe (1.197 %), Sr (0.636 %), Zn (0.591 %), Cu (0.314 %) and Rb (0.242 %) respectively. Ca and K was more abundant in Sat-le-pyat leaves and stems. The results are described in Table 3. The element calcium is essential part of bones

and teeth. We need to consume a certain amount of calcium to build and maintain strong bones and healthy communication between the brain and other parts of body. Potassium is an extremely important element in human body. Without potassium, the never cell couldn't send those message to brain.

Antimicrobial activity of six crude extracts of Sat-le-pyat (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 good diameter, shows the degree of antibacterial activity. The larger zone diameter shows the higher activity on the test bacteria. Among these extracts, EtOAc extract of stems and leaves gave the highest activity against all species of the tested microorganisms (zone of inhibition ranging12~18mm) in leaves and (zone of inhibition ranging 20~30mm) in stems. H_2O extract showed no activity on *Bacillus pumilus* in stems. The antimicrobial activity of leaves and stems of Sat-le-pyat is due to the bioactive constituents present in it. The results of antimicrobial activity are shown in Table 4.

The antioxidant activity of 95 % ethanol and aqueous extracts from Sat-le-pyat (leaves and stems) was studied by DPPH free radical scavenging assay method. The results are summarized in Table 5 and Figures 4, 5 and 6.

From the results, the antioxidant properties were observed in the increasing order of aqueous extract (IC_{50}=15.09 µg/mL) > ethanol extract (IC_{50}=19.59 µg/mL) in leaves, ethanol extract (IC_{50}=11.79 µg/mL) > aqueous extract (IC_{50}=29.34 µg/mL) in stems. The extracts were compared with standard ascorbic acid (IC_{50}=1.90 µg/mL). Among these extracts, the antioxidant activity of ethanol extract of stems showed higher activity than that of leaves. However, it was also found that both extracts have lower the antioxidant activity than standard ascorbic acid (IC_{50}= 1.90 µg/mL). The larger the % RSA showed the higher the antioxidant activity. In contrast, the lower the IC_{50} value showed the more effective antioxidant activity.

According to these observations, it can be inferred that leaves and stems of Sat-le-pyat plant are regarded as one of the best drug in many diseases like Diabetes, Skin diseases etc, which is in use since ages owing to its multiple pharmacological activities. This plant is enriched with many useful phytoconstituents which are responsible for its efficacy.

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

No.	Tests	Extract	Test reagents	Observation	Remark	
					Leaves	Stems
1	Alkaloids	1 %HCl	Mayer's reagent	White ppt	+	+
			Dragendorff's reagent	Orange ppt	+	+
			Wagner's reagent	Reddish brown ppt	+	+
			sodium picrate	Yellow ppt	+	+
2	α-amino acids	H_2O	Ninhydrin	pink spot	+	+
3	Carbohydrates	H_2O	10 % α-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	+	+
8	Tannins	H_2O	2 % NaCl,1% gelatin	Brown ppt	+	+
9	Flavonoids	EtOH	Conc. HCl and Mg turning	Pink colour	+	+
10	Steroids	P.E	Acetic anhydride &conH_2SO_4	Deep green colour	+	+
11	Terpenoids	CH_2Cl_2	Conc. H_2SO_4	Reddish brown colour	+	+
12	Cyanogenic Glycosides	H_2O	Sodium picrate paper	No colour change	-	-
13	Reducing sugar	H_2SO_4	Benedict's solution	No colour change	-	-

(+) Present (−) Absent

Table 2 Nutritional Values of the Sat-le-pyat Leaves and Stems

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

Table 3 Relative Abundance of Elements in Sat-le-pyat (Leaves and Stems) by EDXRF

No.	Elements	Relative abundance (%)	
		leaves	stems
1	Ca	52.1	57
2	K	25.7	36.7
3	Cl	16.9	-
4	Mn	3.28	3.29
5	Fe	0.97	1.2
6	Sr	0.53	0.64
7	Zn	0.43	0.59
8	Rb	0.11	-
9	Cu	-	0.31

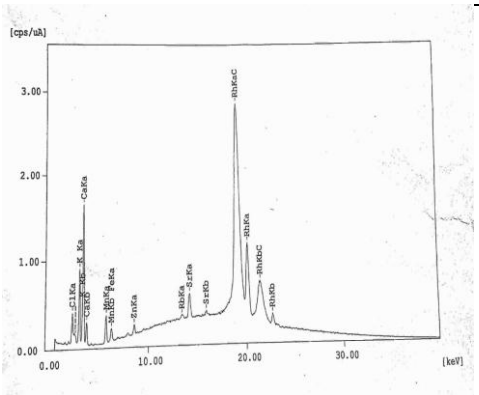

Figure 2 EDXRF spectrum of *Byttneria pilosa* Roxb. (Sat-le-pyat leaves)

