

## DETERMINATION OF SOME PHYTOCONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF FLOWERS OF *Bombax ceiba* L. (LETPAN)

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### Abstract

In this research paper, the flowers of *Bombax ceiba* L. (Letpan) were selected for chemical analysis and antimicrobial activity. The Letpan sample was collected from East Shawbyugone Village, Pyawbwe Township, Mandalay Region. From the experimental results of preliminary phytochemical tests, alkaloids, flavonoids, phenolic compounds, steroids, carbohydrates, tannins, glycosides, terpenes and saponins were present. Nutritional values such as moisture (5.07 %), ash (19.45 %), fats (0.57 %), fibers (25.95 %), proteins (8.24 %), carbohydrates (40.72 %) contents were found to be observed in flowers of Letpan respectively based on dried sample. From the EDXRF results, mineral elements such as K, Ca, Fe, Ti, Zn and Cu were found in Letpan flowers. Antimicrobial activity of various crude extracts (Pet-ether, Ethyl acetate and Ethanol) of Letpan flowers was screened against six microorganisms by Agar Well Diffusion method. Ethanol extract showed antimicrobial activity against all tested microorganisms (18 mm to 21 mm).

**Keywords:** *Bombax ceiba* L. (Letpan), phytochemicals, nutritional values, elemental contents, antimicrobial activity

### Introduction

Medicinal plants are the plants used for centuries remedies for human diseases because they contain components of therapeutic value. About 80 % of world population relies on the value of traditional medicine which is predominantly based on plant material (Gupta *et al.*, 2004). Medicinal plants contain some natural products which perform definite physiological action on the human body and these bioactive substances include alkaloids, carbohydrates, tannins, terpenoids, steroids and flavonoids (Sachin *et al.*, 2013).

*Bombax ceiba* L. (Family: Malvaceae), is a large, deciduous tree commonly known as Silk Cotton Tree, Indian Red Kapok tree, Semal, Shimul, and Shalmali. It is found throughout India and other parts of tropical and sub-tropical Asia, Australia, and Africa. The plant has both economic and medicinal value. It yields gum and cotton. It is a large and long-living tree species which gives strength to the body, mind and heart (Varier *et al.*, 1997).

The plant is popular among the tribal communities for the treatment of various diseases. Almost every part of the plant, the seeds, flowers, roots and barks of *Bombax ceiba* have a long history of medicinal uses (Rani and Khullar, 2004; Girija *et al.*, 2010).

*Bombax* flowers have been used as a care for sexual impotency, hemorrhoids, swelling, and boils, and barks for cough, indigestion and stomach ache (Chopra *et al.*, 1956). It has been widely used in both Chinese and Indian traditional medicine for the

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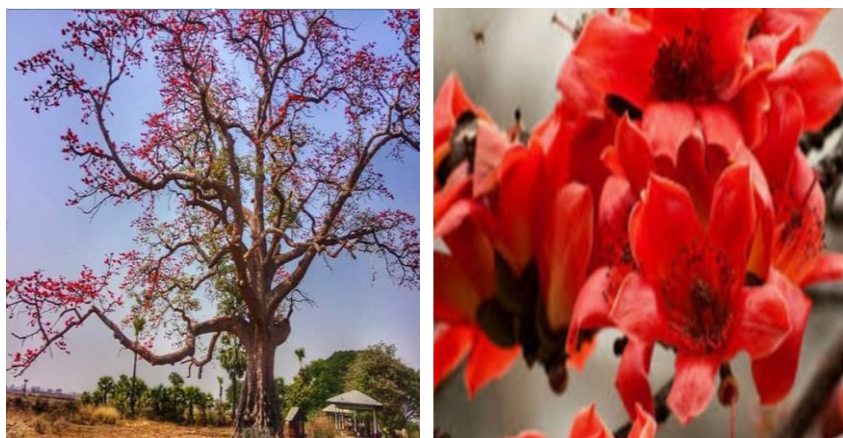
treatment of diarrhea, fever, chronic inflammations muscular injury and catarrhal affection (Wu *et al.*, 2008). The various extracts of *Bombax ceiba* have shown analgesic hypotensive, hypoglycemic, antimicrobial, antioxidant and antiangiogenic activity (You, 2003).

