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Comprehensive Evaluation of Medicinal Fruit Plants *Carica Papaya and Psidium Guajava*

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ABSTRACT: This study investigates the biological activities of aqueous extract from the leaves of *Carica papaya* (*C. papaya*) and *Psidiumguajava* (*P. guajava*), focusing on their phytochemical composition and therapeutic potential, including antioxidant, anti-diabetic, anti-arthritic, anti-microbial, and wound healing properties. Phytochemical analysis revealed the presence of bioactive compounds such as alkaloids and phenols. Anti-oxidant screening demonstrated significant free radical scavenging activity for both the extracts. Both *C. papaya* and*P. guajava* exhibited promising anti-diabetic activity through notable inhibition of the enzyme alpha-amylase. Furthermore, the anti-arthritic potential of the extracts was evident from their ability to inhibit protein denaturation. However, anti-microbial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* revealed no zone of inhibition. In wound healing assays, *C. papaya* extract significantly enhanced cell migration in L929 fibroblast cells, whereas *P. guajava* extract exhibited a slower rate of cell migration. These findings underscore the therapeutic potential of*C. papaya* and *P. guajava* leaf extracts as natural remedies with multifaceted biological activities, warranting further investigation for clinical applications.

KEYWORDS: *Carica papaya,Psidiumguajava,*Anti-oxidant, Anti-diabetic, Alpha-amylase inhibition, Anti-arthritic.

INTRODUCTION

Plants are remarkable organisms exhibiting immense diversity in form, function, and adaptability, enabling their survival across diverse ecosystems. From towering trees in ancient forests to tiny mosses in rocky crevices, plants are fundamental to life on earth, serving as a source of oxygen, food, and ecological balance (Schaal, 2005). The evolutionary transition of plants from aquatic to terrestrial habitats marked a pivotal point in life's history, with cyanobacteria playing a critical role in oxygenating the earth's atmosphere (Graham *et al*., 2000). Plants also produce a variety of bioactive compounds, known as phytochemicals, which contribute to their ecological and medicinal significance. These compounds, including phenolics, flavonoids, alkaloids, and terpenoids, have been widely recognized for their pharmacological properties (Meißner, 1819; Gerhardt, 1841; Freud, 1936).

Medicinal plants like *Carica papaya* (papaya) and *Psidiumguajava* (guava) are well-documented for their therapeutic applications in traditional and modern medicine. *C. papaya* is renowned for its rich phytochemical composition, including papain, alkaloids, and vitamins, which exhibit anti-oxidant, anti-inflammatory, and wound-healing properties (Joshi *et al*., 2000; Oloyede, 2017). Similarly, *P. guajava* is recognized for its high content of flavonoids, tannins, and essential vitamins, which contribute to itsanti-microbial, anti-diabetic, and anti-cancer effects (Ojewole, 2005; Chan *et al*., 2016). These plants have been utilized in traditional systems

of medicine for managing a wide range of ailments, including gastrointestinal disorders, diabetes, arthritis, and microbial infections (Karunamoorthi *et al*., 2014; Singh *et al*., 2020).

The biological activities of *C. papaya* and *P. guajava* leaf extracts have been attributed to their phytochemical constituents, which exhibit anti-oxidant, anti-diabetic, anti-arthritic, anti-microbial, and wound-healing properties. For instance, anti-oxidants in these plants help to mitigate oxidative stress by neutralizing reactive oxygen species, thereby reducing inflammation and preventing cellular damage (Sarin *et al*., 2015). Furthermore, their potential to enhance insulin sensitivity and modulate glucose metabolism has been reported in diabetic models (Singh *et al*., 2017). The wound-healing properties of these plants are primarily due to their ability to promote collagen synthesis and tissue regeneration (Nayak & Pereira, 2006).

This study aims to comprehensively evaluate the phytochemical composition of aqueous leaf extracts of *C. papaya* and *P. guajava* and to explore their therapeutic potential through anti-oxidant, anti-diabetic, anti-arthritic, anti-microbial, and wound-healing assays. The findings of this study will provide valuable insights into the pharmacological applications of these medicinal plants and contribute to the development of plant-based therapeutic agents.

MATERIALS AND METHODS

Sample Collection

Fresh leaves of *C. papaya* and *P. guajava* were collected from local areas of Thiruvananthapuram, Kerala, India. The leaves were washed thoroughly under running tap water multiple times to remove dirt and contaminants. Subsequently, they were air-dried under sunlight for few hours and further dried in a hot air oven at 100°C for 30 minutes. The dried leaves were grounded into a fine powder using an electric grinder and stored in an airtight container for further analysis.

