International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Quantitative Phytochemical Analysis and DNA Barcoding Studies of *Artocarpusheterophyllus*

Charishma C Gowda[1], Dr. Shruthi SD[2]

[1]Department of Biotechnology, Ramaiah Institute of Technology, Bangalore, Karnataka, India

[2]Microbiology and Molecular Biology lab, BioEdge Solutions, Bangalore, Karnataka, India
Corresponding author Email: *sdshruthi[at]gmail.com*
services[at]bioedgesolutions.com
Phone: +919482774014

Abstract: ***Artocarpusheterophyllus, commonly known as jackfruit, is a healthful source that contains various phytochemicals, vitamin C, copper, potassium, manganese, magnesium and riboflavin. This fruit improves immunity and thus have desirable pharmacological activities such as anti - inflammatory, anti - diabetic, anti - angiogenic, anti - oxidant, anti - cancerous, anti - cholesterolemic and wound healing applications. The objectives of the study include performing DNA Barcoding studies, anti - ulcer activity and to estimate various phytochemicals in jackfruit leaves. DNA barcoding technique was conducted to authenticate the jackfruit species. The total phenolic content and the flavonoids were estimated by plotting gallic acid and quercetin standard curve. The antiulcer activity was tested by performing antibacterial test using well - diffusion method where in the zone of inhibition was measured. The ratio of A260/A280 was found to be 16.667 and the concentration of DNA was found to be 3µg/ml. Various phytochemicals were analysed including total phenolic content (484±0.15 µg/ml), flavonoid content (1996.3±0.02 µg/ml), ursolic acid (23±0.02 µg/ml), saponins, triterpenoids and steroids. The zone of inhibition was found in higher scale with Pseudomonas aeruginosa than Staphylococcus aureus. From the results, it was evident that the leaf extract possessed high activity against gram - negative bacteria in comparison with gram - positive bacteria.***

Keywords: flavanoids, pharmacological activities, phytochemicals, well - diffusion, ursolicacid

1. Introduction

Artocarpusheterophyllus, well - known as jackfruit is a tropical fruit that is native to Western Ghats of India and commonly available in Asia, Africa and few parts of South America. It is the highest fruit that is edible in the world. Jackfruit has good amount of nutrients such as carbohydrates, vitamins, proteins, phytochemicals andminerals. It is considered to be a non - seasonal fruit and contributes majorly to the food supply of the people and their livestock when there were food shortages. Hence, it is well known as poor man's food [1].

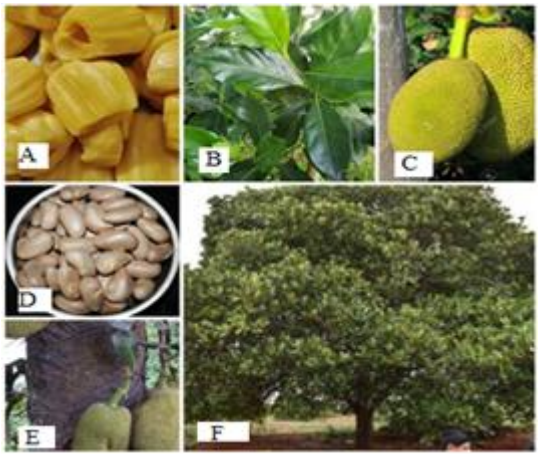


Figure 1: Different parts of jackfruit tree
(A) Ripen jackfruit; (B) Jackfruit leaves; (C) Edible jackfruit; (D) dried seeds of jackfruit; (E) Bark of jackfruit tree and (F) Jackfruit tree bearing fruits.

DNA barcoding is a tool for identification of species which makes use of protocols that are internationally agreed and uses various regions of DNA to create a global database of living organisms [6, 15]. The significance of plant DNA barcoding is authentication of living organisms such as animals or plants. During the DNA barcoding, genetic sequences are obtained and can be used to create phylogenetic trees. These applications depend upon certain regions of DNA that have been identified between species without being variable within certain species [8 - 9].

A mouth ulceration, also called as an oral ulcer, or mucosal ulcer is an ulcer occurring on the mucous membrane in the oral cavity [10]. They form round or oval sores that are painful in the mouth, usually inside the cheeks or lips [11]. Mouth ulcers, also known as aphthous ulcers, can be painful while eating, drinking or brushing teeth [12, 15]. Mouth ulcer can be classified as minor, major and herpetiform based on the size and number. Various Dosage Form used for the Treatment of Mouth Ulcers include pastes, mouthwashes, buccal tablet, buccal patch, pharmaceutical gel [13]. Nowadays, mouth ulcers are very common, and they occur in association with many diseases and by different mechanisms, but usually there is no serious underlying cause [12]. Nutritional deficiencies such as iron, vitamins especially B12 and C, poor oral hygiene, infections, stress, indigestion, mechanical injury, food allergies, hormonal imbalance, skin disease etc., are the most common causes of the mouth ulcer [13].

