

# Anatomical and Physiological responses of Citrus megaloxycarpa Lush.: A Cryptic Species of North-East India.

Arun Jerang

university of science and technology, meghalaya <https://orcid.org/0000-0002-0924-7857>

Sony kumari

University of Science and Technology, meghalaya

Madushmita Borthakur (✉ [mborthakur58@gmail.com](mailto:mborthakur58@gmail.com))

University of Science and Technology, Meghalaya

Shahbaaz Ahmed

University of Science and Technology, meghalaya

---

## Research Article

**Keywords:** Cryptic, Antioxidant, Antimicrobial, Anticancerous, Therapeutic

**Posted Date:** July 6th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-653063/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

## Abstract

In the historical mysteries and present pandemic situation, the use of citrus fruits makes it rise high among other fruits. Citrus has a significant role in dietary and medicinal purposes from time immemorial and widely acknowledged for its therapeutic properties. *Citrus megaloxycarpa* Lush. is an unspecified sibling of the citrus family. The present work highlights the use of the cryptic species of North-East India with its efficient biochemical, antimicrobial, anticancerous properties activity. The aim of this study was to characterize analyzed various biochemical, antioxidant, anticancerous parameters. The peel and pulp extract of ripening large citrus sample indicated as P(L<sub>1</sub>) and Pu(L<sub>2</sub>) respectively and unripe small peel and pulp extracts was indicated as P(S<sub>1</sub>) and Pu(S<sub>2</sub>) respectively. The extract of the Pu(L<sub>2</sub>) has the highest total soluble protein whereas the extract of P(L<sub>1</sub>) demonstrated high in carbohydrate concentration. On quantifying the free amino acid value, the extract of P(L<sub>2</sub>) showed higher in quantification. The total DPPH scavenging activity was compared for the extracts, where the extract of Pu(L<sub>2</sub>) exhibit high IC<sub>50</sub> value. The present work concluded the use of *Citrus megaloxycarpa* Lush. peel and pulp extracts to be an efficient therapeutic drug.

## Introduction

Citrus contains many phytochemicals that are essential for carrying out different cellular activities to perform various physiological and enzymatic functions to prevent certain ailments in our busy lifestyle. Citrus peel found to display extensive antimicrobial, antioxidant, and anticancer activity (Burt 2004; Ortuno et al. 2006). Phytochemicals found in citrus are having high nutritive value and helps in inhibiting many diseases causing organisms.

Citrus is believed to be originated from the tropical and sub-tropical regions of Asia and the Malay Archipelago and slowly spread across the world. The use of citrus is mentioned in Sanskrit and Chinese literature of early 800 B.C. Himalayan region and South China are known to be the place of origin for most citrus fruit, around 78 Species of Rutaceae are found as a native of India.

Citrus is used as a traditional remedy from ancient times for restorative and ritual purpose around the globe. According to the world health organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries (Saharan et al. 2011). Citrus sinensis is the most widespread grown crop in the state. Higher adaptability of citrus in different climatic conditions, govern its successful growth rate in tropical, subtropical, and in some temperate regions of the world. Their nutritional and medicinal values have made them remarkably important. Lemon belongs to the family Rutaceae family and is rich in sources like vitamin C, Flavonoids, and Essential oils which have antimicrobial and anticancer properties (Maruti et al. 2011). Citrus is used as an ethnic medicine in different parts of the world. Citrus medica Fruits and leaves are used in asthma, diarrhea, dysentery, fever, headache, intestinal disorder, jaundice, piles, pulmonary, skin disease, vomiting, worm infestation, etc (Khalid et al. 2014). Citrus contains hesperidin, as well as diosmin, which is known to improve venous blood flow and promoting stability to the capillary vessels (Roger 2002; Economos 2012). Citrus jambhiri

is used as anti-diarrheal and used in digestion dysfunction. Citrus has many therapeutic attributes in Ayurveda for purification, levigating procedures while preparing different ayurvedic formulations and for incineration purposes (Chaudhari et al. 2016).

Considerably North-East India is paradise of many indigenous citrus species. It is considered as one of the important fruit crops. Citrus crops are booming in North-East Region due to its wide-ranging weather and rich soil condition, although production is lesser compare to other states. North-east falls under different tropical and sub-tropical regions that support the growth of different citrus species. The chief citrus growing belts in North-East India are Meghalaya (Garo, Jaintia, Khasi, Dusha), Nagaland, Manipur, Arunachal Pradesh & Assam. Wild flora of North-East is rich in indigenous and semi indigenous citrus fruit. Citrus is highly known for its nutritive value in North-East India, The acidic nature of lemon makes it a preferable flavouring agent in dishes like vegetable, fish, meat, and salads, it is used in many North East Indian cuisine, particularly in Manipur peels of sour pummelo are used for cooking purpose. Citrus megaloxycarpa Lush. is rare, endanger species of citrus found in North-Eastern part of Indian. The citrus crop is propagated by vegetative or seeds (Hore and Barua (2004), it is of high importance to the horticultural community of North-East India. Citrus Medica, commonly known as Memang Narang by Garo tribe are reportedly growing in the wild and virgin forest of Naga hills, in and around the national park, Kaziranga of Assam and in the Garo hills of Meghalaya. Citrus latipes (Swingle) is found growing wild in sacred groves of Meghalaya. Some of the citrus fruit ingenious to North-East region as reported by Sasanka and Shanta (1956) are Citrus limon Brum, C. medica Linn., C. jambhiri Lush, C. inchangenis Swing, C. latipes Tanaka, C. macroptera Montr, C. assamensis, C. indica Tanaka, C. grandis L., and C. megaloxycarpa Lush. Kinnow (C. nobilis and C. deliciosa) is hybrid citrus between king orange and willow leaf Mandarin thriving in low lying areas of North-East above sea level. The family Citrus is known for its diversified biochemical and pharmacologically potential activities. So, the present work aims in characterising and analysing various photochemical, physiological, properties of unexplored variety of Citrus species, Citrus megaloxycarpa Lush which is prevalent mostly in the North eastern part of India.