### Materials and Methods

#### Sample Collection and Preparation

The flowers of Letpan were collected from East Shawbyugone Village, Pyawbwe Township, Mandalay Region from December 2021 to January 2022. The scientific name of the Letpan flowers was identified at Department of Botany, University of Meiktila (Hundley and Chit Ko Ko, 1987; Kress *et al.*, 2003). After cleaning, the flowers were cut into tiny pieces and dried in the air. The air dried pieces were made powder by using blender and then stored in the air tight container to prevent moisture changes, other contamination and further research work.

|                 |   |                        |
|-----------------|---|------------------------|
| Scientific name | - | <i>Bombax ceiba</i> L. |
| Myanmar name    | - | Letpan                 |
| English name    | - | Red silk cotton tree   |
| Family          | - | Malvaceae              |
| Part used       | - | Flowers                |



**Figure 1** Plant and flower of *Bombax ceiba* L. (Letpan)

#### Preliminary Phytochemical Investigation of Flowers of *Bombax ceiba* L. (Letpan)

Preliminary phytochemical screening of the flower extracts from *Bombax ceiba* L. (Letpan) was carried out by standard procedures (Harborne, 1998).

#### Test for alkaloids

About 10 mL of 1 % hydrochloric acid was added to 2 g of the dried powder sample and boiled in water-bath for 10 minutes and then filtered. The filtrate was divided into three portions and they were tested with 1 mL of Mayer's reagent, Dragendorff's reagent and Wagner's reagent respectively.

Observation of a colored precipitate indicated the presence of alkaloid.

**Test for flavonoids**

2 g of dried powder sample was extracted with 20 mL of ethanol. The solution was filtered. 1-2 drops of concentrated hydrochloric acid and then small pieces of magnesium turnings were added into the filtrate.

Observation of a change in the color of the solution with 10 minutes indicated the presence of flavonoids.

**Test for phenolic compounds**

2 g of sample powder was mixed with 10 mL of distilled water and heated in a water bath for 30 minutes. The solution was filtered through a cotton filter. 3 drops of a mixture of 1 mL of 1%  $\text{FeCl}_3$  and 1 mL of 1%  $\text{K}_3[\text{Fe}(\text{CN})_6]$  were added into filtrate.

The greenish blue solution was observed.

**Test for steroids**

2 g of sample powder was mixed with 30 mL of ethanol and boiled with water bath for 10 minutes. The solution was filtered. The filtrate was placed into a test tube and was treated with 7 drops of acetic anhydride and gently warmed, and then a few drops of concentrated sulphuric acid were added.

The reddish brown color solution was observed.

**Test for carbohydrates**

2 g of sample powder was obtained with 10 mL of distilled water for about 20 minutes and filtered. The filtrate was placed in a test tube and a few drops of 10%  $\alpha$ -naphthol were added and shaken. The test tube was inclined an angle of  $45^\circ$  and about  $1 \text{ cm}^3$  of concentrated sulphuric acid was slowly introduced along the side of the test tube. The appearance of brown ring formed between the two layers indicated the presence of carbohydrate.

**Test for tannins**

2 g of dried powder sample was heated with 10 mL of distilled water for 30 minutes in a water bath and the solution was filtered. 3 mL of 2% NaCl solution was added to the filtrate and then 1 mL of 1%  $\text{FeCl}_3$  solution was added to it.

The green precipitate was formed. Therefore, it contained tannins in the sample of *Bombax ceiba* L.

**Test for glycosides**

(a) 2 g of dried powder sample was heated with 10 mL of distilled water for 30 minutes in a water bath and 10% sodium hydroxide solution was added. The yellow solution was observed.

(b) 2 g of dried powder material was extracted with 10 mL of ethanol.

The extract was added to the 10% lead acetate solution. The green precipitate was observed.

**Test for terpenes**

2 g of dried powder samples were warmed with 20 mL of per-ether for a few minutes and filtered. The filtrate was treated with 3 drops of acetic anhydride, four drops of chloroform ( $\text{CHCl}_3$ ) and the mixture was shaken. Then a few drops of concentrated sulphuric acid were carefully added and shaken.

Observation of the colour of the solution changed to a yellowish green color solution indicated the presence of terpenes.

### **Test for saponins**

5 mL of distilled water was added into the dried powder sample 2 g and shaken vigorously.

The permanent frothing in solution was observed. So, it indicated the presence of saponins in the flower of *Bombax ceiba* L.