The current work is done to explore phytochemicals present in jackfruit leaves, anti - ulcer activity by performing DNA Barcoding.

2. Literature Survey

Several parts of jackfruit tree are such as fruits, leaves and bark have been extensively used in traditional medicine due to its anticarcinogenic, antimicrobial, antifungal, anti - inflammatory, wound healing and hypoglycemic effects. Figure 1 represents various pharmacological activities of *Artocarpusheterophyllus* [3]. It is said in Ayurveda that the raw fruit increases vata and kapha whereas ripened fruit alleviates vata and pitta [2]. The jackfruit seeds have high zinc content than the jackfruit pulp and leaves [3 - 5].

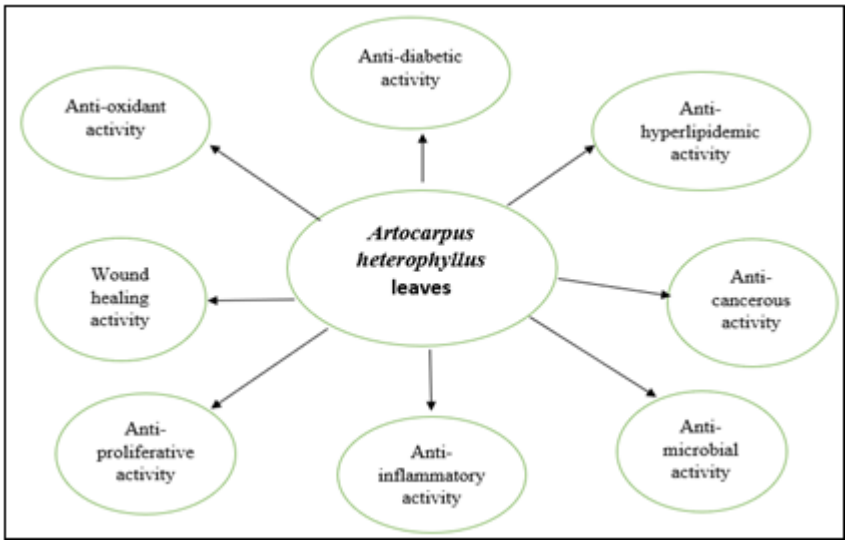


Figure 2: Pharmacological activities of jackfruit leaves.

Paul Hebert of University of Guelph first proposed the term 'DNA barcode' in 2003, and is also termed as 'taxon identifiers'. The basic idea of this system is very simple, that the variation in sequence of the DNA barcode should be very high between the species, so that discrimination from one another is quite easy. If there is a match of the sequence from the unknown organism to one of the reference sequences can produce an instinct and consistent identification [9]. Multi - locus markers like ITS gene with rbcL, matK, trnH are assumed to be more victorious in the identification of species [20].

The other researches were also done to determine the phenolic and flavonoid contents in the extracts and fractions of jackfruit peel and to check their potential as antioxidants. According to Yamin, *et al.*, 2021, The total phenolic content was determined by the Folin - Ciocalteu method, the flavonoid content using the aluminum chloride complex colorimetric method. The ethyl acetate fraction had high phenolic and flavonoid contents, which were 49.667±1.508 g GAE/100g of sample and 70.199±0.374 g of quercetin equivalent/100 g [17].

The antimicrobial activity of *Artocarpusheterophyllus* leaves extracts on *S. enterica* and *E. coli* and their interaction with conventional antimicrobial were studied bySousa, et al., 2021. The antimicrobial test was done against *Escherichia coli*, ATCC 25922, *E. coli* EPEC, CDC 086H35, and *Salmonella enterica* serotype Enteritidisphagotype 4 through Minimal Inhibition Concentration and Minimal Bactericidal Concentration [18].

3. Materials and Methods

Chemicals and reagents

Cetyltrimethylammonium bromide (CTAB), chloroform, isoamyl alcohol, cold isopropanol, wash buffer, elution buffer, 1X TAE buffer, agarose, gel loading dye, DNA ladder, Gel solubilization buffer, TE buffer, ethanol, EDTA, HiDiFormamide.