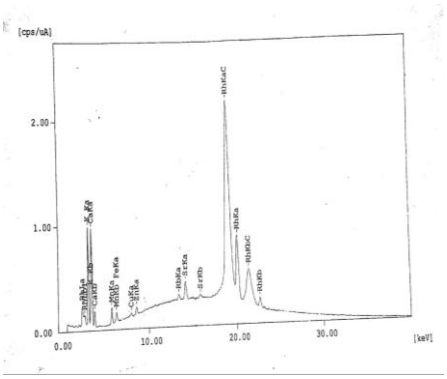

Figure 3 EDXRF spectrum of *Byttneria pilosa* Roxb. (Sat-le-pyat stems)

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

Sample	Extracts	Inhibition zone diameters (mm) of different extracts against tested microorganism					
		I	II	III	IV	V	VI
Leaves	PE	12 (+)	12 (+)	12 (+)	17 (++)	15 (++)	15 (++)
	$CHCl_3$	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_2O	12 (+)	11 (+)	11 (+)	12 (+)	12 (+)	11 (+)
Stems	PE	13 (+)	11 (+)	12 (+)	11 (+)	12 (+)	12 (+)
	$CHCl_3$	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_2O	11 (+)	11 (+)	11 (+)	-	11 (+)	12 (+)

Table 5 Oxidative % Inhibition and IC_{50} Values of Crude Extracts of Sat-le-pyat Leaves and Stems

Samples	Extracts	% Inhibition (mean ±SD) In different concentration (µg/ mL)							IC_{50} (µg/mL)
		6.25	12.5	25	50	100	200	400	
Leaves	Aqueous	45.98 ± 2.85	47.79 ± 8.23	58.43 ± 2.08	82.93 ± 3.42	87.95 ± 0.60	88.75 ± 0.35	89.95 ± 0.92	15.09
	EtOH	22.48 ± 0.79	35.71 ± 3.93	60.90 ± 1.28	72.43 ± 2.41	81.47 ± 3.72	89.04 ± 1.53	93.90 ± 2.1	19.59
Stems	Aqueous	7.12 ± 3.36	20.73 ± 2.80	46.54 ± 7.75	66.46 ± 4.26	85.57 ± 2.46	86.79 ± 0.93	91.66 ± 0.93	29.34
	EtOH	21 ± 2.60	53.72 ± 3.20	65.57 ± 0.73	73.50 ± 0.42	79.85 ± 5.72	90.35 ± 1.80	93.04 ± 2.40	11.79

Table 6 Percent Oxidative Inhibition and IC_{50} Values of Standard Ascorbic Acid

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

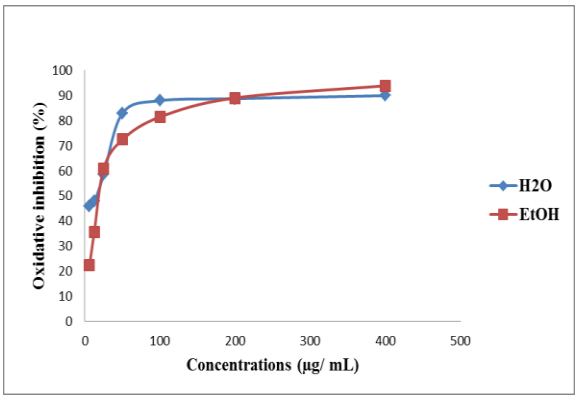

Figure 4 Plot of % oxidation inhibition vs concentrations (µg/mL) of aqueous and ethanol extracts of leaves

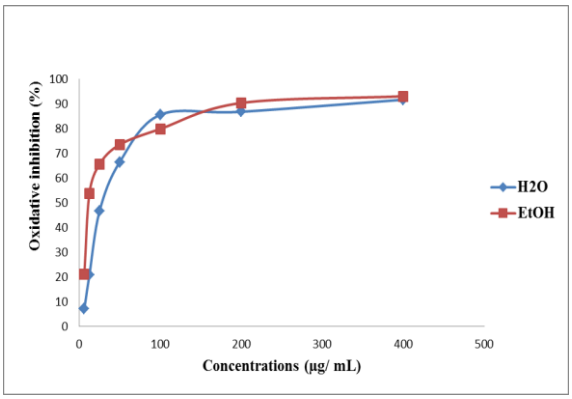

Figure 5 Plot of % oxidation inhibition vs concentrations (µg/mL) of aqueous and ethanol extracts of stems