Extraction

The powdered leaves of *C. papaya* and *P. guajava* were subjected to Soxhlet extraction using an aqueous solvent. Approximately 9 g of dried powder from each plant sample was weighed and placed into the thimble ofSoxhlet apparatus. Extraction was performed for 6 hours, maintaining a solvent volume of 100 mL and temperatures of 60°C for *C. papaya* and 80°C for *P. guajava*. After extraction, the solvent was evaporated to obtain crude extracts, which were weighed and stored at room temperature in an airtight container until further use.

Phytochemical Analysis

Phytochemical screening was conducted to identify the presence of flavonoids, alkaloids, and phenols in the aqueous extracts of *C. papaya* and *P. guajava*. Standard methods were employed for the qualitative analysis.

Test for Flavonoids

The *C. papaya* and *P. guajava* extract was mixed with 2% sodium hydroxide solution. Subsequently, a few drops of dilute hydrochloric acid were added. A color change from yellow to colorless confirmed the presence of flavonoids.

Test for Alkaloids

Wagner's reagent was added to *C. papaya* and *P. guajava* extracts. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

Test for Phenols

Ferric chloride solution was added dropwise to *C. papaya* and *P. guajava* extracts. The appearance of a bluish-black color for *C. papaya* extract and dark green color for *P. guajava* extract confirmed the presence of phenolic compounds.

Quantitative Analysis of Phytochemicals

Estimation of Alkaloids

Extract concentrations of *C. papaya* and *P. guajava* ranging 5, 50, 100, 250, and 500 µg/mL were prepared using distilled water. Quercetin dissolved in methanol was used as standard. 5 ml phosphate buffer and 5 ml bromocresol green (BCG) reagent were added to the test tubes. . Each reaction mixtures were then transferred to separating funnel followed by the addition of 3ml chloroform for phase separation. The lower layer was collected and made upto a known volume. The absorbance was measured at 470 nm using a spectrophotometer. Alkaloid content was calculated using the linear equation derived from the standard-Quercetin curve.

Estimation of Phenols

Extract concentrations of *C. papaya* and *P. guajava* ranging 5, 50, 100, 250, and 500 µg/mL were prepared, and gallic acid served as standard. 2 ml Folin - Ciocalteu reagent (FC) was added and shaken well. 4 ml sodium carbonate (Na_2CO_3) were added and mixed properly. After 30 minutes of incubation at room temperature in the dark, absorbance was measured at 760 nm. Phenol content was calculated using the linear equation from the standard - Gallic acid curve.

Anti-oxidant Activity

Ferric reducing antioxidant power (FRAP) Assay

Extract concentrations of *C. papaya* and *P. guajava* ranging 5, 50, 100, 250, and 500 µg/mL were prepared, and ascorbic acid was used as standard. 2.5 ml sodium phosphate buffer and 2.5 ml potassium ferrocyanide were added to the test tubes and incubated at 50°C for 20 minutes. After incubation 2.5 ml of trichloroacetic acid (TCA) was added. From each tube, 2 ml of supernatant was mixed with 4 ml distilled water. To these tubes, 0.5ml of ferric chloride ($FeCl_3$) was added and incubated at room temperature for 10 mins. Absorbance was measured at 700 nm. The anti-oxidant activity of the samples was determined by calculating the percentage inhibition using the following formula:

$$\%\ \text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Anti-diabetic Activity

Alpha-Amylase Inhibitory Assay

Extract concentrations of *C. papaya* and *P. guajava* ranging 5, 50, 100, 250, and 500 µg/mL were prepared, and ascorbic acid served as standard. Into each test tube, added 100µL of phosphate buffer and 100µL of alpha-amylase solution and was incubated at 30°C for 10 mins. Added 1ml of 1% starch solution to each tube and incubated again at 30°C for 10 mins. After incubation, added 1 ml DNSA reagent and kept in boiling water bath for 10 mins. Added 100µl distilled water to each tube and the absorbance was measured at 540 nm. Inhibitory activity was calculated using the formula:

$$\%\ \text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Anti-Arthritic Activity