Collection of samples

The jackfruit leaves were harvested from a local farm located at Jindal, Bangalore – North. The leaves were separated from the tree using sterile knife, then were sliced, washed with distilled water and stored in the refrigerator further laboratory evaluation.

Isolation of Genomic DNA:

100mg *Artocarpusheterophyllus* was weighed in an Eppendorf tube and 1ml of CTAB extraction buffer was added to it. The mixture was vortexed thoroughly and transferred to 60℃ waterbath for 30 minutes. Centrifugation was performed at 14, 000rpm for 5 minutes and equal volume of chloroform: isoamyl alcohol was added. After vortexing for 5 seconds, the sample was centrifuged again for 5 minutes at 14, 000rpm. The upper aqueous layer was taken to a fresh tube and DNA was precipitated by adding 0.7ml cold isopropanol.

After one - night incubation, it was transferred to a silica - based DNA column and spun at 10, 000rpm for 1 minute. The constituents other than DNA were removed using wash buffer and 20µl elution buffer was added to elute DNA and centrifuged at 10, 000 rpm for 1 minute.

Agarose Gel Electrophoresis

1% agarose was weighed and dissolved in 50ml 1X TAE buffer. The gel was poured on a casting plate keeping the comb and allowed to solidify. It was then placed in electrophoretic tank and remaining buffer was slowly poured

on it.10µl of eluted DNA sample along with 2µl of gel loading dye were loaded onto the wells of agarose gel along with DNA ladder. The setup was turned on for 40 minutes to 90V, 250mA.

Quantification of DNA
Spectrophotometric estimation of DNA – 1ml of TE buffer was taken in a cuvette and calibrated at 260nm as well as 280nm.10µl of DNA sample was added to 900µl of TE buffer and mixed well. Absorbance was noted at 260nm and 280nm. The ratio of OD260/OD280 was calculated and the total amount of DNA was known by using the formula:

$$\text{DNA concentration (µg/ml)} = \frac{OD_{260} \times 100 \text{ (dilution factor)} \times 50 \text{ µg/ml}}{1000}$$

DNA Barcoding
The sample is then subjected to PCR amplification and the obtained PCR Product is run on gel and compared the gene size with standard ladder.

Gel Purification
The required DNA band on gel was cut and 400µl of Gel solubilization buffer was added. It was then heated at 55°C until gel dissolves completely and 200µl of Isopropanol was added. The solution was mixed thoroughly and transferred to DNA column. Spin at 12000 rpm for 1 minute was performed and 700µl of wash buffer was added twice. Elution Buffer of 20µl was added andspun at 12000 rpm for 1 minute.

Sanger Sequencing - DNA
Sanger's method, which is also referred to as dideoxy sequencing or chain termination, is based on the use of dideoxy nucleotides (ddNTP's) in addition to the normal nucleotides (NTP's) found in DNA.

Post Sequencing and PCR Purification
125 Mm 2.5µl EDTA was added to each well and was given a short spin. Afteradding 35µl of Ethanol using multichannel pipette, vortex for 10 minutes at 2000 rpm was done and was also centrifuged at 3510 rpm for 30 minutes. Using tissue bed decant ethanol at 300 rpm (for 30 seconds invert the plate), 40µl of 80% Ethanol was added to the wells and centrifuged at 3510 rpm for 12 minutes. The above - mentioned invert spin was repeated and air dried for 30 - 45 minutes covering the plate with lint free tissue.13µl of HiDiFormamide was added and given a short spin. It was then denatured at 95°C for 5 minutes. The plate was placed in a sequencer.

4. Phytochemical Analysis

Preparation of plant material
10g of jackfruit leaves were immersed in 50ml absolute ethanol and mixed well. The solution was allowed to stand for two days. The plant extract was obtained by filtering the solution.

Determination of Total Phenolic Content
To 0.5ml of plant extract, 1.5ml of Folin - Ciocalteau (FC) reagent was added and left for 5 minutes.1.5ml of 7% sodium carbonate solution was added and the volume was made up to 10ml with distilled water. The solution was allowed to stand for 90 minutes and absorbance was measured at 750nm. To estimate the concentration of phenolic content, gallic acid curve was used as a standard [14].

Determination of Flavonoid content
1ml of sample along with 4ml distilled water was taken in a test tube and 0.3ml of 10% aluminum chloride was added to it. After 6 minutes of incubation at room temperature, 1ml of sodium hydroxide was added. The volume was made up to 10ml with distilled water and absorbance was noted at 510 nm. The quercetin curve was used as a standard for the estimation of flavonoid content.

