Quantitative Determination of Carbohydrate, Lycopene Contents and Antimicrobial Activity of Ripe Fruit of *Lycopersicon Esculentum* Mill.

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Abstract

In this research work, one of the Myanmar vegetable fruits *Lycopersicon esculentum*(tomato), Myanmar named Kha-yan-chin-thee was chemically analyzed. The ripe fruit of *Lycopersicon esculentum*(tomato) was collected from Lay-Taing-Sin Village, Myothit Township, Magway Region in January, 2022. The main aim of this research is to investigate phytochemical constituents, carbohydrate, lycopene contents, antimicrobial activity, and elemental composition from the ripe fruit of *Lycopersicon esculentum*. Firstly, preliminary phytochemical tests were determined by test tube method. The collected fruit contained valuable phytochemicals such as α-amino acid, alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, terpenes, and tannin compounds. Water, ash, and fiber contents of the collected fruit were determined by AOAC methods. Water, ash and fiber contents of the selected fruit were found to be 92.13 %, 2.58 %, and 1.40 % respectively. Qualitative determination of carbohydrate from ripe fruit of *Lycopersicon esculentum* was examined by Molisch's, Fehling's, Benedict's, Barfoed's, Moore's, Seliwanoff's, Rapid furfural and Bial's tests. Carbohydrate content was examined by using iodometric technique. Carbohydrate (reducing sugar) content of *Lycopersicon esculentum* was found to be 2.45 %. Lycopene content of selected fruit was determined by spectrophotometric method. Lycopene content from ripe fruit of *Lycopersicon esculentum* was found to be 6.14 mg/100g. Antimicrobial activity of various solvent extracts (water, methanol, ethanol, ethyl acetate, *n*-hexane) of selected fruit was determined by agar well diffusion method and tested on six microorganisms. Among the various solvent extracts water, ethanol, and ethyl acetate extracts of selected fruit possessed more effective antimicrobial activity than other extracts. From EDXRF analysis, high composition of potassium, calcium, phosphorus, iron, sulphur, zinc, copper, and manganese consisted in selected fruits. Therefore, the ripe fruit of *Lycopersicon esculentum* is good sources of minerals, carbohydrate, lycopene and possesses effective antimicrobial activity.

Keywords **-** antimicrobial activity, carbohydrate, lycopene, *Lycopersicon esculentum*

Introduction

Lycopersicon esculentum (Family-Solanaceae) belong to the vegetable class. Tomato is a bright red colored fruit, which is not only good taste but also very healthy. Tomato is a natural source of vitamins A, B, C, and D and minerals such as calcium, phosphorus, potassium, sulphur, and iron that are essential for the normal functions of the body. It is also used in the productions of several cosmetic, skin and beauty products, and certain ketchup (Kim *et al.*, 2014). Tomato helps in digestion, improves weight loss, regulates menstruation, prevents infections, reduces inflammatory, and improves heart health. It has anticancer properties and helps in skin care. Tomato is rich in antioxidant nutrients such as carotenes, flavonoids, and vitamin C, as well as vitamin B. It is a good source of fiber. Together, these nutrients help to improve cardiovascular health and protect against colon cancer (Moco *et al.*, 2006). In this research, preliminary phytochemical screening, qualitative and quantitative determination of carbohydrate, lycopene content, elemental compositions,

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and antimicrobial activity were investigated from ripe fruit of *Lycopersicon esculentum*.

Botanical description of *Lycopersicon esculentum*

Figure 1. Ripe fruit of *Lycopersicon esculentum*

Family - Solanaceae
Botanical name - *Lycopersicon esculentum*
Common name - Tomato
Myanmar name - Kha-yan-chin-thee
Part used - Ripe fruit

Materials and methods

Sample Collection and Preparation

The ripe fruit of *Lycopersicon esculentum* was collected from Lay-Taing-Sin Village, Myothit Township, Magway Region in January, 2022. The peel pulp of collected fruit was cut into small pieces and air dried at room temperature for about one months. Then, the air-dried sample was stored in a well stopper bottle, and used throughout the experiment.

Preliminary Phytochemical Tests for Ripe Fruit of *Lycopersicon esculentum*

Preliminary phytochemical screening was done using standard qualitative methods. Tests for presence or absence of alkaloids, α-amino acid, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoid were carried out.