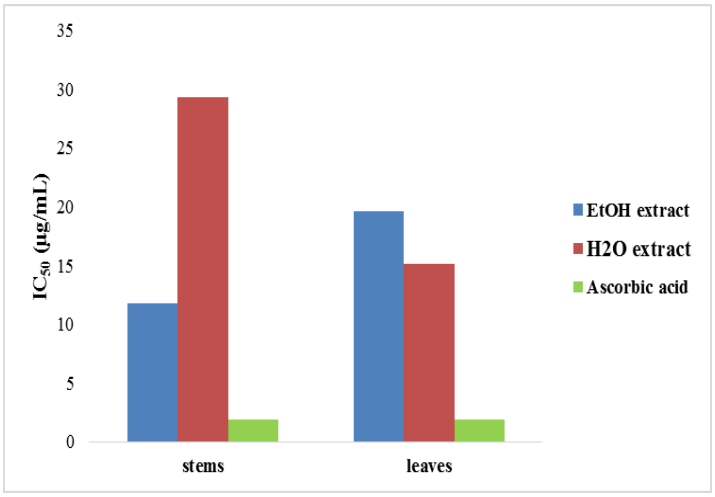

Figure 6 Bar graph of IC_{50} values of aqueous and 95% ethanol extracts compared with ascorbic acid

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 α- amino acids. From the results of nutritional values determination, Sat-le-pyat (leaves and stems) have higher carbohydrates and fiber content than other nutrients. The results of antimicrobial activity revealed that EtOAc extracts gave significant activity against all species of microorganisms (12∼ 30 mm) but H_2O 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 (leaves and stems), it was found that ethanol extract of stems (IC_{50}=11.79 µg/mL) showed the highest activity followed by aqueous extract of leaves (IC_{50}=15.09 µg/mL) respectively. According to these observations, it can be concluded that the Sat-le-pyat plant possessed antibacterial and antioxidant activities.

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References

AOAC. (2000). *Official and Tentative Methods of Analysis, Association of Official Analytical Chemist*. Washington D.C: 11th Ed., pp 978

Cruickshank, R. (1975). *Medical Microbiology*. London: 1st Edn., E.S. Living Stone Ltd.,

Evans, G.M. and Furlong, J.C. (2003). *Environmental Biotechnology Theory and Application*. England: John Wiley and Sons Ltd., pp 11-31

Finegold, S.M., Martin, W.J. and E. G., Scott. (1978). *Diagnostic Microbiology*. London: 5 th Ed., C.V Mosby Co. Ltd., pp 586-588

Heywood, V.H., Mode, D.M., Richardson, I.B.K. and Stearn, W.T. (Eds.) (2007). Flowering Plants of the World. London: Oxford University Press.

Harbone, J.B. (1993). *Methods in Plant Biochemistry*, London: Academic Press, vol. 8, pp 397

Harborne, J.B. (1984). *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis*, New York: 2nd Ed., Chapman and Hall Ltd., pp 120-126

Ibrahim, M. Y., Ansari, P., Sultana, M., Zhumur, N.A. and Shafi, S.M. (2015). " Evaluation of Thrombolytic and Cytotoxic activities of an Ornamental medicinal plant: Byttneria pilosa", *American Journal of Biomedical Research* , vol. 3 (2), pp 35-39

Jain, S.R., Jain, P.R. and Jain, M.R. (1974). "Antibacterial Evaluation of Some Indigenous Volatile Oils". Planta Medica, vol. 26, pp196

Kress, J.W., Robert, A.D., Farr, E. and Yin Yin Kyi, (2003). A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar, Department of Systematic Biology- Botany, National Museum of Natural History, Wishington DC, USA

Mar Mar Nyein, Chit Maung, Mya Bwin and Tha, S.J. (1991). "*In Vitro* Testing of Various Indigenous Plant Extracts on Human Pathogenic Bacteria, Myanmar Health". *J, Sci-Res*., vol. 3, 89

Marini-Bettolo, G.B., Nicolettic, M. and Patamia, M. (1981). "Plant Screening by Chemical and Chromatographic Procedure under Field Conditions". *J.Chromato*., vol. 213, pp 121

Raghuramulu, N., Madhavan Nair, K. M. and Kalyanasun Daram, S. (1983). "A Manual of Laboratory Techniques". National Institute of Nutrition, *Indian Council of Medical Research*, India, pp 31-33

Robinson, T. (1983). *The Organic Constituents of Higher Plants*. North America : 5th Edn., Cordus Press, pp 285-286

Shriner, R.L., Fuson, R.C., Curtin, D.Y. and Morrill, T.C. (1980). *The Systematic Identification of Organic Compounds*. New York: 6 th Ed., A Laboratory Manual, John Willey and Sons,

M-Tin Wa. (1972). "Phytochemical Screening, Methods and Procedures". *Phytochemical Bulletin of Botanical Society of America*, Inc., **5**(3), pp 4-10

Trease, G.E. and Evans, W.C. (1978). *Phytochemistry: Introduction and General Methods*. Pharmocognosy. London: Bailliere Tindall, pp227-247

Vogel, A.I. (1956). *A Text Book of Practical Organic Chemistry*. London: Longmans Green & Co. Ltd.,, pp454

Zaman, R., Parvez, M., Sekendar Ali, Md. and Islam, M. (2015). "Possible Anti-Obesity Activity of Methanol Extract of *Byttneria pilosa* Roxb. Leaves". *Middle- East Journal of Scientific Research*, vol. 23 (8), pp 1585-1589