QUALITATIVE AND QUANTATIVE PHYTOCHEMICAL ANALYSIS OF LEAVES OF *ARTOCARPUS LAMELLOSUS* BLANCO AND ITS ANTIMICROBIAL ACTIVITY

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Abstract

The plant *Artocarpus lamellosus* Blanco is an unrecorded species in Myanmar, belong to the family Moreaceae. It was collected from University of Yangon Campus, Kamayut Township. The aim of the present study on qualitative and quantitative phytochemical screening of the *Artocarpus lamellosus* Blanco and its microbial activity. In preliminary phytochemical analysis, the three different extracts such as HCL, ethanol and water extracts of leaves were found alkaloids, flavonoids, glycosides, phenolic compounds, saponins, starch, reducing sugars, carbohydrates, proteins, tannins and α-amino acid. In quantitative analysis, moisture content, acid-insoluble ash, water soluble ash. The extractive values with different solvents and ash values were also analyzed and recorded. The elemental analysis and the presence of the toxic elements of the leaves were analyzed by using Atomic Absorption Spectrophotometer (AAS). According to the result, it was revealed that moisture content (7.62%), total ash (6.24%), acid insoluble ash (75. 06%) and water-soluble ash (9.73%). According to the extractive value result, the phytoconstituents of *Artocarpus lamellosus* Blanco were the highest soluble in petroleum ether whereas the least soluble in water or methanol. The nutritional value of the leaves was analyzed. Antimicrobial activities of *Artocarpus lamellosus* Blanco were investigated with seven extracts of different solvents from the powdered leaves on eight test organisms by agar well diffusion assay. Among them, antimicrobial activity on pathogenic microorganism, *Salmonella typhi* and the weakly antifungal activity was found on *Escherichia coli*.

Keyword: Artocarpus lamellosus Blanco (leaves) qualitative and Quantitative phytochemical analysis and antimicrobial activity

Introduction

The genus *Artocarpus* (Moraceae) consists of 50 species, mainly distributed over tropical regions of Asia. There are 15 species growing in southern China. Some *Artocarpus* members are known for their medicinal value. *Artocarpus* species have been exploited in various countries and revealed the medicinal variation possibility. As noted earlier, the most common ethnomedicinal usage was to treat wounds and ulcers as well as some skin problems. Extensive literature reported different pharmacological tests involved with the extracts and the isolated phytochemicals which exhibited anti-inflammatory, antioxidant, antimicrobial, gastroprotective, cytotoxic, anti-proliferative activities and acted as selective enzymes inhibitors. Different parts of the *Artocarpus* species are used in traditional folk medicinal practices. *Artocarpus* species as a source of essential nutrients and bioactive phytochemicals which categories it as a bifunctional food. This review compiles traditional, phytochemical and pharmacological data on different *Artocarpus* species that the plants possess pharmacological properties like, antioxidant, anti-inflammatory, antibacterial, anticarcinogenic, Immunomodulatory, antifungal, hypoglycaemic effects, inhibits melanin biosynthesis. (Journal of Pharma Search Vol. 9 (1), 2014:7). The *Artocarpus* plants are proclaimed to be rich in phenolic compounds such as flavonoids, stilbenoids, and arylbenzofurons (Yen, *et al.*, 2017). The *Artocarpus* genus has provided diverse

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information to determine their phytochemical compounds and pharmacological activities. The relating bioactive compounds can potentially treat infection caused by microorganisms and diseases like diabetes, cancer, and malaria. *Atrocarpus lamellosus* Blanco extract is as a pancreatic lipase inhibitor, and has antiobesity action, can be used for diseases such as prevention or treatment of obesity (Journal of Pharma Search Vol. 9 (1)2014:7).

The objectives of this paper are to investigate the chemical constituents of *Artocarpus lamellosus* Blanco (leaves), to examine its elemental analysis and nutritional value, and to study antimicrobial activity of the various crude extracts from the leaves of *Artocarpus lamellosus* Blanco.

Materials and Methods

Collection of the plant samples

Artocarpus lamellosus Blanco was collected from University of Yangon campus, Kamayut Township. The samples were washed, cut into small pieces and then air-dried at room temperature. When constant weight was obtained, the dried samples were pulverized by grinding machine and stored in an air tight container.