## Materials And Methods

### Sample collection and preparation

The Sample was collected from Pasighat, East Siang district of Arunachal Pradesh, located at 28.07°N 95.33°E with an average elevation of 502 feet. Collected samples were zipped and brought to laboratory condition and all the necessary morphological data was recorded. The peel and pulp extract of ripening large citrus sample were indicated as P(L1) and Pu(L2) respectively and unripe small peel and pulp extracts were indicated as P(S1) and Pu(S2) respectively .The samples were washed, peeled and dried in hot air oven at a temperature of 40°C for 6-8 days. A part of dried samples was stored at -20°C for its further analysis.

### Sample Extraction

The samples were extracted following the method described by Maruti et al. 2011 with little modifications. Ground dried sample (10 g) was extracted by stirring with 100 mL of hexane at 150 rpm at 25°C for 24 hours using shaker incubator and then filtered through Whatman No.4 filter paper. The residue was again extracted with 100ml of hexane. The combined extracts were evaporated at 40°C to dryness and redissolved in hexane at a concentration of 100 mg/mL and stored at 4°C for further use

#### Determination of Biochemical Properties

##### Total Soluble Sugar

Estimation of total soluble sugar was carried out with the process as described by Clegg (1956) with minor modifications. The extract was mixed with sulphuric acid and Anthrone reagent. The solution was boiled until the reaction was completed. The solution was cooled and its absorbance was measured at 620nm.

##### Total Soluble Protein

Total soluble protein was estimated as per the method of Abdul (1949) with minor modifications. The extract was treated with 5ml of solution C, 50ml solution A (2% sodium carbonate in 0.1N NaOH) + 1ml solution B (0.5% CuSO<sub>4</sub> in 1% sodium potassium tartarate) followed by addition of 0.5ml Folin-Ciocalteu reagent. The mixture was mixed and incubated in dark for half an hour then absorbance was measured at 660nm.

##### Total Carbohydrate

The estimation of total carbohydrate was carried out as per the method given by Clegg (1956). The extract was digested with 2N HCl and neutralized followed by centrifugation at 8000 rpm for 5 min. The supernatant was collected and treated with Anthrone reagent. The Absorbance was read at 630 nm.

##### Free Amino Acid

Free amino acid estimation was carried out using Ninhydrin method with some slightest modification on the protocol by Moore and Stein (1948). The sample was mixed with the Ninhydrin reagent and heated at a temperate of 85°C for 7mins.

##### Free Fatty Acid

Estimation of Free Fatty acid was carried using the protocol of Cox and Pearson (1962). The sample was mixed with the neutral solvent and was titrated against 0.1N potassium hydroxide. The acid value of the extract was calculated as follows:

$$\text{Acid value } (\mu\text{g KOH/ml}) = (\text{Titrate value} \times \text{Normality of KOH} \times 56.1) / \text{Amount of sample}$$

##### Antioxidant assay

## 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The scavenging activity of free radical 2, 2- diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of kekuda et al. (2015). Various concentrations of hexane

extracts of the sample (0.3 ml) were mixed with 2.7 ml of solvent sample containing DPPH radicals. The mixture was left undisturbed in dark for 60 min until stable absorption was obtained. The DPPH radical reduction was monitored for the decreased absorption at 517 nm.

The radical-scavenging activity (RSA) was later calculated as a percentage of discolored DPPH using the formula:

$$\text{Scavenging activity (\%)} = [(A-B)/A] \times 100$$

where A- absorbance of DPPH solution, B- absorbance of DPPH when the extract was added. The assays were carried out for triplicate and the mean value  $\pm$  standard deviations were accepted. IC<sub>50</sub> (50% inhibition) was determined from the graph against the extracts where Ascorbic acid was considered as a standard.

## Total Antioxidant Capacity (TAC)

The total antioxidant capacity is determined following the procedure described in Pietro et al.(1999). As per the protocol 50  $\mu$ l of hexane extracts were taken and the volume was adjusted up to 500 $\mu$ l adding distilled water. To this mixture 4.5 ml of phosphomolybedenum reagent was added and vortexed. The tubes were capped properly and incubate at 95°C for 90 minutes. The absorbance was recorded at 695 nm against the blank and the control. Ascorbic acid (0.25 mg/ml) was used as standard. Concentration Total antioxidant capacity was calculated using the following formula:

$$\text{Total antioxidant capacity (\%)} = [(As-Ac)/ (Aaa-Ac)] \times 100$$

Where Ac- Control absorbance, As- Sample absorbance, Aaa- Ascorbic acid absorbance.

## Evaluation of Antimicrobial Activity

This method involves the measurement of bioactivity of the test material used to induce effect on selected organism under controlled conditions. To carry out the experiment Glass wares were sterilised in autoclave at 121°C at 15 Psi for 20 min.

The test organism used in this study includes both gram negative and gram positive bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Bacillus subtilis). Series of dilution was prepared containing same amount of inoculated test organism i.e., 1ml. the test drug was prepared using serial dilution method (i.e. the concentration of drug in 1st tube is 45 $\mu$ l the 2nd tube will 40 $\mu$ l and

so on), distilled water is used tube serving as control. The tubes were incubated for 24hrs at 37°C. The tubes were observed for the growth of microorganism which is indicated by the turbidity in the tubes.

#### Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration is the minimum concentration in which the test drug will inhibit growth of test microorganism. Turbidimetric assay to test Minimum inhibitory concentration of drug used against the microbial population by measuring turbidity of the suspension by Bansode and Chavan. Where 9 ml of nutrient broth is added into 6 culture tubes and 1 ml of citrus was added to each tubes with 0.1 ml of microorganism is inoculated into each tube. The tubes were incubated overnight at 37°C and the development of turbidity is measured at 625 nm on U.V spectrophotometer.