### **Extraction of Sample Powder**

The dried powdered sample (500 g) was extracted with 1 liter of ethanol, pet-ether and ethyl acetate for one month. Then, the extracted solutions were filtered through Whatmann No.1 filter paper and the residues were washed with small volume of ethanol, pet-ether and ethyl acetate and filtered again. The filtrates were concentrated at room temperature and crude extracts were obtained.

### **Determination of Nutritional Values of Flowers of *Bombax ceiba* L.**

Nutrients compositions in flowers of *Bombax ceiba* L. (Letpan) were quantitatively determined according to AOAC methods (AOAC, 2000).

#### **Determination of moisture content**

3 g of powdered sample was placed in a pre-weighed porcelain crucible in an oven at 100 °C. It was cooled in a desiccator and then weighed again. The process of heating, cooling and weighing was repeated until a constant weight was achieved. The crucible containing dried sample was weighed again and the loss in weight was recognized as the moisture content in the sample. The moisture content of sample is recorded in Table 2.

#### **Determination of ash content**

3 g of dried powdered sample was placed in a porcelain crucible and ignited in furnace at 500 °C until the substance turned into ash. After ashing, crucible was cooled in a desiccator and weighed. The procedure was repeated until a constant weight was obtained and the percent content of ash in the sample could be calculated. The ash content of sample was recorded in Table 2.

#### **Determination of proteins content**

3 g of powder sample, 50 mL of distilled water, 8 g of catalyst mixture and 20 mL of concentrated sulphuric acid were added into a Kjeldahl digestion flask. It was completed and almost clear solution was obtained. The digested solution was cooled and added 400 mL of distilled water and large pieces of granulated zinc into it. To receive conical flask, 50 mL of boric acid solution (2 %) and a few drops of methyl red indicator were added. The distillation apparatus was set up with the delivery tube dipping below the boric acid solution. The diluted digest was made alkaline with 75 mL of 50 % sodium hydroxide solution. It was then distilled the ammonia into the boric acid solution. After about 300 mL had distilled over, the distilled was treated with 0.1 M sulphuric acid solution. The blank determination was carried out at the same manner. Finally, the percentage of nitrogen content in the sample was calculated and multiplying this value with 6.25 gave the protein % in Table 2.

### **Determination of fibers content**

3 g of powder sample was extracted three times by stirring, setting and decanting with petroleum ether. The extracted sample was air-dried and transferred to a dry 1000 mL of conical flask. 200 mL of 0.255 M sulphuric acid was added to the sample and it was heated to boiling point within 1 minute. The solution was boiled gently for exactly 30 minutes, maintaining a constant volume. The acid mixture was allowed to stand for 1 minute and filtered through a Bunchner funnel. The insoluble matter was transferred into a dried weighed crucible and dried at 100 °C to a constant weight.

Finally, the contents in the crucible were completely incinerated to an ash at a dull red heat. The difference in weight of the contents in crucible before and after incineration gave the weight of fiber. The fiber content of the sample was recorded in Table 2.

### **Determination of fats content**

The dried powder sample (3 g) was put in a cloth bag and it was then placed in Soxhlet extractor. The petroleum ether was poured into the extractor until some of it overflowed into the flask. After the extraction was completed (usually it takes about 12 h), the petroleum ether was dried at 100 °C in an oven to constant weight and weighed. The fat content of the sample is described in Table 2.

### **Determination of carbohydrates content**

The carbohydrates present in the sample include starch (glycogen in animal tissue), dextrin and disaccharides. The total carbohydrates content can be obtained as the difference between 100 and the sum of percentages of protein, fat, ash, moisture and fiber. The resultant carbohydrate content percent is shown in Table 2.

### **Determination of Elemental Analysis of Sample by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry**

In order to determine the elements in the sample, dried powder flowers of *Bombax ceiba* L. was determined by EDXRF method at the Department of Physics, Meiktila University.

Pellet of the sample (2.5 cm diameter) was first made by using a pellet making machine. X-ray spectrometer permits simultaneous analysis of light element to heavy element. Energy dispersive X-ray fluorescence spectrometer (Rigaku EDX-700) can analyze the elements from Na to U under vacuum condition. X-ray fluorescence was X-ray to excite an unknown sample. The individual elements comprising in the sample emit their own characteristic X-rays. They are detected by using semiconductor detector [Si(Li)] that permits simultaneous analysis of multi elements within the sample. In this way, EDX-700 spectrometer determines the elements that are present in the sample. It can perform two kinds of quantitative analysis: Fundamental Parameter (FP) Method and Calibration Curve Method. In FP method, theoretical results can be calculated even when standard samples are not available. It can be applied to most samples. Although limited samples can be applied, the accuracy is high. In the present study, FP method was applied for the elemental analysis. The result was reported and discussed in Table 3.