Determination of Some Nutritional Values from Ripe Fruit of *Lycopersicon esculentum*

1. Determination of water content: Sample (2 g) was exactly weighed and introduced into a pre-dried and weighed moisture dish. Then it was placed in an oven at 105 ºC for 2 hours. Then it was removed from oven and cooled in a desiccator and weighed. The procedure was repeated until constant weight was obtained. From the resulting data, the water content was calculated.

2. Determination of crude fiber: 1 g sample powder was extracted with pet-ether. The extracted sample was air dried and transferred into a 250 mL of round-bottomed flask. Then 100 mL of 0.1 M sulfuric acid solution was added. The flask was connected to the condenser and refluxed for about 30 minutes and it was filtered through a filter paper. The residue was washed with distilled water and the washing continued until the final washing was free from acid. The residue was then washed down into the flask with 100 mL of 0.3 M sodium hydroxide solution. The flask was again refluxed for about 30 minutes and filtered through the filter paper. The residue was then washed with boiling distilled water, 1 % weight by volume hydrochloric acid and again with boiling water until it is free from acid. It was then washed twice with ethanol and three times with ether. The residue was transferred into the crucible and dried to a constant weight (A) at 100 ºC. After drying residue was weight and recorded. Then the residue was ignited until the carbonaceous matter has been

removed. The contents of the crucible were cooled and weighed. Heating, cooling and weighing was repeated until the constant weight (B) was obtained. The loss in weight was taken as crude fiber.

3. Determination of ash content: About 3 g dry sample powder was weight and put into a pre-dried, cooled and weighed porcelain crucible. The crucible was heated in a muffle furnace at 550-600 ºC. The heating was continued until the resultant ash was white in color and free from carbon. Then the crucible containing residue was cooled in a desiccator and weighed. Heating, cooling and weighing were repeated until a constant weight was obtained. The weight of the residue remaining after incineration under specific conditions is taken as the ash content of the sample.

4. Qualitative determination of carbohydrate: Molisch's, Fehling's, Benedict's, Barfoed's, Moore's, Seliwanoff's, Rapid furfural and Bial's tests were performed for the qualitative determination of carbohydrate.

5. Quantitative determination of carbohydrate (reducing sugar): The outer peel of tomato was removed. Then 10 g of tomato was weighted and grinded in a motor. The ground mass was boiled with distilled water and through a piece of white cloth or cotton was filtered after cooling. The value of the filtrate was made to 250 mL in a volumetric flask with distilled water (stock solution). From the stock solution 12.5 mL was taken and a mixture of 10 mL of 5 % $ZnSO_4$ and 10 mL of 5 % Ba $(OH)_2$ were purified by adding to the solution. It was filtered and the volume of the filtrate was made to 100 mL with distilled water. 5 mL of purified sugar solution and 5 mL of Somogyi's reagent were taken in a borosil test tube. Three such test tubes with the mixture was to be taken (marked as sample). Another such set of three test tubes were to be prepared by taking 5 mL of Somogyi's reagent and 5 mL of distilled water (marked as "blank"). Covering with a glass stopper immersed all the six tubes in a boiling water bath for 20 minutes. After the required time, it was noticed that the mixture of the "sample" tubes turns brown and those of the tubes marked "blank" remained blue. The tubes were cooled and it was titrated with 0.005 M sodium thiosulfate.

Procedure for titration: A burette (100 mL) was filled with 0.005 M sodium thiosulfate solution. 2 mL of 10 % NaOH was added to the thiosulfate solution to prevent atmospheric oxidation. The solution of the tubes was taken in a conical flask (100 mL) and 2 mL of previously prepared KI solution was prepared. After a few minutes 1.5 mL of 2 M H_2SO_4 was to be added to the solution. Followed by immediate addition of a few mL of thiosulfate from the burette prevent the liberation of I_2 from KI solution. The color of the mixture was turned brown. 2 drops of starch indicator were added, the solution was turned to blue. The solution was titrated against the thiosulfate until the blue color solution disappears. The amount of thiosulfate required was noted for neutralization. The procedure was repeated for the remaining tubes.

Calculation - 1 mL of thiosulfate = 0.135 mg of reducing sugar

Percentage of glucose (reducing sugar)

$$\text{Percentage of glucose} = \frac{(\text{Blank reading} - \text{Sample reaidng}) \times 0.135 \times 100}{5 \times 0.5 \times 10}$$

= ... mg of reducing sugar.