Qualitative analysis of *Artocarpus lamellosus* Blanco (Leaves)

In this investigation, the powdered sample of leaves from *Artocarpus lamellosus* Blanco was tested to find out the presence or absence of chemical constituents such as alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, reducing sugars, saponins, starch, steroids/terpenoids and tannins compounds. Qualitative and quantitative analysis were carried out in the Phytochemistry Laboratory of Botany Department, University of Yangon according to the methods of B.P. (1968); Marini Bettolo, *et al*., (1981); Central Council for Research in Unani Medicine (1987); Trease and Evans (2002).

Test for Alkaloids

The powdered sample (3 g) was boiled with 1% HCL (50 ml) for about 20 minutes and filtered off. The filtrate was divided into two portions and test with modified Mayer's reagent, and Wagner's reagent. Treatment with the mentioned alkaloid reagents furnished alkaloid precipitates.

Test for α-amino acids

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. An aqueous portion was drop onto a filter paper, allowed to dry and sprayed with Ninhydrin reagent. The plate was allowed to dry at room temperature and then kept in an oven at 110 ºC for a few minutes; purple colored spot appeared on the filter paper, indicating the presence of α-amino acids.

Test for Carbohydrates

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. The filtrate was added with (1 ml) of α-napthol reagent solution and a few drops of concentrated sulphuric acid. A red or brick precipitate was formed which indicate the presence of carbohydrates.

Test for Flavonoids

The powdered sample (2 g) was boiled with 95% ethanol (25 ml) for about 20 minutes and filtered. The filtrate was introduced into a test tube added a small piece of magnesium and a few drops of concentrated hydrochloric acid. Boil the solution for few minutes. The presence of a pink or yellowish-brown color indicates the presence of flavonoids.

Test for Glycosides

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. The filtrate was treated with 10% lead acetate solution. A yellow color indicates the presence of glycosides.

Test for Phenolic Compounds

The powdered sample (2 g) was boiled with 1% HCL (25 ml) for about 20 minutes and filtered. The filtrate was added with 10% ferric chloride solution. Observation was made to see if dark green color appeared.

Test for Proteins

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. The filtrate was added (1 ml) of distilled water and 5 drops of Millon's reagent. A white precipitate is formed which turns red on heating which indicate the presence of proteins.

Test for Reducing Sugars

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. To this filtrate a mixture of Benedict's solution was added and boiled for a few minutes on boiling water bath. Brick red precipitate was formed which indicate the presence of reducing sugars.

Test for Saponins

The powdered sample (2 g) was put into a test and some distilled water was added. Then the mixture was vigorously shaken for a few minutes. Observation was made to see if frothing took place.

Test for Starch

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered, 2 drops of iodine solution were added to filtrate. Yellowish brown precipitate which indicates the presence of starch.

Test for Steroids/ Terpenoids

The powdered sample (2 g) was boiled with 95% ethanol (25 ml) for about 20 minutes and filtered. The filtrate was added (2 ml) of chloroform and (1 ml) of concentrated sulphuric acid, formation of reddish-brown color indicates the presence of steroids.

Test for Tannins

The powdered sample (2 g) was boiled with 95% water (25) ml for about 20 minutes and filtered. The filtrate was added a few drops of 5% ferric chloride solution. Black color precipitate is produced indicating the presence of tannins.

Quantitative analysis of *Artocarpus lamellosus* Blanco (**Leaves**)

Physicochemical investigation was determined according to British Pharmacopecia (1965).

1. Moisture content
2. Total ash content
3. Acid insoluble ash content
4. Water soluble ash content
5. Aqueous soluble matter content
6. Soluble matter content in different solvents petroleum ether, acetone, methanol, ethanol and water.

Determination of moisture content

The moisture content of the powdered sample of the leaves was determined on an oven. The powdered samples 5 g were weighed accurately, placed in a weighed a clean dry beaker and kept in an oven at 110° C for one hour. The beaker was then removed from the oven and cooled in a desiccator at room temperature and weighed. This procedure was repeated until a constant weight of the sample was obtained.