### Screening of Antimicrobial Activity of the Flowers of *Bombax ceiba* L. by Agar Well Diffusion Method

*Bacillus subtilis*, *Bacillus pumilus*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus*

Nutrient agar was boiled and 20-25 mL of the medium was poured in the conical flask and plugged with cotton wool and autoclave at 100 °C for 45 mins in an autoclave. After this, the flasks were cooled down at room temperature and 0.2 mL of spore suspension were added into it and poured into the sterilized petri-dish. The agar was allowed to set for 30 mins after with 8 mm plate agar-well was made with the help of sterilized cork borer. After that about 0.2 mL of the sample was introduced into the agar-well and incubated at 25 °C for 24 h. The inhibition zone (dear zone) which appeared around the agar-well, indicated the presence of antimicrobial activity.

### Results and Discussion

#### Preliminary Phytochemical Tests for Flowers of Letpan Samples

The phytoconstituents by the standard method were shown in Figure 2 and Table 1.

**Table 1** Preliminary Phytochemical Investigation of Flowers of *Bombax ceiba* L. (Letpan)

| No | Test of compounds  | Extract solvent | Reagent used  | Observation                             | Result      |
|----|--------------------|-----------------|---|---|-------------|
| 1  | Alkaloids          | 1 % HCl         | Mayer's reagents<br>Dragendorff's reagents<br>Wagner's reagents | White ppt.<br>Orange ppt.<br>Brown ppt. | +<br>+<br>+ |
| 2  | Flavonoids         | EtOH            | Conc: HCl and<br>Mg turnings                                    | Pink color solution                     | +           |
| 3  | Phenolic compounds | DW              | 1 % $K_3[Fe(CN)_6]$ solution and<br>1 % $FeCl_3$ solution       | Greenish blue solution                  | +           |
| 4  | Steroids           | EtOH            | Acetic anhydride and<br>conc: $H_2SO_4$                         | Reddish brown color                     | +           |
| 5  | Carbohydrates      | DW              | 10 % $\alpha$ -naphthol,<br>conc: $H_2SO_4$                     | Brown ring                              | +           |
| 6  | Tannins            | DW              | 2 % NaCl solution +<br>1 % $FeCl_3$ solution                    | Green ppt.                              | +           |
| 7  | Glycosides         | DW,<br>EtOH     | 10 % NaOH solution<br>10 % lead acetate                         | Yellow solution<br>Green ppt.           | +<br>+      |
| 8  | Terpenes           | Pet-ether       | Acetic anhydride,<br>chloroform and<br>Conc: $H_2SO_4$          | Yellowish green color solution          | +           |
| 9  | Saponins           | Distilled water | Shaken vigorously   | Frothing solution                       | +           |

Note : (+) = Presence , ppt. = Precipitate



**Figure 2** Phytochemical tests of flowers of *Bombax ceiba* L. (Letpan)

### Determination of Nutritional Values in Letpan Flowers

The nutritional values such as moisture, ash, protein, fiber, fat and carbohydrates content for flowers of *Bombax ceiba* L. (Letpan) were reported in Table 2.

As a result, it was found that the moisture content of the flower of *Bombax ceiba* L. (Letpan) was found to be 5.07 %. The ash content was found to be 19.45 % and the protein content was found to be 8.24 %. The main work of protein is to build the body and to repair the warm tissue, but any protein, eaten in excess of the amount required by the body can be used to provide energy (Ruth, 1997). The fiber content of Letpan was found to be 25.95 %. The fat content was found to be 0.57 % for Letpan sample. The Letpan sample contains low fat content, so it benefits for human health.

In the present work, the carbohydrates content in Letpan was found to be 40.72%. The Letpan sample of high source was carbohydrate. When carbohydrate was deficient, it prevents the unnecessary usage of protein and allows it to be used for body building process.