Determination of Steroid and Triterpenoid
To 1ml of sample, 2 - 3 drops of acetic acid was added and stirred slowly until it dries.1 - 2 drops of concentrated sulphuric acid was added and the color change was observed. Red or purple color indicates presence of triterpenoids and green - blue color indicates the presence of steroids.

Determination of Saponin
1ml of sample was taken in a test tube and hot water was added to it. The sample was then cooled by shaking for 10 seconds. Few drops of 2N hydrochloric acid was added and the appearance of foam was observed.

Estimation of ursolic acid
To 200µl of sample, 16ml of concentrated sulphuric acid was added and thermostatic at 70°C for 1 hour. The solution was cooled and the volume was made up to 20 ml with sulphuric acid. Absorbance was taken at 520 nm and the concentration of ursolic acid was noted by ursolic acid standard curve.

Antibacterial study
There are various reasons for mouth ulcer formation and one of them are ulceration by microorganisms. Few include *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [19]. Luria - Bertani agar was used for antibacterial study. The agar plate was inoculated with microorganisms; one with *Staphylococcus aureus* and the other with *Pseudomonas aeruginosa*. On the agar media, four wells were punched. The first well was added with 100µl of antibiotic Amoxycillin, second well with 100µl of 70%ethanol, third well with jackfruit leaf extract and fourth well with 1: 1 dilution of the leaf extract. The plates were incubated for 24 hours at 37°C and the zone of inhibition was measured.

5. Results

DNA Barcoding

DNA barcoding is basically performed for authentication of any taxa or a species. It aims to use the information of one or a few regions to identify all species of life.

Agarose gel electrophoresis was executed to confirm the presence of DNA in the sample.

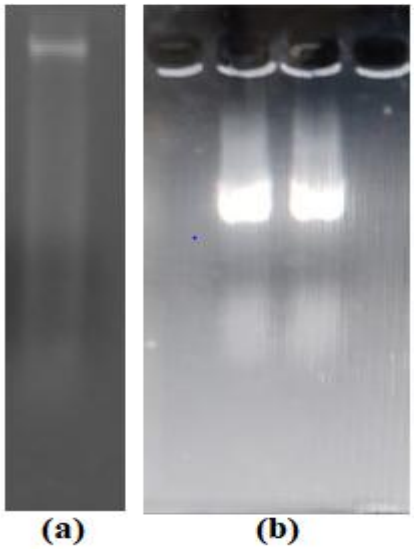


Figure 3 (a): DNA run on agarose gel electrophoresis; 3 (b): DNA run on agarose gel electrophoresisafter PCR amplification.

The ITS2 is a phylogenetic marker and this region of DNA is the best candidate for DNA barcoding in plants which has about 700 base pairs.

DNA was further quantified by UV - spectrophotometry by measuring the absorbance at 260nm and 280nm. The readings noted were applied to the formula. The OD value of the sample at 260 nm was 0.6 and at 280 nm was 0.036. The ratio of A260/A280 was found to be 16.667. The concentration of DNA was found to be 3µg/ml. Hence, 1µg concentration was used for running PCR due to good amount of DNA.

Phylogenetic analysis

After performing Sanger's sequencing, the electrogram obtained was converted to FASTA sequence and was pasted in NCBI BLAST. The ten different species with maximum similarity percentage and minimum e - value were considered. The following result was obtained.

Hit: *Artocarpusheterophyllus* voucher DMB5 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer
Percent identity: 98.52%
E value: 0.0
Query coverage: 99%
Accession no.: MG847193.1