The percentage of the moisture content was calculated by the following formula,

$$\% \text{ of total moisture } = M\text{-}m \diagup W \times 100$$

M = weight of sample of weighting beaker in g before in g before drying

m = weight of sample of weighting beaker in g before in g after drying

W = weight of taken in g

Determination for Weight of Total Ash

The powered sample 10.0 g was weighted in a crucible and placed in a muffle-furnace at 550 °C until substance turned into ash. After that, the crucible was cooled and weighted. This procedure was repeated until a constant weight was obtained and the percentage of total ash was calculated.

$$\% \text{ of total ash } = M\text{-}m \diagup W \times 100$$

Determination of Acid-Insoluble Ash

For the acid insoluble ash determination, the above ash was boiled for 5 minutes in 10 ml of 6% HCl. The insoluble ash was collected by filtration with filter paper. The insoluble ash was washed with water until free of acid. The acid insoluble residue together with the crucible were dried in an oven at 110 °C and weighed again. The percentage of insoluble ash was calculated.

Determination of Water- Soluble Ash

The total ash was boiled with 10ml of water for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with water and ignited to a constant weight. This

weight or insoluble matter was substrated from the weight of the total ash, to give weight of the water-soluble ash.

Determination of Soluble Matters

The powdered rhizomes 5.0 g was weighed and placed in a conical flask. It was soaked with 50 ml of water in a flask for 72 hours and overnight. Then it was filtered and the filtrate was evaporated to dryness in a previous weighed container was weighed. This procedure was repeated until a constant weight was obtained.

Antimicrobial Activities

The powered leaves were extracted with petroleum ether, chloroform, acetone, ethyl acetate, ethanol, methanol, and distilled water. The various solvent extracts were tested on eight pathogen microorganisms. Antimicrobial activities were conducted by using agar well method (Collins, 1965) at the Microbiology Laboratory of Botany Department, University of Yangon.

Test agar plates

Test organisms were *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Pseudomonus aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. Each test organism (50μl) was added to 100 ml of assay (SM: sucrose 1.0g, malt extract 0.3 g, NaCl 0.05 g, $CaCO_3$, 0.01 g, agar 1.8 g, distilled water 100 ml, pH 7 poured into plates.

Agar well diffusion assay

The agar plates containing test organisms were punched to make the wells (8 mm in diameter) using sterile cork borer and filled with the stock solution (0.2 ml) and then these plates were incubated at room temperature for 24 hrs. After incubation, the diameters of the growth inhibition zones surrounding the wells were measured in mm. These zones indicate the presence of antimicrobial activities which inhibit the growth of test organisms selectively (Collins, 1965).

Table 1. Test organisms and diseases

Test organisms	Diseases
Agrobacterium tumefaciens	Plant tumor cell
Bacillus subtilis	Food spoilage and fever
Candida albicans	Vaginal 207andidiasis, alimentary tract infection, skin infection, sinus irritation, intense itching and sores
Escherichia coli	Diarrhoea and vomiting dysentery and urinary tract infection
Malassezia furfur	Dandruff and skin infection
Pseudomonus aeruginosa	Chronic lung, urinary tract infection, inflammation in bone and joints, gastrointestinal and blood infections
Salmonella typhi	Typhoid
Staphylococcus aureus	Skin diseases, wound, burns, staphylococcal pneumonia, toxic shock syndrome, bacteraemia, meningitis, osteomyelitis, endocarditis and sepsis

Results

Qualitative Analysis

In this experiment alkaloids, glycosides, phenolic compounds, steroids/ terpenoids, flavonoids, saponin, starch, reducing sugars, tannins, carbohydrates, proteins and α-amino acids were observed in the leaves. The results were shown in Table 2.

Table 2. Qualitative analysis of *Artocarpus lamellosus* Blanco

Sr.No.	Test	Extract	Test reagent	Observation	Results
1	Alkaloids	1%HCL	Mayer's reagent Wagner's reagent	orange color Reddish brown color	 -
2	Glycosides	water	10%lead acetate solution	brown ppt	+
3	Phenolic compounds	1%HCL	3% Ferric chloride solution	Reddish brown ppt	+
4	Steroids/Terpenoids	EtOH	$CHCL_3$ + conc:H_2SO_4	Reddish brown color	+
5	Flavonoids	EtOH	Conc:HCL/Mg	Yellowish brown ppt	+
6	Saponin	Water	Distilled water	Frothing	+
7	Starch	Water	Iodine solution	Brown ppt	+
8	Reducing sugars	Water	Benedict's solution	Yellowish brown ppt	+
9	Tannins	Water	5% ferric chloride solution	Black ppt	+
10	Carbohydrates	Water	10% α-napthol & conc:H_2SO_4	Red ppt	+
11	Proteins	Water	Millon's reagent	Red ppt	+
`12	α-amino acid	Water	Ninhydrin reagent	purple spot appear	+