#### Cell Cytotoxicity Assay (MTT Assay)

The test was outsourced and performed in Department of Biotechnology and Bio-engineering, Institute of Technology, Gauhati University. The method of Mosmann, 1983 was followed for the cytotoxicity assay. 10  $\mu$ l of MTT solution was added in each well except blank to get a final concentration of 0.5 mg/ml. incubated at 37°C for 1-4hrs. The formazan crystals and triturate are dissolved by adding 100  $\mu$ l of solubilizing solution. Incubated at room temperature (37°C) for 3 hrs to ensure complete solubilisation of formazan crystals the the absorbance was measured at 750nm. The cell viability is indicated by higher absorbance value, while lower value indicates cell cytotoxicity.

$$\% \text{ Viability} = (\text{A sample} / \text{A Blank}) / (\text{A Control} / \text{A Blank}) \times 100$$

$$\% \text{ Cytotoxicity} = 100 - \% \text{ Viability}$$

Where A=Absorbance.

## Results And Discussion

The Sample was collected from North-Eastern state of India, Arunachal Pradesh, Pasighat, East Siang district located at 28.07°N 95.33°E with an average elevation of 502 feet. Collected sample was morphologically diverse and largest of all citrus species, fruits were globose and light green to pale yellow in colour, moderately pear shaped and have bumpy fruit surface. Exocarp contains aromatic oil glands with thick mesocarp, carpel is small and contains no juicy vesicles or seeds. The sample fresh weight was 4.25 kg and 1.62 kg and size 36×14(1/2) ×7 cm. The morphology and cross section appearance has been shown in Fig 1 and Fig 2 respectively. The whole plant of Citrus megaloxycarpa lush has been shown in Fig 3.

Over the past decade, a considerable amount of work has done to study the various potential of citrus fruit. Citrus megaloxycarpa Lush is a little-known sibling of genus citrus and family Rutaceae with an unknown origin. This study formulated for the assessment of biochemical and antioxidant activities of Citrus megaloxycarpa Lush. The statistics support bioactive compounds presence combined with

Antioxidant, Antimicrobial, and Anticancer activity that further emulates with the result obtained from different studies.

The pulp extract of ripening large sample exhibits highest total soluble sugar (TSS) with a significance level of ( $P<0.0001$ ) at 95% Confidence interval (Table I). Total soluble protein (TSP) of  $8.074 \pm 0.0567$   $\mu\text{g/ml}$  obtained in Peel extract of unripe smaller sample to be highest (Table I), However Ayona, et al. (2017) confirm *Citrus limonum* to have  $0.021 \pm 0.014$  mg followed by *Citrus aurantium* with  $0.019 \pm 0.013$  mg which shows that the above studied sample have significant amount of total soluble protein. Total Carbohydrate was estimation shows  $8.326 \pm 0.003844$   $\mu\text{g/ml}$  on peel extract of ripening large sample with significance ( $P< 0.0001$ ) (Table I), However Athira et al. (2017) revealed *Citrus maxima* with carbohydrate content of  $3.690 \pm 0.705$  mg/ml followed by *Citrus limonum*  $3.073 \pm 0.001$  mg/ml. The free amino acid (FAA) of the sample was has a concentration of  $24.35 \pm 0.0225$   $\mu\text{g/ml}$  shown by peel extract of ripening large sample and Free fatty acid value of 373.9 ( $\mu\text{g KOH/ml}$ ) is reported in pulp extract of ripening large sample (Table I), reportedly Valencia orange found to have free fatty acid of  $0.27 \pm 0.03$  meq/kg (Caroline et al. 2013). The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity computed to be 73.80% highest among the extracts used (Table II and Fig 4), by comparing the standard (Ascorbic acid – 14%) it is found that citrus extracts are having higher scavenging activity other studies conducted shows 85% of scavenging activity on *Citrus reticulate* (Vinita et al. 2016). The IC<sub>50</sub> value was computed for all the samples (Table II) in comparison with IC<sub>50</sub> of 107.48  $\mu\text{g/ml}$  on acetone extract and 278.24  $\mu\text{g/ml}$  in methanolic extract reported by Sheila et al. (2017). The total antioxidant capacity was found to be highest in peel extract (L1) and minimum in peel extract (L2) and was recorded as  $150 \pm 0.333$  and  $75 \pm 0.265$  as represented in the Fig 5. Thus the observed result shows MIC at 45%, 35%, 30% (v/v) for (*Salmonella typhi*), and 40% (v/v) for (*Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*), and at 35% for *Salmonella typhi* as represented in Table III and Fig 6. In conclusion with the experimental data obtained by (Oikeh et.al. 2016) lime juice shows promising result against *Pseudomonas aeruginosa* (12.5 $\mu\text{g/ml}$ ). The MTT assay shows cell viability of 94.32% and percentage cytotoxicity of 5.67%.

## Conclusion

The bioactive compound studied has a vast potential in the field of therapeutic drugs that can benefit the pharmaceuticals industries. Unlike other states North-East India is rich in diverse species of wild citrus fruit; amid those *Citrus megaloxycarpa* Lush is exceptional. The species showed a wide range of properties in terms of Nutritional, Antioxidant, Antimicrobial and Anticancer activities. Despite having an enormous potential of this plant, it is still an endangered species of citrus. Making available data about its significance will vastly intercept species extinction furthermore introducing the market into different sectors will help in the upliftment of the socio-economic condition of the ethnic tribe of the region.

## Declarations

### Acknowledgement

I hereby acknowledge University of Science and technology, Meghalaya and for providing the entire requisite needed for completion of this work. Authors also acknowledge GUIST, Department of Biotechnology and Bioengineering for carrying out the Anti-cancerous assays.

**Ethical Approval-** Not applicable.

### **Consent to Participate**

The author gives his full consent to participate, with regarding to the underlining procedure for Journal Applied Biochemistry and Biotechnology.

### **Consent to Publish**

The author gives his full pledge consent to Journal Applied Biochemistry and Biotechnology for publishing this piece of work for the future prospects of researchers and Institutional bodies.

### **Author's contribution**

Mr. Arun jerang and Shahbaaz Ahmed has meticulously carried out Biochemical and Antioxidant assay whereas the anticancer study was outsourced and performed in Guwahati University. The work was completed under the guidance of Dr. Sony kumari, and Dr. Madushmita Borthakur supportively helped in collection of data and compiling it.