Where, the values

100 = Total volume of the purified sugar

5 = Volume of sugar solution taken for estimation

0.5 = Weight of tomato present in 12.5 mL sugar solution taken for purification from the stock solution (250 mL); prepared from 10 g tomato

10 = Percentage conversion into mg

Extraction and Estimation of Lycopene from Fruit of *Lycopersicon esculentum*

Principle: Lycopene is extracted in acetone and then taken up in petroleum ether. The extracted lycopene is measured at 503 nm in a spectrophotometer.

Requisites**:** Acetone, Petroleum ether (40-60ºC), 5 % sodium sulfate, anhydrous sodium sulfate

Extraction Procedure: 10-20 g of fresh red tomato fruit was weighted and with about 50 mL of acetone was crushed. It was centrifuged and the supernatant was taken in a beaker and the extraction was repeated with acetone until the residue was colorless. All the acetone extract was transferred to a separating funnel containing an equal amount of petroleum ether. 40 mL of distilled water (10 mL at a time) was added to this mixture side of the funnel. Then 20 mL of 5 % sodium sulfate solution was added and the funnel was gently shaken. The lower layer was washed by adding more petroleum ether until all the color moved to the upper petroleum ether layer. The lower acetone-water layer was discarded. The extract was washed with a little distilled water. The washed petroleum ether was poured into an amber cultured bottle. About 10 g of anhydrous sodium sulfate was added and it was allowed to stand for about an hour. After the required time, the extract of petroleum ether was filtered through cotton placed in a Buchner funnel into a 100 mL volumetric flask. The residue of sodium sulfate was washed with petroleum ether until it was colorless. The washing was transferred to the same flask and the volume was made up with petroleum ether (100 mL). The absorbance of the extract was read at 503 nm against the reagent blank (petroleum ether).

Calculation: The amount of lycopene present in the sample could be calculated by the following methods. The extinction coefficient of lycopene has been reported (Britton, 1985) at 3450 in light petroleum.

$$\text{Amount of lycopene in gm} = \frac{\text{O.D} \times \text{Total volume of the extract (mL)}}{\text{Extinction coefficient} \times 100}$$

O.D = absorbance of extract

Confirmation of Lycopene

The observed lycopene could be confirmed by melting point determination and solubility tests.

Determination of Antimicrobial Activity from Ripe Fruit of *Lycopersicon esculentum*

Antimicrobial activity of various solvent extracts from the ripe fruit of *Lycopersicon esculentum* was carried out by agar well diffusion method test on six microorganisms such as *Bacillus subtilis, Staphylococus aureus, Psedomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* respectively.

Samples: The crude extracts of the sample were prepared by extracting the sample with different solvents like water, methanol, ethanol, ethyl acetate and *n*-hexane by percolating method. The extracts (1 g of each) were introduced into sterile petri-dishes and 1 mL of their respective solvent.

Procedure: The antimicrobial activity of the crude extracts from selected sample was determined against six strains microorganisms by agar-well diffusion method. The extract 1 g was introduced into sterile petri-dish and dissolved. The bacteria suspension from trypticase soy broth was done evenly onto the surface of the trypticase soy agar slant immediately after hardening of the agar-well were made with a 10 mm sterile cork borer from each extract agar. After inoculums had dried for 5 minutes, the agar discs were removed and the wells were filled with sample to be tested. And then, the plates were incubated at 37 ºC. After overnight incubation at 37

ºC the diameter of inhibition zone including 10 mm wells was measured. This method was used to test antimicrobial action of the extracts on 24 hours broth culture of the organism used. The extracts from sample were tested with six microorganisms. The observation was done and the inhibition zone diameter and measurements were recorded.

Determination of Elemental Composition from Ripe Fruit of *Lycopersicon esculentum*

The elemental composition in ripe fruit of *Lycopersicon esculentum* was determined by EDXRF technique.

Results and discussion

Phytochemical Tests for the Ripe Fruit of *Lycopersicon esculentum*

According to preliminary phytochemical tests, the ripe fruit of *Lycopersicon esculentum* consisted of alkaloids, α-amino acid, carbohydrate, flavonoids, glycosides, steroids, starch, saponins, phenolic compounds, terpenes, reducing sugar, and tannins respectively. These phytochemicals are secondary metabolites and protect substances causing diseases. Among them flavonoids, polyphenols, and phenolic compounds possess antioxidant, anticancer, and anti-inflammatory properties.