(+) = present (-) = absent ppt = precipitate

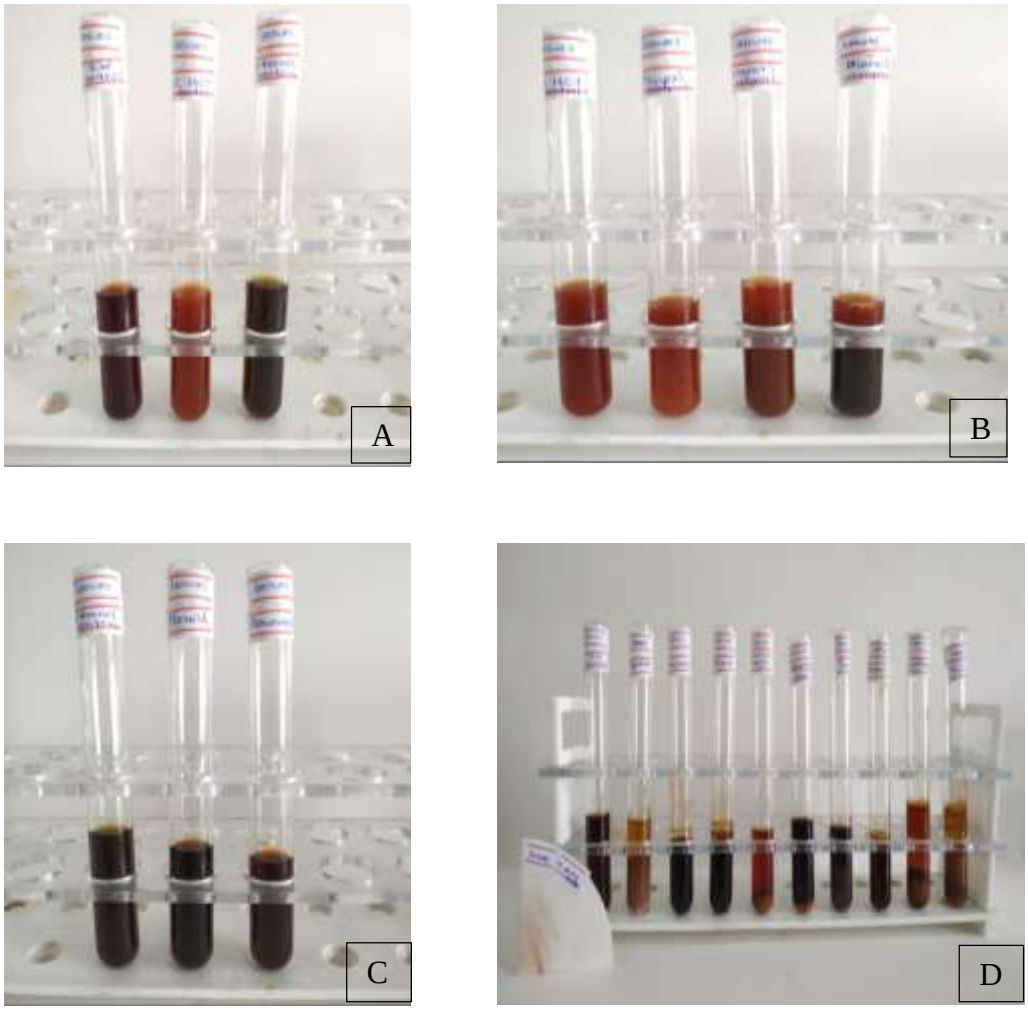

Figure 1. Preliminary Phytochemical Test of *Artocarpus lamrellosus* Blanco Powdered leaves

A. DW control, 1% HCL control and ethanol control,

B. Test for Alkaloids and Phenolic compounds,

C. Test for Steroid and Flavonoids and

D. Test for Glycoside, Saponin, Starch, Reducing sugar, Tannins, Carbohydrate, Proteins and α-amino acids

Quantitative Analysis

In quantitative analysis, the solubility of the powdered leaves was the best in petroleum ether and acetone, and was moderately soluble in ethanol, and methanol. That of the powdered samples was the least in water as shown in Table 3.