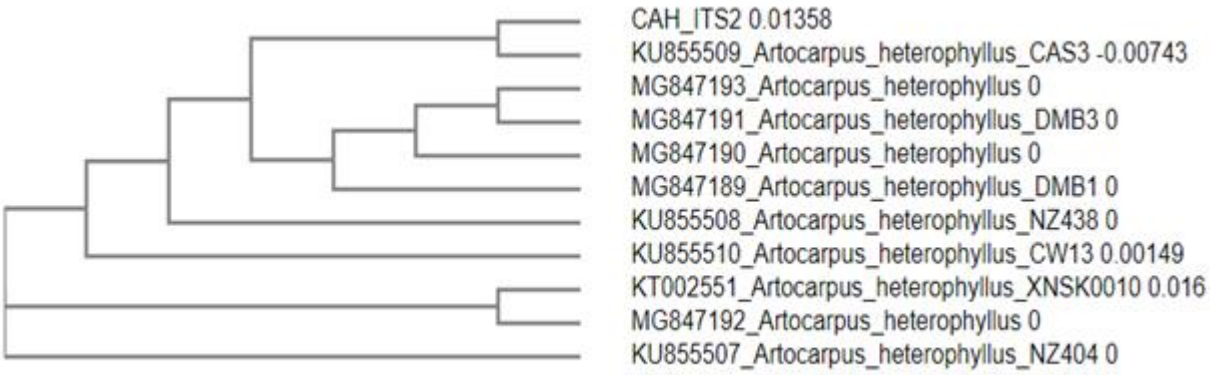


Figure 4: Results of Phylogenetic analysis using Clustal Omega

From the phylogenetic tree (Figure 4), we can say that our sample is almost similar to KU85509_*Artocarpus heterophyllus*_CAS3. It also has similarities with other jackfruit species suchas - MG847193_*Artocarpus_heterophyllus*, MG847191_*Artocarpus_heterophyllus*_DMB3, KT002551_*Artocarpus_heterophyllus*_XNSK0010, and MG847192 _ *Artocarpus_heterophyllus*.

Phytochemical Analysis

The extract was prepared by adding 10g of leaves to 50ml of absolute ethanol.

Total Phenolic Content

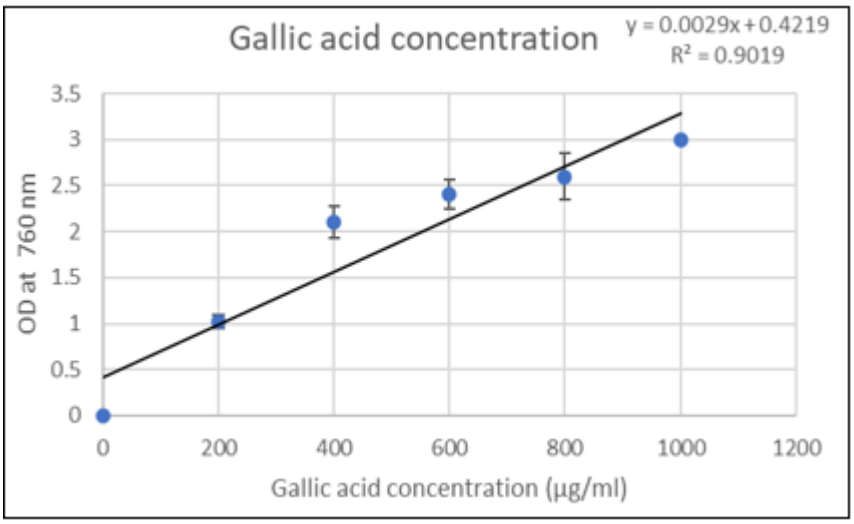


Figure 5: Gallic acid standard curve

The gallic acid standard curve was plotted for various concentrations of gallic acid and the concentration of the gallic acid in jackfruit extract was found to be 484.172±0.15 µg/ml by substituting the values of x and y in the equation of figure5.

Flavonoid content

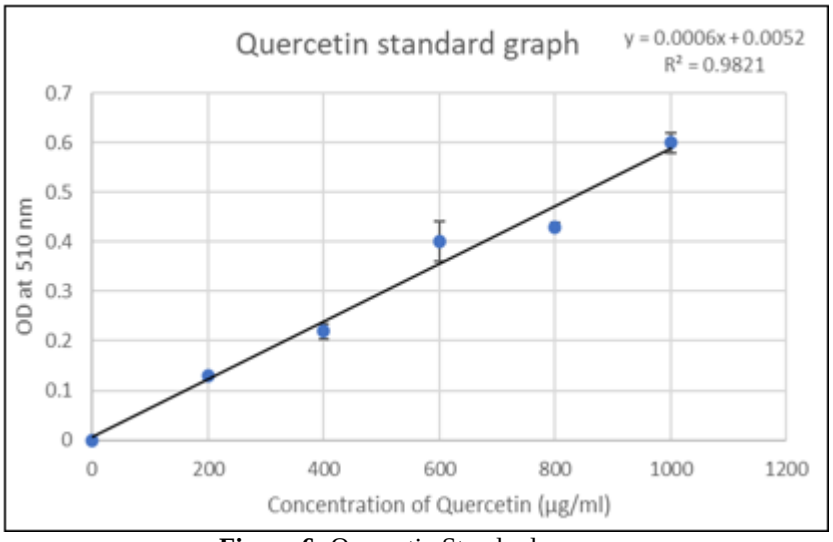


Figure 6: Quercetin Standard curve.