Table 1. Phytochemical Screening of Ripe Fruit of *Lycopersicon esculentum*

No.	Tests	Extract	Test Reagent	Observation	Results
1	Alkaloids	1 % HCl	Wagner's reagent	Yellowish brown ppt	+
			Dragendorff's reagent	Orange ppt	+
2	α- Amino acid	Distilled water	Ninhydrin reagent	Purple spot	+
3	Carbohydrates	Distilled water	10 % α- naphthol, conc: H_2SO_4	Red ring	+
4	Cyanogenic glycosides	Distilled water	Picric acid and 5 % Na_2CO_3 solution	No colour change (in paper)	-
5	Flavonoids	95 % EtOH	Conc : HCl, and Mg ribbon,	Pink colour solution	+
6	Glycoside	Distilled water	10 % lead acetate	White ppt	+
7	Phenolic compounds	Distilled water	10 % $FeCl_3$ solution	Green color solution	+
8	Reducing sugars	Distilled water	Benedict's solution	Brick red ppt	+
9	Saponins	Distilled water	Distilled Water	Froth formation	+
10	Starch	Distilled water	1 % I_2 solution	Deep blue colour solution	+
11	Steroids	Petroleum ether	Acetic anhydride, conc: H_2SO_4	Reddish brown colour solution	+
12	Tannins	Distilled water	10 % $FeCl_3$, dil:H_2SO_4,	Pale green ppt	+
13	Terpenoids	95 % EtOH	Acetic anhydride, $CHCl_3$, conc: H_2SO_4	Reddish brown colour solution	+

(+) = presence (-) = absence (ppt) = precipitate

Some Nutritional Values Present in Ripe Fruit of *Lycopersicon esculentum*

Determination of some nutritional values containing in the selected fruit were carried out in Department of chemistry, University of Magway. The resulting data are shown in Table 2. The resulting carbohydrates are glucose and fructose by the qualitative determination of sugar tests. Both simple sugars (glucose and fructose) these are quickly digested by the body, supporting us with energy. Glucose is the source of energy for our brain and nervous system.

Table 2. Some Nutritional Values Containing the Ripe Fruit of *Lycopersicon esculentum*

No.	Nutrient	Observed value (%)
1	Water Content	92.13
2	Fiber	2.58
3	Ash	1.40
4	Carbohydrate (Reducing sugar)	2.45

Lycopene Content in Ripe Fruit of *Lycopersicon esculentum*

Lycopene content in ripe fruit of *Lycopersicon esculentum* was determined by spectrophotometric method. The observed data were described in Table 3. Lycopene content of the selected fruit was found to be 6.14 mg based upon 100 g sample. Lycopene may promote good oral health, bone health, and regulate blood pressure. Many health benefits of lycopene provide such as a cancer fighting agent and a powerful antioxidant.

Confirmation of lycopene

The observed lycopene could be confirmed by melting point determination and solubility tests.

Table 3. Melting Point and Solubility Tested on Observed Lycopene

	Melting point
Observed lycopene	172.2ºC
Literature value	172-173ºC
Solvents	Solubility
Water, Methanol, Ethanol	Insoluble
Chloroform, Ether, *n*-hexane	Soluble

Figure 2. Observed lycopene

Antimicrobial Activity of the Ripe Fruit of *Lycopersicon esculentum*

Antimicrobial activities crude extracts in five solvent systems were measured by agar well diffusion methods and tested on six microorganisms. The resulting data are shown in Table 4. According to the results of antimicrobial activity, ethanol extract responded high activity (inhibition zone diameter 24 mm) on *Candida albicans* and medium activity (inhibition zone diameter range 16-19 mm) on other tested microorganisms. And then water and ethyl acetate extracts responded medium activity (inhibition zone diameter range 15-19 mm) on all tested microorganisms. Methanol extract responded high activity (inhibition zone diameter 23 mm) on *Candida albicans*, medium activity on *Escherichia coli* and low activity on other tested microorganisms. In addition, *n*-hexane extract responded low activity on tested microorganisms except *Bacillus subtilis*. Therefore water, ethanol, and ethyl acetate extracts are more useful than methanol and *n*-hexane extracts for production of antimicrobial drug.