Table 3. Quantitative analysis of *Artocarpus lamellosus* Blanco

Sr.No.	Physio-chemical characters	Quantity determined in %
		Rhizomes
1.	Moisture content	7.62
2.	Total ash	6.24
3.	Acid-insoluble ash	75. 06
4.	Water-soluble ash	9.73
5.	Petroleum ether soluble matter	76.30
6.	Ethanol soluble matter	69.90
7.	Methanol soluble matter	64.31
8.	Acetone matter	72.58
9.	Water soluble matter	46.79

Elemental Analysis of *Artocarpus lamellosus* Blanco leaves by using Energy Dispersive X-ray Fluorescence (EDXRF) Spectrophotometer

The results of elemental analysis of *Artocarpus lamellosus* Blanco leaves revealed that Calcium (Ca) and Potassium (k) were contained in highest amount whereas Manganese (Mn), Iron (Fe) and Strontium (Sr) were present in moderate amount. Sliver (Ag) and Zinc (Zn) were found in trace amount. The results were shown in Table 4

Table 4. The relative concentration of elements in the sample powder of *Artocarpus lamellosus* Blanco

Sr.No.	Element	Concentration
1.	Calcium (Ca)	67.994 %
2.	Potassium (K)	15.605 %
3.	Manganese (Mn)	9.228 %
4.	Iron (Fe)	4.695 %
5.	Strontium (Sr)	1.105 %
6.	Silver (Ag)	0.865 %
7.	Zinc (Zn)	0.508 %

Toxic elements Analysis of *Artocarpus lamellosus* Blanco leaves by using Atomic Absorption Spectrophotometer (AAS)

According to the results, Chromium (Cr), Cadmium (Cd), Arsenic (As) and Lead (Pb) were found in small amount. The results were shown in Table 5.

Table 5. Toxic elements analyzed by Atomic Absorption Spectrophotometer (AAS)

Sr. No	Test Parameter	Result	Unit	Detection Limit
1	Arsenic (As)	0. 0031	mg/L	0.08 ppb
2	Cadmium (Cd)	0.023	mg/L	0.009 ppb
3	Chromium (Cr)	0.058	mg/L	0.0011 ppm
4	Lead (Pb)	0.357	mg/L	0.00 63 ppb

mg/L = milligram per litter*

Nutritional Value of leaves from *Artocarpus lamellosus* Blanco

In nutritional value, protein, fiber, fat and carbohydrate were observed in the leaves. According to the experiments, the amount of fat and carbohydrate were greater than protein and fiber. The results were shown in Table 6.

Table 6. Present chemical analysis resulted by Nutritional Value

No.	Experiment	Present Chemical Analysis Results
1	Protein (%)	6.41
2	Fiber (%)	2.54
3	Fat (%)	11.9
4	Carbohydrate (%)	10.4

Antimicrobial Activities of *Artocarpus lamellosus Blanco* (leaves)

In this experiment, the petroleum ether, chloroform and acetone extracts showed no activity and the ethyl acetate, methanol, ethanol and aqueous extracts indicated high activity on *Agrobacterium tumefaciens, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli*. The petroleum ether, chloroform and acetone extracts showed no activity and the ethyl acetate extract showed moderate activity and the methanol, ethanol and aqueous extracts *Candids albicans.* The petroleum ether and chloroform extracts showed no activity and the acetone extract exhibited weak activity on *Malassezia furfur, Salmonella typhi* and *Staphylococcus aureus*. The ethyl acetate, methanol, ethanol and aqueous extracts indicated high activity on *Malassezia furfur* and *Salmonella typhi.* The ethyl acetate and methanol extracts indicated moderate activity whereas the ethanol and aqueous extracts showed high activity on *Staphylococcus aureus.* The petroleum ether, chloroform extracts of *Artocarpus lamellosus* Blanco showed no activity, but the ethanol and aqueous extracts showed high activity on eight pathogenic organisms. The acetone extract exhibited weak activity on *Malassezia furfur, Salmonella typhi* and *Staphylococcus aureus*. The results of antimicrobial activities were shown in Table 7 and Figures 4-11.