From the graph (Figure6), the flavonoid content of jackfruit leaves was found to be 1996.3±0.02 µg/ml.

Steroid and Triterpenoid test

Triterpenes constitute a significant portion of the lipid substances of all plants. Both triterpenes and steroids occur free, as glycosides, or in other combined forms.

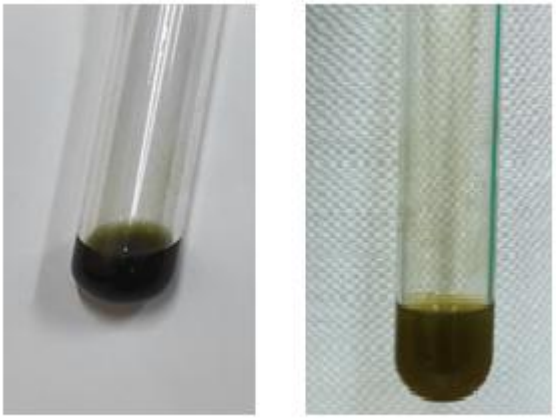


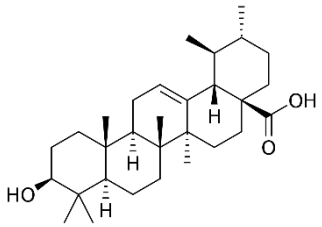
Figure 7: (A) - represents positive results of Steroids test and Figure7 (B) - represents negative results of saponin test

The jackfruit leaf extract showed green color indicating the presence of steroids as shown in the Figure7 (A).

Saponin Test

When few drops of 2N hydrochloric acid was added, there was no appearance of foam. This showed that the sample does not contain saponin as indicated in Figure7 (B).

Estimation of Ursolic acid



Ursolic Acid is a pentacyclic triterpenoid found in various fruits, vegetables and medicinal herbs, with a variety of potential pharmacologic activities including anti - inflammatory, antioxidative, antiviral, serum lipid - lowering, and antineoplastic activities. Upon administration, ursolic acid may induce apoptosis and prevent cancer cell proliferation through multiple mechanisms.

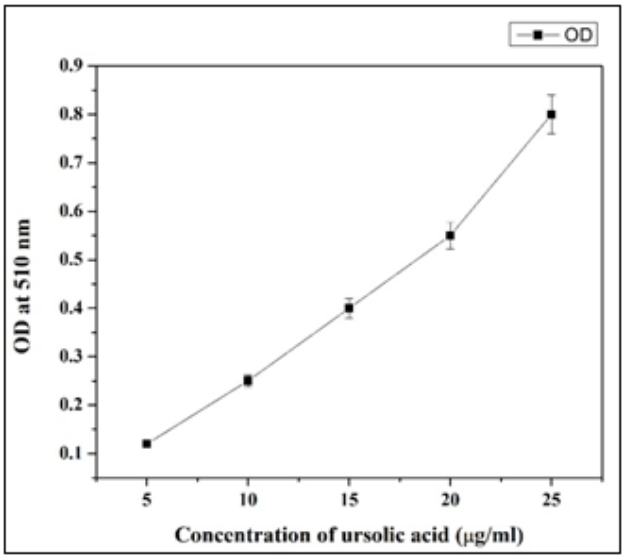


Figure 8: Standard curve of ursolic acid

The ursolic acid content from the graph was found to be 23±0.02 µg/ml from the figure8.

Antibacterial Activity

This test was carried out by agar - diffusion method. Two different microorganisms, *Staphylococcus aureus* (gram positive) and *Pseudomonas aeruginosa* (gram negative) were used as test organisms which were also one of the causes for mouth ulceration.