Table 4. Results of Antimicrobial Activity on the Ripe Fruit of *Lycopersicon esculentum*

Sample	Solvent Extracts	Inhibition Zones Diameter (mm)					
		I	II	III	IV	V	VI
Lycopersicon esculentum	*n*-Hexane	-	11 (+)	11 (+)	12 (+)	12 (+)	12 (+)
	EtOAc	15 (++)	18 (++)	16 (++)	17 (++)	19 (++)	18 (++)
	EtOH	18 (++)	16 (++)	17 (++)	16 (++)	24 (+++)	19 (++)
	MeOH	13 (+)	14 (+)	12 (+)	14 (+)	23 (+++)	17 (++)
	Water	16 (++)	15 (++)	15 (++)	17 (++)	19 (++)	18 (++)

agar-well – 10 mm
(+) low activity (10 mm~14 mm)
(++) medium activity (15 mm~19 mm)
(+++) high activity (20 mm above)

Organisms
I. *Bacillus subtilis* (N.C.T.C-8236)
II. *Staphylococcus aureus* (N.C.P.C-6371)
III. *Pseudomonas aeruginosa* (6749)
IV. *Bacillus pumilus*(N.C.I.B-8982)
V. *Candida albicans*
VI. *Escherichia coli* (N.C.I.B-8134)

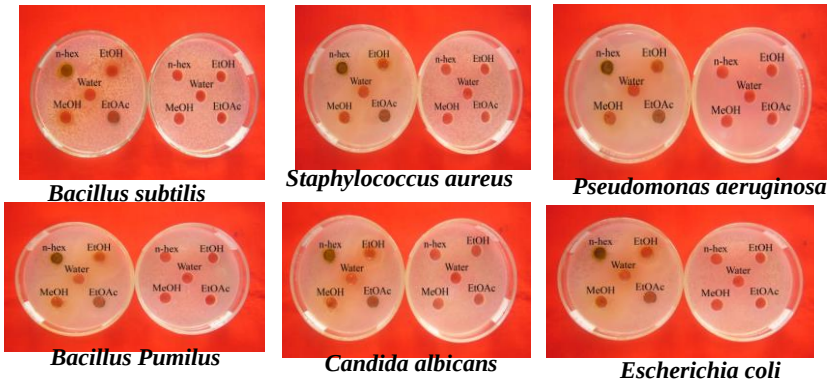

Figure 3. Inhibition zones of the antimicrobial activity of various extracts from ripe fruit of *Lycopersicon esculentum*

Elemental Compositions of the Ripe Fruit of *Lycopersicon esculentum*

Elemental compositions from the ripe fruits of *Lycopersicon esculentum* was determined by EDXRF technique. The observed data were described in Table 5. According to the observed results, potassium, magnesium, calcium, phosphorus, copper, zinc, manganese, and iron are high contents among the inorganic nutrients. These elements are essential elements for metabolism of our body. Potassium, calcium, phosphorous and sulfur are macro-elements. Iron, zinc, manganese and copper are microelements. These elements play in important role of biochemical reaction.

Table 5. Relative Abundance of Elements Present in *Lycopersicon esculentum*

No.	Element	Relative Abundance (Percent)
1.	K	53.523
2.	Ca	16.232
3.	P	12.824
4.	Fe	11.027
5.	S	5.649
6.	Zn	0.303
7.	Mn	0.291
8.	Cu	0.151

Conclusion

In this study, the ripe fruit of *Lycopersicon esculentum was* chosen for chemical analysis. Firstly, preliminary phytochemical screening of the selected fruit was determined. According to these results, the selected fruit contains the valuable phytochemical constituents which have good health benefits to human. These phytochemicals are secondary nutrients and protect substances causing diseases. The water content of the selected fruit was found to be 92.13 %. So, the ripe fruit of *Lycopersicon esculentum* should be consumed for the healthy, smooth and fresh skin. The fibre content was found to be 2.58 %. The ash content *Lycopersicon esculentum* was found to be 1.40 %. The carbohydrate content was found to be 2.45 %. Lycopene content of the selected fruit was found to be 6.14 mg based upon 100 g sample. Lycopene promotes weight loss, regulates menstruation, prevents infections, relief inflammatory, and improves heart health. From antimicrobial activity determination, ethanol extract showed high activity on *Candida albicans*, medium activity on other tested microorganisms. Water, methanol, and ethyl acetate extracts responded significant activity on all tested microorganisms. Therefore, the selected fruit sample should be consumed for antimicrobial drug especially the infection of candidiasis, which results from overgrowth of the fungus and dysentery. Potassium, calcium, phosphorus, iron, sulphur, copper, zinc, and manganese are highly consisted in the ripe fruit of *Lycopersicon esculentum*. Therefore, the ripe fruit of *Lycopersicon esculentum* is good sources of minerals, lycopene, and it possesses effective antimicrobial activity. So, the ripe fruit of *Lycopersicon esculentum* should be consumed for maintaining human health and may be used as a variety of medicinal purposes.

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