Table 7. Antimicrobial activities of various solvents extract for the leaves of *Artocarpus lamellosus* Blanco

Test Organisms	Solvents						
	Petether	Chloroform	Acetone	Ethyl Acetate	Ethanol	Methanol	Water
Agrobacterium tumefaciens	-	-	-	18	26	22	20
Bacillus subtilis	-	-	-	20	24	20	20
Candida albicans	-	-	-	16	22	18	22
Escherichia coli	-	-	-	18	20	20	20
Malassezia furfur	-	-	10	18	22	22	22
Pseudomonas aeruginosa	-	-	-	20	22	18	22
Salmonella typhi	-	-	10	20	26	24	24
Staphylococcus aureus	-	-	10	14	20	16	22

Agar well size = 8 mm, - = no activity, 10 – 12 mm weak activity, 13 – 17 mm = moderate activity, > 18 mm = high activity

Antimicrobial activities of various extracts from the leaves of *Artocarpus lamellosus* Blanco

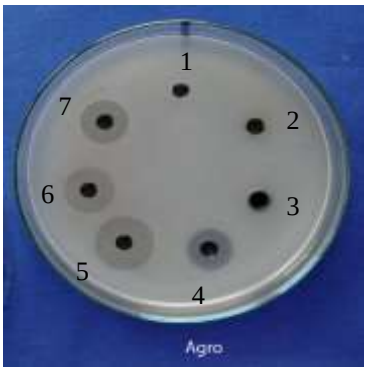

Figure. 4. Inhibitory zones against *Agrobacterium tumefaciens*

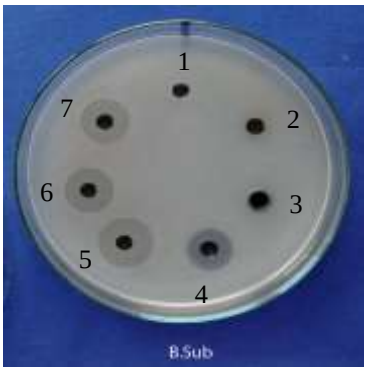

Figure. 5. Inhibitory zones against *Bacillus subtilis*

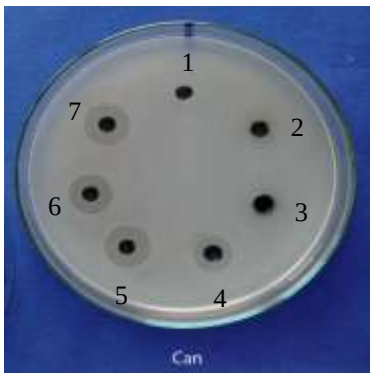

Figure. 6. Inhibitory zones against *Candida albicans*

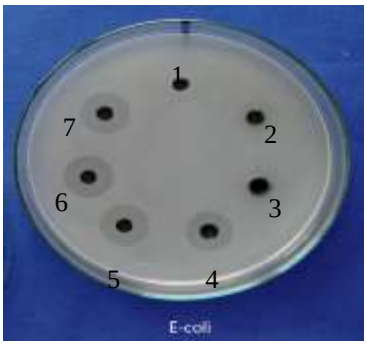

Figure.7 Inhibitory zones against *Escherichia coli*

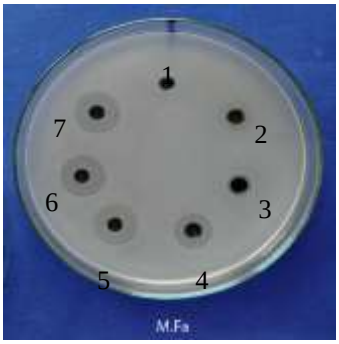

Figure. 8. Inhibitory zones against *Malassezia furfur*

Figure.9. Inhibitory zones against *Pseudomonas eruginosa*

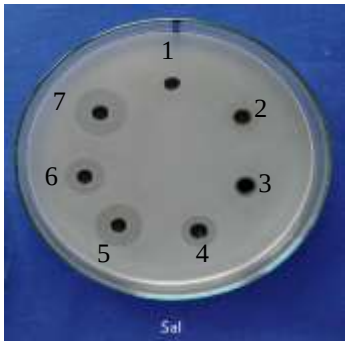

Figure. 10. Inhibitory zones against *Salmonella typhi*

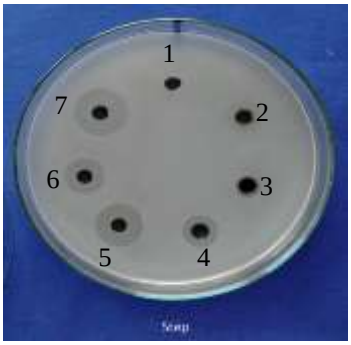

Figure 11. Inhibitory zones against *Staphylococcus aureus*

1 = Petroleum 2 = Chloroform extract 3 = Acetone extract

4 = Ethyl acetate 5 = Ethanol extract 6 = Methanol extract

7 = Aqueous extract

Discussion and Conclusion

In this research, the qualitative and quantitative phytochemical analysis of *Artocarpus lamellosus* Blanco had been conducted. In the preliminary phytochemical study, it was revealed that the presence of twelve different physicochemicals in three different extracts. According to the results of phytochemical investigation, it indicated that the alkaloids, α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, saponins, starch, steroids/terpenoids, tannins and reducing sugars are presented in the leaves. *Artocarpus* species are known to produce phenolic compounds and flavonoids.

In quantitative analysis, five different solvents were used. Powdered leaves were mostly soluble in petroleum ether (76.30%) and acetone (72.58%) while they were moderate soluble in ethanol (69.90%) and methanol (64.31%), and was the least in water (46.79%). In the elemental analysis, AAS data, potassium (K), phosphorus (P), calcium (Ca) and sulphur (S) are found as macronutrient elements whereas manganese (Mn), iron (Fe), titanium(Ti), silver (Ag), Zinc (Zn) and rubidium (Rb) elements. According to this study, potassium is found to be the highest concentration in the leaves of this plant and lead is also highest concentratiion among heavy metals. These characters are agreed with those of WHO, 1998.