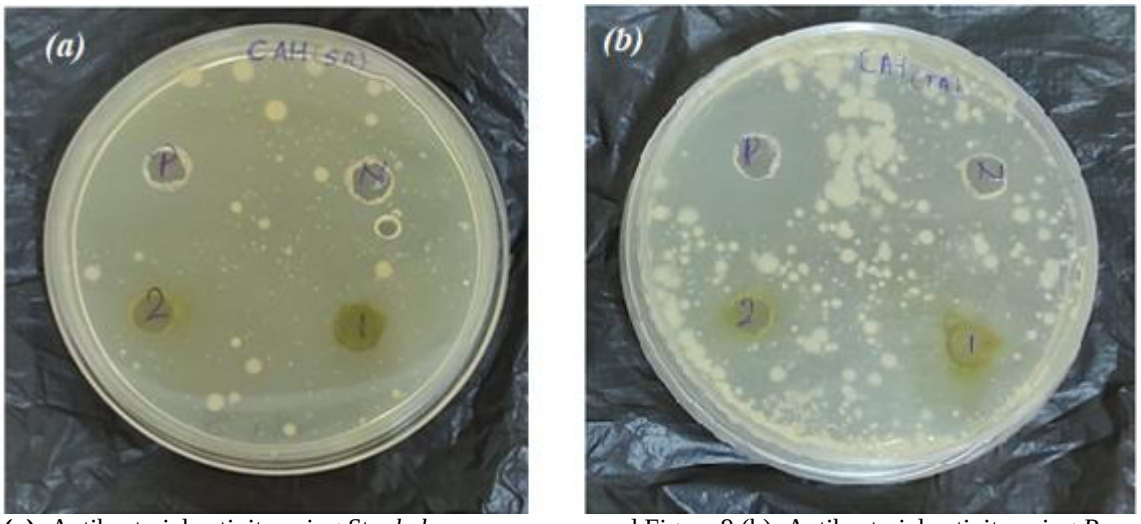


Figure 9 (a): Antibacterial activity using *Staphylococcus aureus* and Figure 9 (b): Antibacterial activity using *Pseudomonas aeruginosa*.

Four wells were made as shown in Figure 10. Well P was loaded with 10µl ampicillin; Well N loaded with 10µl of 70% ethanol; Well 1 with crude leaf extract and Well 2 with 1: 1 dilution of the extract (1ml sample: 1ml absolute ethanol)

Table 1: Zone of inhibition by (a) *Staphylococcus aureus*and (b) *Pseudomonas aeruginosa*

Contents	Zone of Inhibition by (a) (cm)	Zone of Inhibition by (b) (cm)
Positive control	3	3
Negative control	-	-
1	2.5	3.5
2	2	2.5

As mentioned earlier, the microorganisms such as gram positive and gram - negative bacteria are one of the causes for mouth ulceration. From these results of Table 1 (a) and (b), we can conclude that the jackfruit leaves possess anti - ulcer activity.

6. Discussion

This study explains that phytochemical analysis usually has a vital role in determining bioactive activities and it is because of the presence of flavonoids, saponins, phenolic content, tannins, triterpenoids, and steroids in the jackfruit leaf extracts. The total phenolic content was expressed as 0.484 mg/ml in *Artocarpusheterophyllus* leafextract which is

higher than 0.125 mg/ml as per [15]. The flavonoid content in leaf extract was found to be 1.9963 mg/ml from our experiment, whereas the maximum flavonoid content was 3.91 ±0.08 mg/100g [17]. According to Turkiewicz, *et al*., [16]ursolic acid in leaves were about 0.22 mg/g but we obtained ursolic acid of about 23±0.02 µg/ml which is comparatively less. The flavonoid and phenolic content were approximately 70.199g/100g of sample and 49.667g/100g of sample respectively in Yamin*et al*., 2021 [18], which were higher than our findings. The antibacterial test was found to be higher for gram positive *Staphylococcus aureus* than gram negative *Pseudomonas aeruginosa*. These two microorganisms are one of the causes for mouth ulceration. This activity also showed that the leaf extract exhibited anti - ulcer activity.

7. Conclusion

In this study, we used jackfruit leaves to check the phytochemicals present in it and also a biomedical application named anti - ulcer activity. The leaf extract was used to evaluate total phenolic content as 0.484±0.15µg/ml using gallic acid as standard and, flavonoids as 1.9963±0.02µg/ml using quercetin as standard. The ursolic acid content was estimated using concentrated sulphuric acid method and was found to be 23µg/ml. The *Staphylococcus aureus* and *Pseudomonas aeruginosa* exhibited excellent zone of inhibition for crude extract of leaves compared to the diluted leaf extract. On consuming jackfruit leaves, occurrence of various metabolic disorders can be reduced, thus helping the society with good health. On consuming leaves of jackfruit, there will be definite reduction in asthma, gall stones as well as diabetes. In future, the jackfruit leaves can be extracted in powder forms of nanosized and can be infused as a band aid in wound healing or can be used as spray for reducing pain.

8. Future Scope

Artocarpusheterophyllus is made use as an ayurvedic tree for the treating the wounds, Bell's palsy, increasing sperm count, improve the body strength, diarrhea, skin diseases and in cases of poisoning. These leaves can be extracted in powder forms of nanosized and can be infused as a band aid in wound healing or can be used as spray for reducing pain.