**Table 2** Nutritional Values in the Flowers of *Bombax ceiba* L. (Letpan)

| No. | Nutrients     | Content (%) |
|-----|---------------|-------------|
| 1   | Moisture      | 5.07        |
| 2   | Ash           | 19.45       |
| 3   | Protein       | 8.24        |
| 4   | Fiber         | 25.95       |
| 5   | Fat           | 0.57        |
| 6   | Carbohydrates | 40.72       |

### Measurement of Elemental Contents of Flowers of *Bombax ceiba* L. (Letpan)

Quantitative determination of the elements in *Bombax ceiba* L. (Letpan) sample was carried out by EDXRF method. It was found that the elements such as Ca, K, Fe, Ti, Zn, and Cu were detected in the sample by the EDXRF method. The

elemental values showed that the amount of Ca and K are large amounts. Fe and Ti contents were in a significant amount. Moreover, Zn and Cu were observed as trace elements.

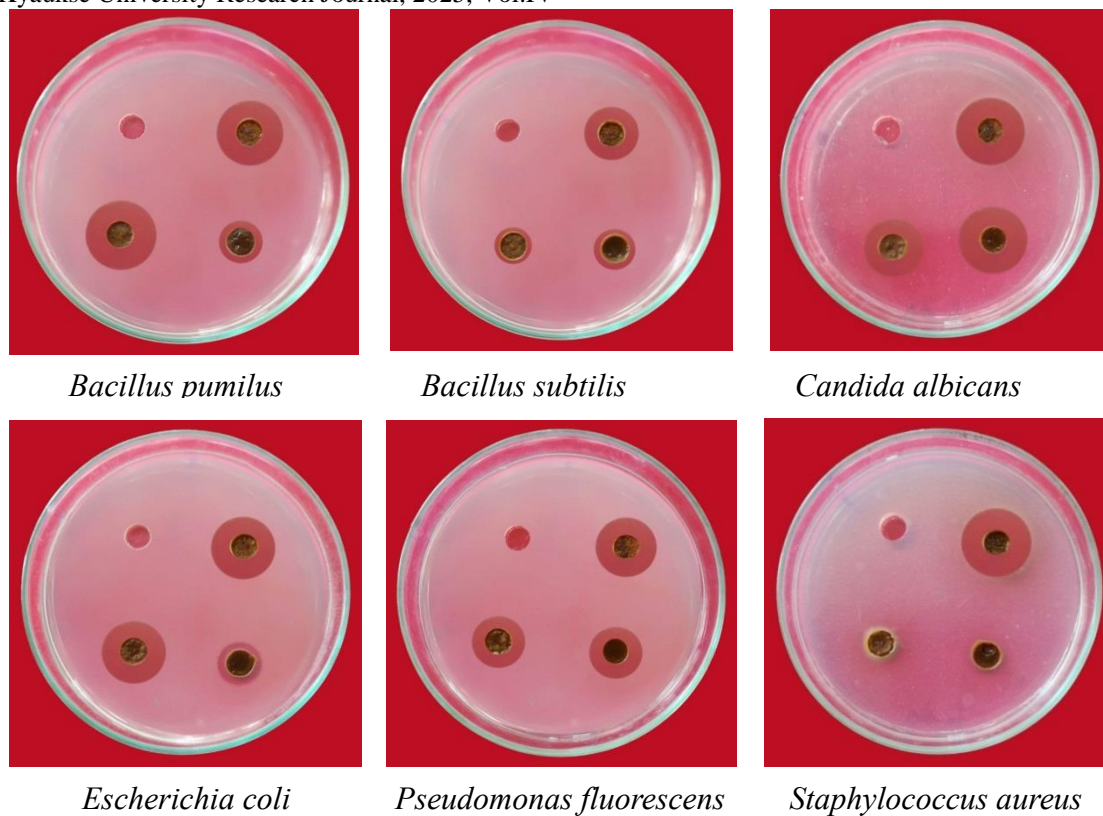
Calcium is important to bone growth and formation, blood clotting, nerve and muscle functioning. It also helps regulate the passage of nutrients in and out of the cell walls, and lower blood pressure. Calcium is important to normal kidney function that reduces blood cholesterol levels. The element potassium is an extremely important element in the human body. Potassium is important in controlling of the heart, muscles, and nervous. Without potassium, the nerve cell couldn't send those messages to the brain. Iron is used by the body to make tendons and ligaments. It can be found that these elements can be very effective for human health.