Egg Albumin Protein Denaturation Assay

Extract concentrations of *C. papaya and P. guajava* ranging 5, 50, 100, 250, and 500 µg/mL were prepared in duplicate and made upto 2ml using distilled water. Diclofenac sodium in methanol was used as standard. The reaction mixture contained 0.2 ml egg albumin and 2.8 ml phosphate-buffered saline (PBS), incubated at 37°C for 15 mins, and then heated at 70°C to induce protein denaturation. Absorbance was measured at 660 nm, and the percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Antimicrobial Activity

Agar Well Diffusion Method

Extracts of *C. papaya* and *P. guajava* at concentrations of 5, 50, 100, 250, and 500 µg/mL were tested against gram negative bacterium *Escherichia coli* (*E. coli*), gram positive bacterium *Staphylococcus aureus* (*S. aureus*), and fungus *Candida albicans* (*C. albicans*). Positive controls (Gentamicin for bacteria, Amphotericin B for fungi) and a negative control (distilled water) were included. After 24 hours of incubation at 37°C, the zone of inhibition was measured.

Wound Healing Activity

Scratch Assay

L929 (0.3×10^6)cells were cultured in a 6-well plate with 1.5ml of media containing DMEM, 10% FBS, GEN & Abs. Plates were incubated in a CO_2 incubator at 37°C for 24 - 48 hrs to obtain confluency. When confluent growth was observed, a sterile pipette was used to introduce a scratch on the cells. Thedifferentconcentrations of *C. papaya* and *P. guajava* ranging 5µg/ml, 50µg/ml, 100µg/ml, 250µg/ml and 500µg/ml were added to designated wells. A well containing no sample served as control. The plates were then incubated for 24 hrs. The wound healing activity of samples wasphotographed using an inverted microscope. The percentage of wound healing activity was calculated using the Fiji Image J software with following formula:

$$\text{Wound healing percentage} = \frac{\text{reduction percent}}{\text{area initial percent}} \times 100$$

where,

$$\text{Reduction Percentage} = \text{Initial wound area} - \text{The wound area after 24 hrs of scratch}$$

RESULTS

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACT OF*C. PAPAYA* AND *P. GUAJAVA*

The phytochemical analysis of *C. papaya* and *P. guajava*in aqueous extract showed the presence of alkaloids and phenols and absence of flavonoids which was analyzed through various phytochemical tests respectively.

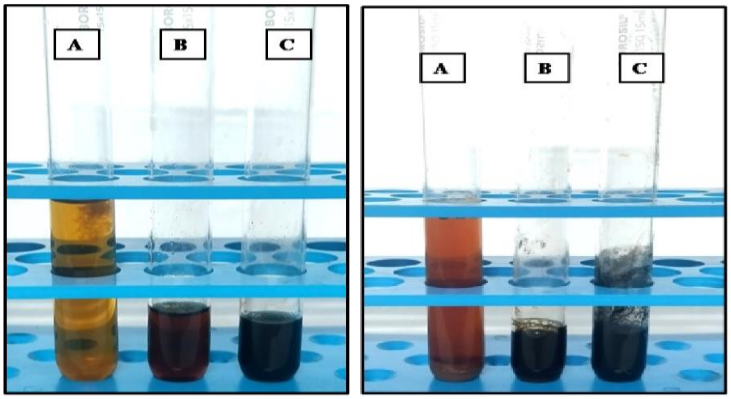


Fig 1: Phytochemical analysis of aqueous extract of *C. papaya*and *P. guajava* A-Test forflavonoids (negative result), B- Test for alkaloids (Brown red precipitate) C- Test for phenols (Bluish black)

AQUEOUS EXTRACT OF *CARICA PAPAYA*	
Phytochemicals	**Results**
Flavonoids	–
Alkaloids	+ + +
Phenol	+ + +

Table 1:- Phytochemical analysis of aqueous extract of *C. papaya*.

EXTRACT OF *P. GUAJAVA*	
Phytochemicals	**Results**
Flavonoids	-
Alkaloids	+ + +
Phenol	+ + +

Table 2:- Phytochemical analysis of aqueous extract of *P. guajava*.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF ALKALOIDS FOR *C. PAPAYA* AND *P. GUAJAVA*

The quantitative approach for alkaloids determined the total alkaloid content in *C. papaya, P. guajava* and the standard - Quercetin results are given below.