**Funding-** Not applicable.

**Competing interests-** Not applicable.

### **Availability of Data and Materials**

The findings and relevant data along with material has been made available for publishing in the Journal Applied Biochemistry and Biotechnology.

## **References**

1. Athira U (2007) Evaluation of carbohydrate and phenol content of citrus fruits species. In J of applied research 3(9):160-164.
2. Ayona J and Athira U (2017) Comparative analysis of nutritional and anti nutritional components of selected citrus fruit species. In J for research in Applied science & engineering technology 5(10):309-312. doi:10.22214/ijraset.2017.10047.
3. Burt S (2004) Essential oils: Their antibacterial properties and potential applications in foods: A review. In J of food microbial. 94(3):223-253. doi:10.1016/j.ijfoodmicro.2004.03.022.

4. Bhattacharya SC and Dutta S (1956) Classification of citrus fruits of Assam. Indian council of agriculture research.
5. Caroline PMA and Neuza J (2013) Physico chemical characterization of seed oils extracted from oranges (*citrus sinensis*). *Food Sci Technol Res.* 19(3):409-415. doi:10.3136/fstr.19.409.
6. Cox HE and Pearson D (1962) The chemical analysis of foods. Chemical publishing Co Inc New York pp 420.
7. Chaudhari SY, Galib R, prajapati P(2016) Ethno medicinal values of citrus genus :A review. In *Med J of Dr. D.Y patil vidyapeeth* 9(5):560-565. doi:10.4103/0975-2870.192146.
8. Clegg KM (1956) The application of the anthrone reagent to the estimation of starch in cereals. *Journal of the science of food and Agriculture.* 7(1), 40-44. doi:10.1002/jsfa.2740070108.
9. Economos C and Clay W (2012) Nutritional and health benefits of citrus fruits. *Food and agriculture.*
10. Hansen MB, Nielsen SE, Berg K (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. In *J Immunol methods.* 119, 203-210. doi:10.1016/0022-1759(89)90397-9.
11. Hedge JE and Hofreiter BT (1962) Methods in carbohydrate chemistry. (Eds Whistler RL and Be Miller JN) Academic press New york.
12. Hore DK and Barua U (2004) Status of citrus culture in North Eastern region of India. A review. 25(1), 1-15.
13. Kekuda P and Onkarappa R (2015) Bioactivities of *Streptomyces* species from soil of Western ghats of Karnataka India. *J of Chemical and Pharmaceutical research* 7(11), 181-189.
14. Khalid H, Nisar M, Majeed A, Nawaz K, Bhatti K (2014) Ethnomedicinal survey for important plants of jabalpur jattan District Gujrat, Punjab, pakistan. In *J Ethnobotanical leaf.* 14, 807-25.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) protein measurement with the folin phenol reagent. 193(1), 265-275.
16. Maruti JD, Chidamber BJ, jai SG and Kailash DS (2011) Study antimicrobial activity of lemon (*Citrus lemon L.*) peel extract. *British journal of pharmacology and toxicology*, 2(3), 119-122.
17. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicityassays. In. *J. Immunol.Meth.* 65, 55-63.
18. Stanford M and William HS (1948) Photometric ninhydrin method for use in the chromatography of amino acid. *J Biol chem.* 176, 367-88.

19. Ortuno AA, Baidez P, Gomez MC, Arcas I, Porras AG, Del RJA (2006) Citrus paradise and citrus sinensis flavonoids: their influence in the defence mechanism against penicillium digitatum. In J Food Chem 98, 351-358. doi:10.1016/j.foodchem.2005.06.017.
20. Prietro PM, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. In J Anal biochem. doi:10.1006/abio.1999.4019.
21. Pallavi M, Ramesh C K, Krishna V, Sameera P, Nanjunda S L (2017) Qualitative phytochemical Analysis and Antioxidant Activities of some Citrus fruits of South India. Asian J Pharmaceutical and Clinical Research. 10(12), 198-05. doi: 10.22159/ajpcr.2017.v10i12.20912.
22. Roger GD (2002) P Encyclopedia of medicinal plants, Educational and health library editorial safeliz SL splam. (1), 153-154,265-267.
23. Suja D, Bhupesh G, Nivya RMV, Ramasamy P, Muthiah NS, Arul AE, Meena KK and Prabhu K (2017) phytochemical screening antioxidant antibacterial activities of citrus limon and citrus sinensis peel extracts. Medwin publishers, michigan, united states. 1 (2), 2576-4472.
24. Sasidharan S, Saravanan D, Sundram KM, Latha LY, Chen Y (2011) Extraction, isolation and characterization of bioactive compounds from plants extracts. In J African journal of traditional, complementary and alternative medicines. 8(1), 1-10.
25. Vinita DA, Nancy SP (2016) Evaluation of skin anti-aging potential of citrus reticulata blanco peel. In J india pharmacognosy Res. 8(3), 160-168. doi:10.4103/0974-8490.182913.

## Tables

**Table I: Biochemical assay of *Citrus megaloxycarpa* lush.**

Biochemical test	Peel extract (L <sub>1</sub> )	Pulp extract (L <sub>2</sub> )	Peel extract (S <sub>1</sub> )	Pulp extract (S <sub>2</sub> )
Total soluble sugar content ( $\mu\text{g}/\text{ml}$ ) $\pm$ SEM	7.823 $\pm$ 0.03553	9.174 $\pm$ 0.006741	6.254 $\pm$ 0.006928	7.496 $\pm$ 0.03017
Total soluble protein content ( $\mu\text{g}/\text{ml}$ ) $\pm$ SEM	6.673 $\pm$ 0.0176	6.537 $\pm$ 0.0318	8.074 $\pm$ 0.0567	6.643 $\pm$ 0.09074
Total carbohydrate content ( $\mu\text{g}/\text{ml}$ ) $\pm$ SEM	8.326 $\pm$ 0.003844	2.352 $\pm$ 0.1159	4.026 $\pm$ 0.0005696	2.240 $\pm$ 0.0008819
Free amino acids content ( $\mu\text{g}/\text{ml}$ ) $\pm$ SEM	24.35 $\pm$ 0.0225	24.22 $\pm$ 0.01289	24.12 $\pm$ 0.00115	24.03 $\pm$ 0.00881
Free fatty acid content ( $\mu\text{g}/\text{ml}$ ) $\pm$ SEM	0.2614 $\pm$ 0.03333	0.3739 $\pm$ 0.05774	0.3552 $\pm$ 0.03333	0.2614 $\pm$ 0.03333

Result are expressed in mean  $\pm$  SEM, n = 3, \*\*\*\* (P< 0.0001) 't' test.