**Table 3** Percent Relative Abundance of Elements in Letpan Flowers by EDXRF

| Method |          |             |
|--------|----------|-------------|
| No.    | Elements | Content (%) |
| 1      | Ca       | 45.577      |
| 2      | K        | 41.353      |
| 3      | Fe       | 6.470       |
| 4      | Ti       | 2.057       |
| 5      | Zn       | 0.475       |
| 6      | Cu       | 0.204       |

#### **Antimicrobial Activity of Crude Extracts from Flowers of *Bombax ceiba* L. (Letpan) Samples**

In the present work, antimicrobial activities of crude extracts (Pet-ether, EtOAc and EtOH) from Letpan, were investigated on six harmful microorganisms which include *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* by agar well diffusion method. The diameter of the agar well is 8 mm. When comparing different antimicrobial agents to know concentrations, the inhibitory zone diameter is taken as a measure of antimicrobial activity. The larger the diameter, the higher the antimicrobial activity of test agents (Fire gold and Martin, 1982). The results of the antimicrobial activity on crude extracts tested on against selected organisms were presented in Figure 3 and Table 4.



**Figure 3** Antimicrobial screening of crude extracts of flowers of *Bombax ceiba* L.(Letpan)

**Table 4** Results of Antimicrobial Activity of Flowers of *Bombax ceiba* L. (Letpan)

| No. | Microorganisms                 | Inhibition zone diameter (mm) |             |      |
|-----|--------------------------------|-------------------------------|-------------|------|
|     |                                | Pet-ether                     | EtOAc       | EtOH |
| 1   | <i>Bacillus pumilus</i>        | 14                            | 21          | 19   |
| 2   | <i>Bacillus subtilis</i>       | 14                            | 12          | 18   |
| 3   | <i>Candida albicans</i>        | 19                            | 17          | 18   |
| 4   | <i>Escherichia coli</i>        | 17                            | 16          | 19   |
| 5   | <i>Pseudomonas fluorescens</i> | 14                            | 20          | 21   |
| 6   | <i>Staphylococcus aureus</i>   | -                             | -           | 18   |
|     | Agar well diameter             | -                             | 8 mm        |      |
|     | 8 mm                           | -                             | 12 mm (+)   |      |
|     | 13 mm                          | -                             | 17 mm (++)  |      |
|     | 18 mm                          | -                             | above (+++) |      |

(-) = No activity

From the result, given in Table 4, it was observed that ethanol extract of flowers of Letpan sample exhibited the antimicrobial activity on all tested organisms. In addition, ethanol extract showed the highest activity on *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens*. Among them, pet-ether extract possessed the highest activity on *Candida albicans*. Ethyl acetate extract had the

highest antimicrobial activity on *Bacillus pumilus*. On *Staphylococcus aureus*, ethyl acetate extract and pet-ether extract did not show antimicrobial activity.

### Conclusion

From the overall assessments of the present work, the following inferences can be deduced.

From the phytochemical tests, the results were the presence of alkaloids, flavonoids, phenolic compounds, steroids, carbohydrates, tannins, glycosides, terpenes, and saponins in this Letpan sample. These results expose that *Bombax ceiba* L. (Letpan) has quite a number of chemical constituents which may be useful for pharmacological actions.

From the nutritional values determination by AOAC method, moisture content (5.07 %), ash content (19.45 %), protein content (8.24 %), fiber content (25.95 %), fat content (0.57 %) and carbohydrate (40.72 %) respectively. Among them, it was found that the amount of carbohydrate, fiber, and ash were highest, and the amount of fat was observed lowest content in the *Bombax ceiba* L. (Letpan). Therefore, the flowers of Letpan should be eaten for nutritions and maintaining health.

In the elemental analysis by EDXRF method, calcium (45.577 %), potassium (41.353 %), iron (6.470 %), titanium (2.057 %), zinc (0.475 %) and copper (0.204 %) were present. High calcium value can build and maintain strong bones and healthy communication between the brain and other parts of the body. The flowers of Letpan control blood clotting, and reduces blood cholesterol levels.

From the antimicrobial results by agar-well diffusion method, it was observed that pet-ether, ethyl acetate and ethanol extracts of Letpan sample exhibited the respective antimicrobial activity inhibition zone diameter. Ethyl acetate extracts of Letpan showed the highest antimicrobial activity in *Bacillus pumilus* (21 mm), ethanol extract showed on *Pseudomonas fluorescens* (20 mm) and pet-ether extracts exhibited on *Candida albicans* (19 mm). In the case of *Staphylococcus aureus*, pet-ether and ethyl acetate extracts did not show antimicrobial activity.

According to the results, it may be concluded that Letpan is very nutritious, maintaining health and pharmacological activity for human consumption. Thus, the flowers of Letpan are indeed remarkably nutritious and have health benefits.

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