Potassium is very important mineral for the proper function of all cells, tissues, and organs in the human body. It helps regulate fluid balance, muscle contractions and nerve signals. A high-potassium diet may help reduce blood pressure and water retention, protect againt stroke and prevent and kidney stones. Calcium is one of the most important minerals for the human body. It helps form and maintain healthy teeth, bones, and it has keys metabolic functions. A proper level of calcium in the body over a life time can help prevent osteoporosis.This minerals help our body grow, develop, and stay health. It is essential that the balance diet should include a wide variety of foods. Some minerals are even used to make hormones or maintain a normal heartbeat. Potassium and calcium are found in *Artocarpus lamellosus* Blanco leaved. So, it may be considered to be useful for medicinal purposes. These characters are agreed with those described by WHO, 1998.

The toxic elementals such as arsenic, chromium, cadmium and lead were found as trace element in toxic levels. Lead (Pb) concentration present in the leaves of *Artocarpus lamellosus* Blanco**.** Is 0.357mg/kg. Lethal dose of inorganic a 70kg (155 pound) in adult, a toxic dose is 450mg or 0.45g for 20 pound child, this works out to 160 mg or 0.16 g. The poisonous level of (Pb) for human is 60mg/kg. These constituents were not harmful on human health. Thus it could be used as traditional medicine. These characters are in agreement with those of Hartley *et al*.,1980.

Nutritional value is important. Nutritional value relates to carbohydrates, fats, proteins, minerals, additives, enzymes, vitamins, sugar intake, cholesterol, fat and salt intake. A healthy diet throughout life promotes healthy pregnancy out comes, supportsnnormal growth, development ageing, helps to maintain a healthy body weight, and reduces the risk of chronic disease to overall health and well-being. These characters are agreed with those of Magd. Zakaria, *et al*., 2020.

In test of antimicrobial activities, the different solvent extracts of leaves inhibited *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. According to the agar well diffusion method, the ethanol and aqueous extracts from the leaves showed highly antimicrobial activities on eight pathogenic organisms. The methanol extract indicated moderate activity on *Staphylococcus aureus* and high antimicrobial activities on seven pathogenic organisms. The petether and chloroform extracts of leaves did not show antimicrobial activities on eight pathogenic organisms. These characters are in agreement with those of Cruickshank, R.J.P. (1975).

In conclusion, qualitative and quantitative analysis, elemental analysis, nutritional values and antimicrobial activities of *Artocarpus lamellosus* Blanco (leaves) were conducted. These findings are crucial to providing immense opportunities for the development of new *Artocarpus lamellosus* -based products in pharmaceutical industries, cosmetics and toxicity. The ethanol, methonol and aqueous extracts possessed higher antimicrobial activities than other solvent extracts in this study. Thus, the methanol extract or the ethanol extract from the laves of *Artocarpus lamellosus* Blanco will be used to produce the bioactive compounds from the leaves.

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