References

[1] Ranasinghe RA, Maduwanthi SD, Marapana RA. Nutritional and health benefits of jackfruit (*Artocarpusheterophyllus*Lam.): a review. International journal of food science.2019 Jan 6; 2019.

[2] Amadi JA, Ihemeje A, Afam - Anene OC. Nutrient and phytochemical composition of jackfruit (*Artocarpusheterophyllus*) pulp, seeds and leaves. International Journal of Innovative Food, Nutrition and Sustainable Agriculture.2018; 6 (3): 27 - 32.

[3] Sreeja Devi PS, Kumar NS, Sabu KK. Phytochemical profiling and antioxidant activities of different parts of *Artocarpusheterophyllus*Lam. (Moraceae): A review on current status of knowledge. Future Journal of Pharmaceutical Sciences.2021 Dec; 7 (1): 1 - 7.

[4] Adan AA, Ojwang RA, Muge EK, Mwanza BK, Nyaboga EN. Phytochemical composition and essential mineral profile, antioxidant and antimicrobial potential of unutilized parts of jackfruit. Food Res.2020 Aug; 4 (4): 1125 - 34.

[5] Moreno - Nájera LC, Ragazzo - Sánchez JA, Gastón - Peña CR, Calderón - Santoyo M. Green technologies for the extraction of proteins from jackfruit leaves (*Artocarpusheterophyllus*Lam). Food Science and Biotechnology.2020 Dec; 29 (12): 1675 - 84.

[6] FišerPečnikar Ž, Buzan EV.20 years since the introduction of DNA barcoding: from theory to application. Journal of applied genetics.2014 Feb; 55 (1): 43 - 52.

[7] Techen N, Parveen I, Pan Z, Khan IA. DNA barcoding of medicinal plant material for identification. Current opinion in Biotechnology.2014 Feb 1; 25: 103 - 10.

[8] Vere ND, Rich TC, Trinder SA, Long C. DNA barcoding for plants. InPlant Genotyping 2015 (pp.101 - 118). Humana Press, New York, NY.

[9] Bruni I, De Mattia F, Galimberti A, Galasso G, Banfi E, Casiraghi M, Labra M. Identification of poisonous plants by DNA barcoding approach. International Journal of Legal Medicine.2010 Nov; 124 (6): 595 - 603.

[10] Mittal S, Nautiyal U. A review: herbal remedies used for the treatment of mouth ulcer. mouth.2019 Jan 30; 8: 9.

[11] Vaishnavi Burley D, Biyani D, Umekar M, Naidu N. Medicinal plants for treatment of ulcer: A review. Journal of Medicinal Plants.2021; 9 (4): 51 - 9.

[12] Bandana K, Kumar S, Sagar MK, Tyagi H. Formulation and evaluation of herbal gel of guava leaves and liquorice roots extract for using mouth ulcer. Journal of Critical Reviews.2020; 7 (18): 3097 - 110.

[13] Shahare N, Chouhan S, Darwhekar GN. Herbs used in treatment of mouth ulcer - a review. International Journal of Pharmacognosy and Chemistry.2021 Jul 29: 68 - 74.

[14] Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, *8* (4), 96.

[15] Turkiewicz IP, Wojdyło A, Tkacz K, Nowicka P. Comprehensive characterization of Chaenomeles seeds as a potential source of nutritional and biologically active compounds. Journal of Food Composition and Analysis.2021 Sep 1; 102: 104065.

[16] Riyanti S, Sunardi C, Falah SN. Isolation of Flavonoid from Jackfruit Leaves (*Artocarpusheterophyllus*Lamk.) and its Antioxidant Activity. ActaPharmaceutica Indonesia.2013; 38 (2): 58 - 61.

[17] Yamin R, Mistriyani S, Ihsan S, Armadany FI, Sahumena MH, Fatimah WO. Determination of total phenolic and flavonoid contents of jackfruit peel and in vitro antiradical test. Food Res.2020; 5: 84 - 90.

[18] SOUSA DF, Campos Filho PC, CONCEIÇÃO AO. Antibacterial activity of jackfruit leaves extracts and the interference on antimicrobial susceptibility of enteropathogen. Food Science and Technology.2021 Jun 11; 42.

[19] Anitha M, Monisha DM, Ramya K, Chinmayee Y, Sulthan AM. A study on the occurrence of bacterial isolation in mouth ulcer patients. Int. J. Adv. Res. Biol. Sci.2016; 3 (10): 188 - 92.
[20] Purty, R. S., & Chatterjee, S. (2016). DNA barcoding: an effective technique in molecular taxonomy. Austin J BiotechnolBioeng, 3 (1), 1059.