**Table II:** IC50 DPPH scavenging activity of *Citrus megaloxycarpa* lush.

Peel extracts (L <sub>1</sub> )	Pulp extract (L <sub>2</sub> )	Peel extract (S <sub>1</sub> )	Pulp extract (S <sub>2</sub> )
logIC50 = 0.6527	logIC50 = 0.6577	logIC50 = 0.5088	logIC50 = 0.5122
IC50 = 89.9	IC50 = 90.94	IC50 = 64.54	IC50 = 65.04

Result shown in % mean, n = 3, \*\*\*\* (level of significance) P<0.0001 by 't' test.

**Table III:** Inhibitory concentration of different standard used.

Antibiotic	Organisms	O.D at 625 nm (100 µl)
Ampicillin	<i>Salmonella typhi</i>	0.162
	<i>Pseudomonas aeruginosa</i>	0.336
	<i>Escherichia coli</i>	0.221
	<i>Bacillus subtilis</i>	0.205
Chloramphenicol	<i>Salmonella typhi</i>	0.267
	<i>Pseudomonas aeruginosa</i>	0.275
	<i>Escherichia coli</i>	0.328
	<i>Bacillus subtilis</i>	0.154

## Figures

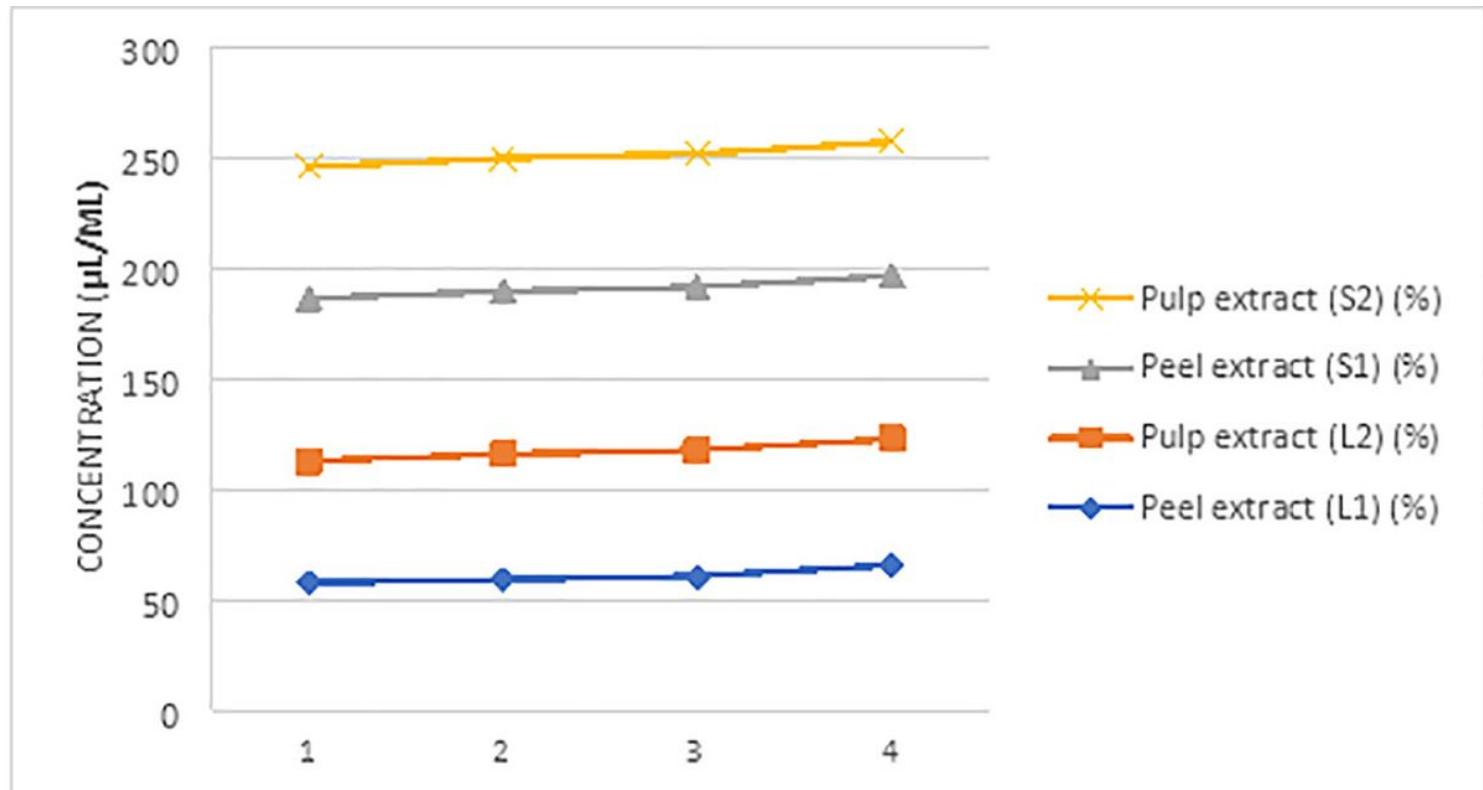
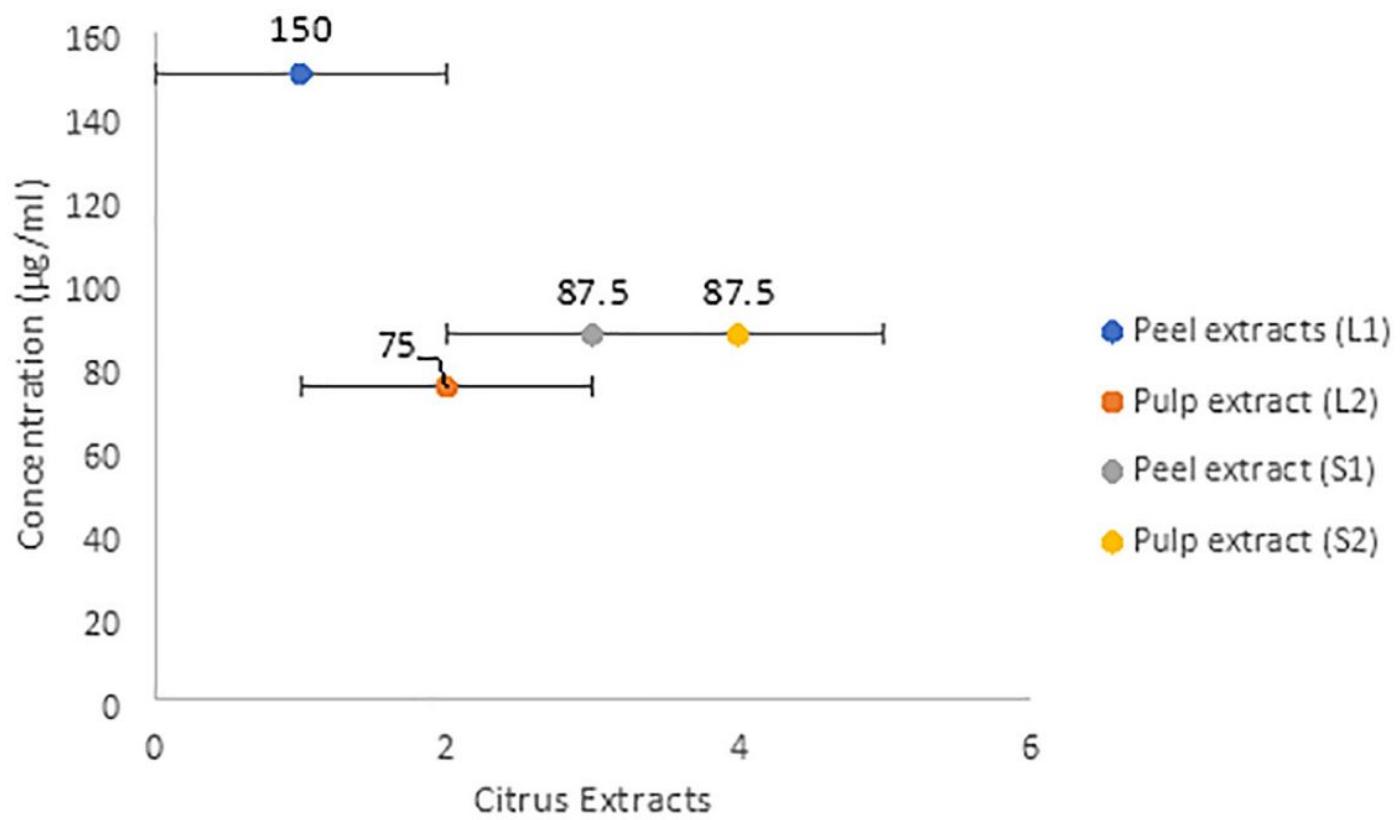


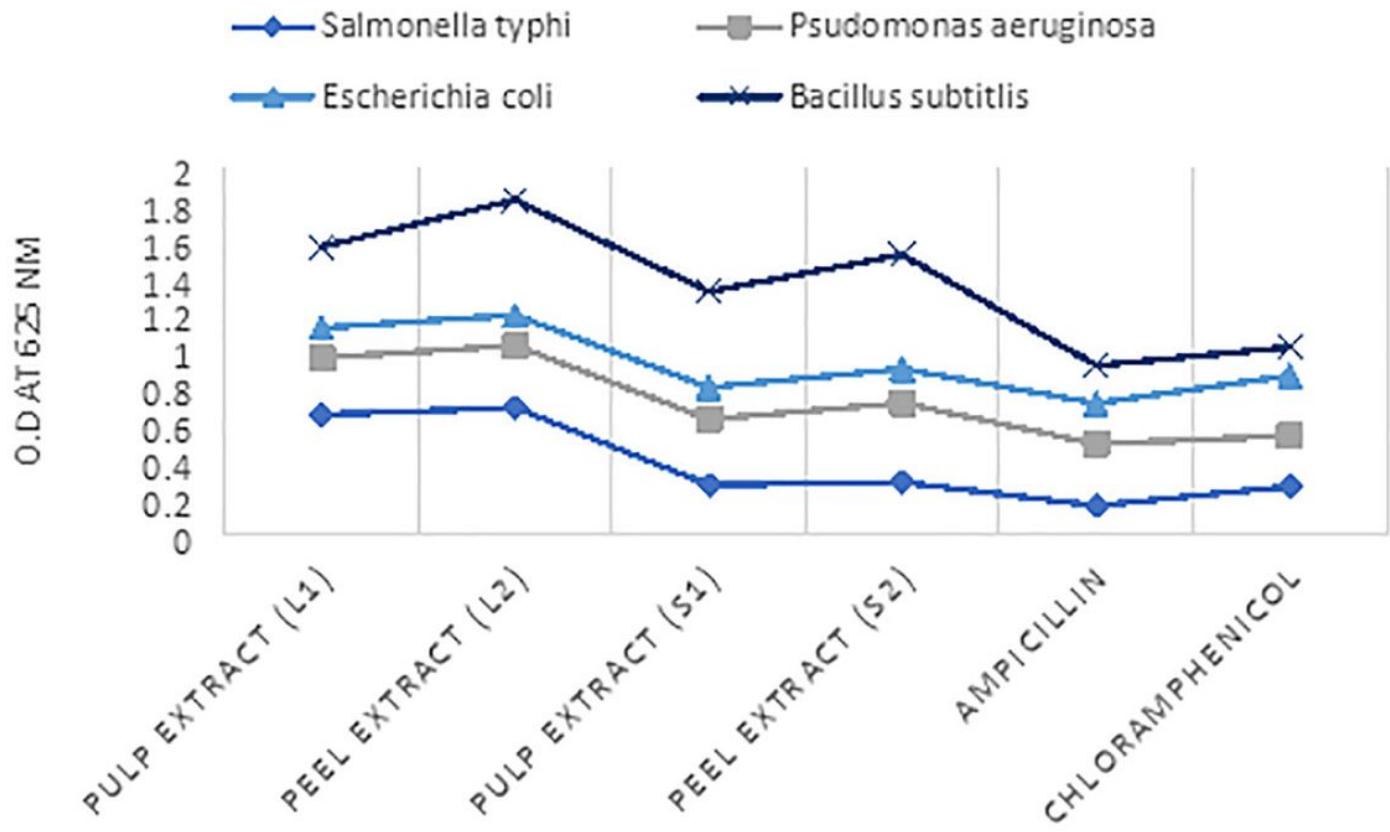
Figure 1

The morphology and cross section appearance



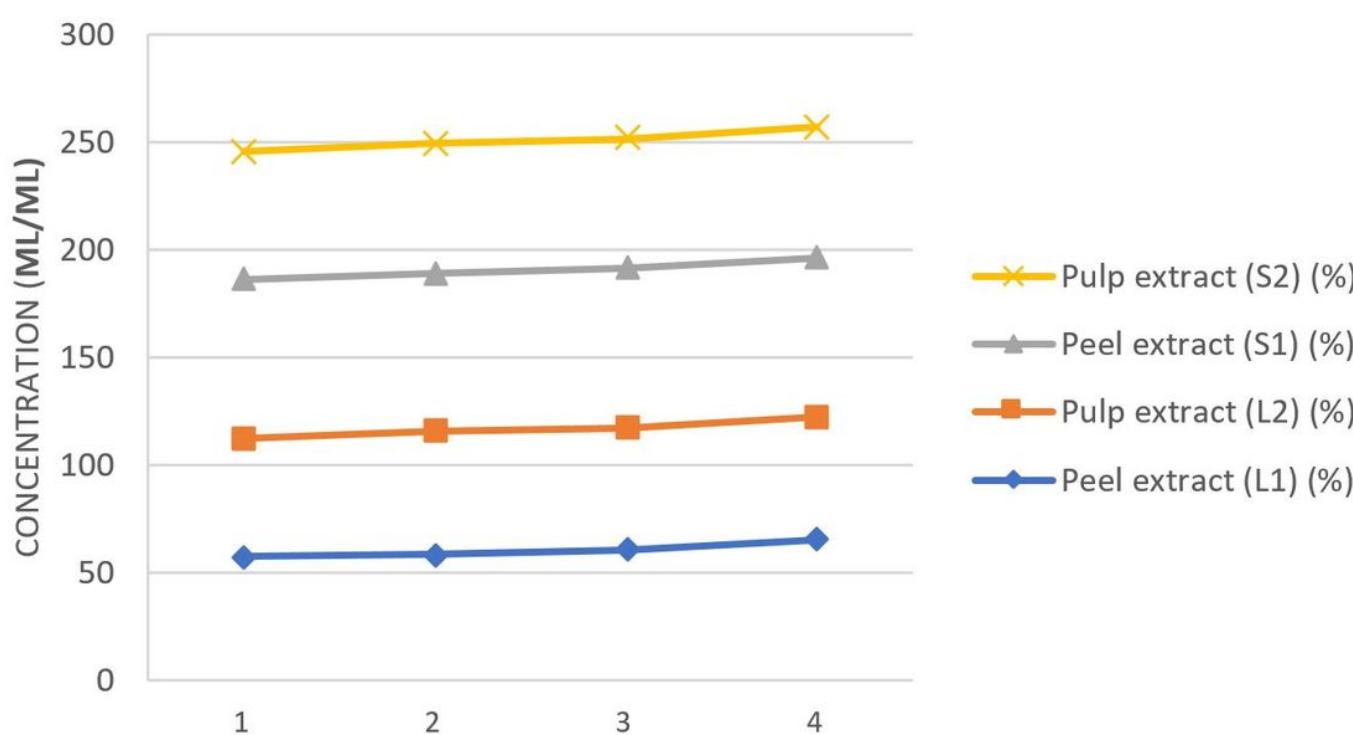
**Figure 2**

The morphology and cross section appearance



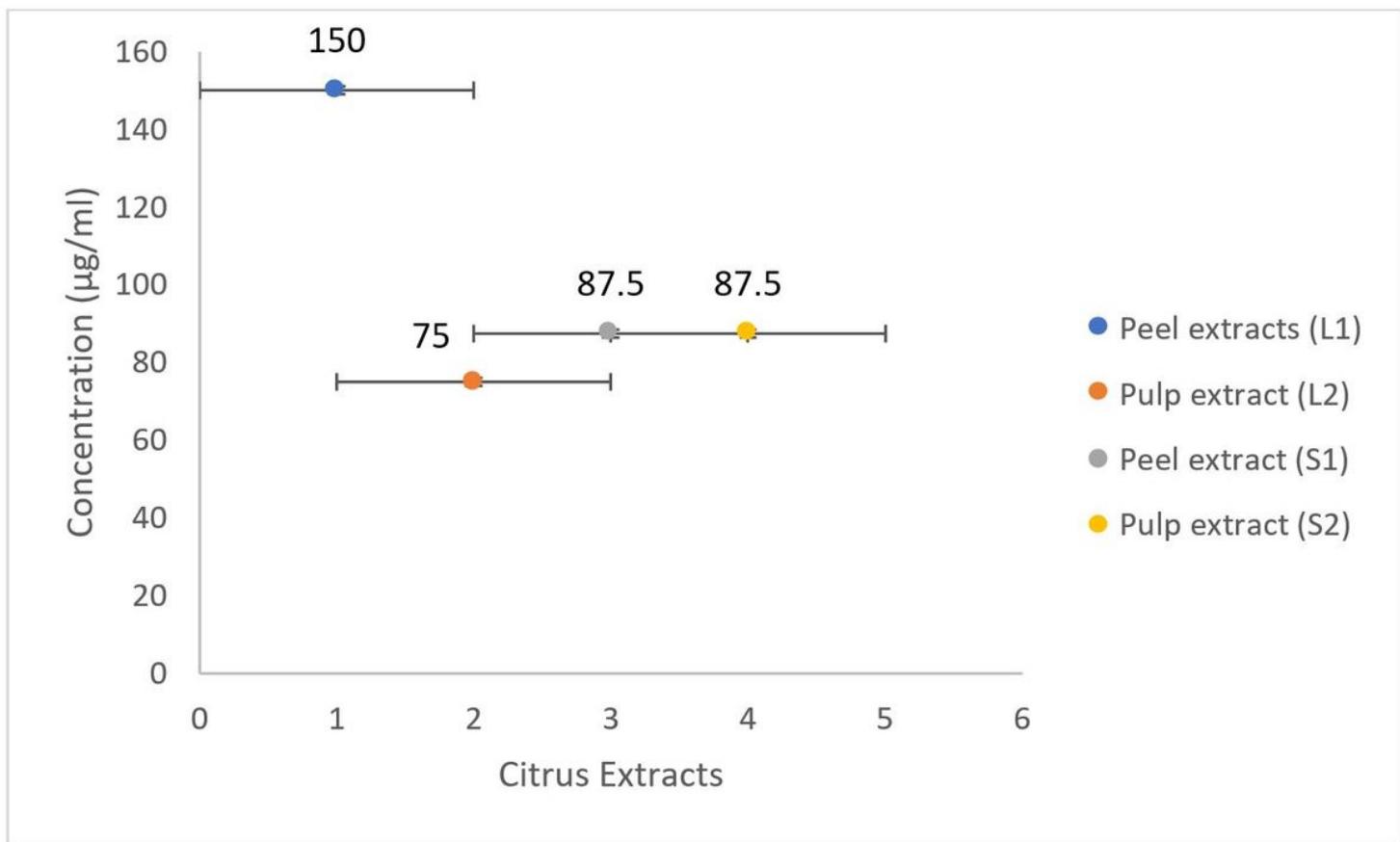
**Figure 3**

The whole plant of *Citrus megaloxycarpa* lush



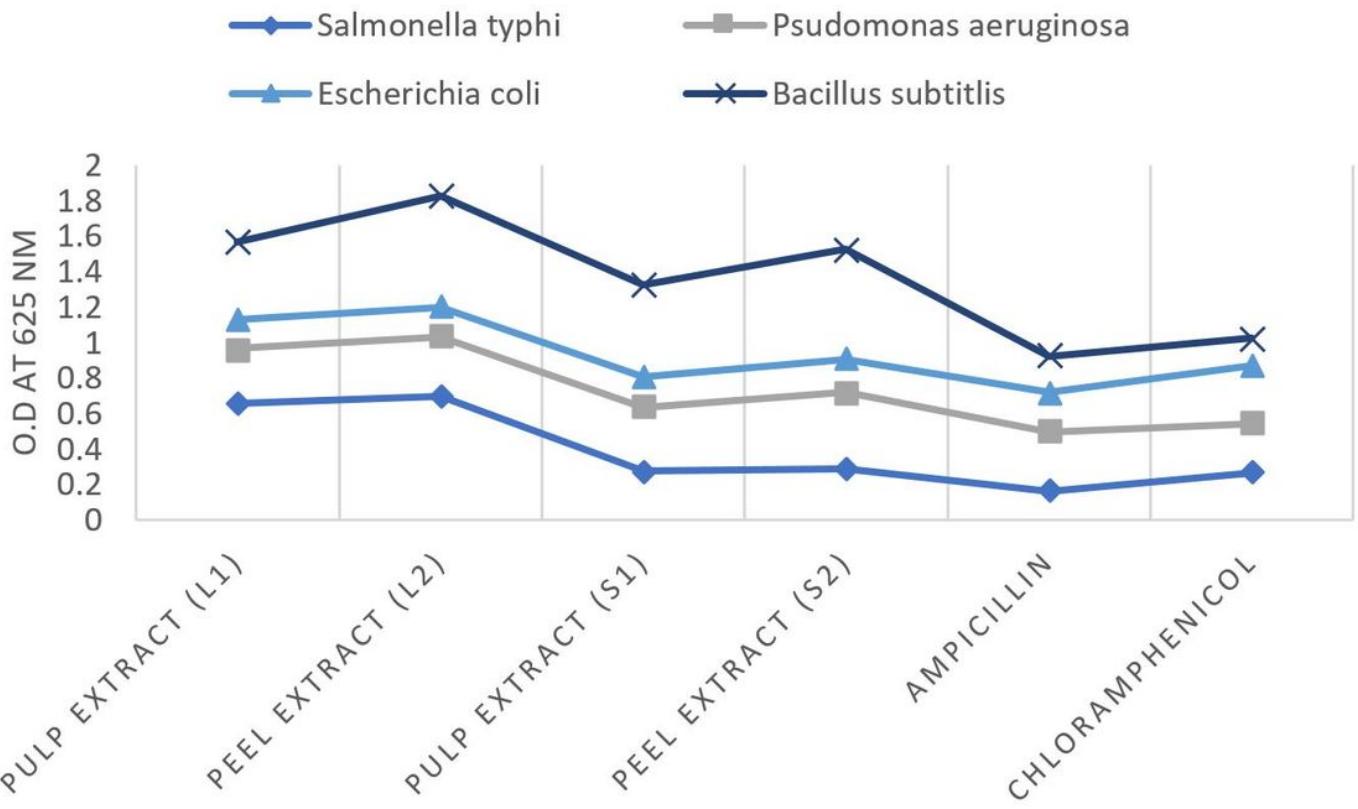
**Figure 4**

DPPH free radical (%) scavenging activity of *Citrus megaloxycarpa* lush extracts.



**Figure 5**

TAC (%) of *Citrus megaloxycarpa* Lush extracts.



**Figure 6**

MIC of Citrus extracts against standard antibiotic used.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplimentarymaterials.docx](#)