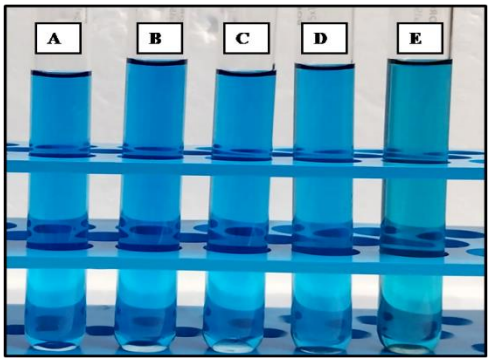
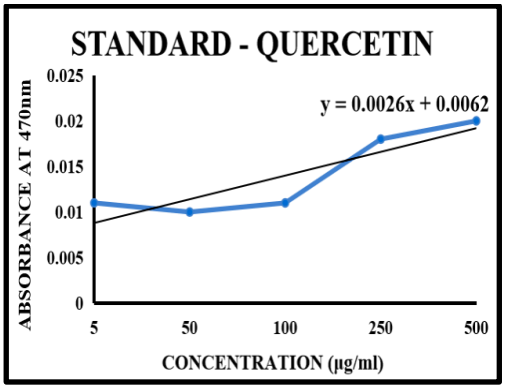


Fig 2:- Quantitative analysis of Quercetin as standard for alkaloids at different concentrations (A-5µg/ml, B-50 µg/ml, C-100 µg/ml, D-250 µg/ml and E-500 µg/ml).

SL.NO.	CONCENTRATION OF STANDARD (µg/ml)	ABSORBANCE OF STANDARD AT 470nm
1	5	0.011
2	50	0.010
3	100	0.011
4	250	0.018
5	500	0.020

Table 3:- Absorbance of Quercetin at different concentrations.



Graph 1:- Graph showing absorbance of Quercetin at different concentrations.

TOTAL ALKALOID CONTENT IN *C. PAPAYA* AND *P. GUAJAVA*

The sample *C. papaya* contains the highest amount of alkaloids at a concentration of 50 µg/ml and lowest at a concentration of 5µg/ml and 100µg/ml, indicating the content was below the quantifiable level at this defined concentration compared to Quercetin. The concentration of alkaloids in the sample *C. papaya* was almost equivalent to Quercetin.The sample *P. guajava* contains the highest amount of alkaloids at a concentration of 250µg/ml and lowest at concentrations of 5µg/ml and 50µg/ml, indicating the content was below the quantifiable level at this specific concentration compared to Quercetin. The concentration of alkaloids in the sample *P. guajava*was lower compared to standard - Quercetin.

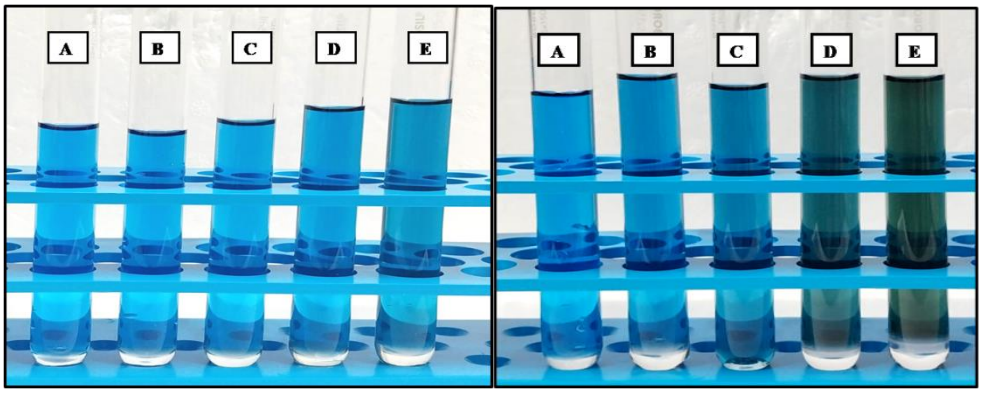


Fig 3:- Quantitative analysis of alkaloids in *C. papaya*and *P. guajava*at different concentrations (A-5µg/ml, B-50µg/ml, C-100 µg/ml, D-250µg/ml and E-500µg/ml).

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE OF SAMPLE AT 470nm	TOTAL ALKALOID CONTENT (µg/ml)
1	5	0.005	-0.5
2	50	0.019	6.5
3	100	0.006	0
4	250	0.007	0.5
5	500	0.012	3

SL. NO.	CONCENTRATION (µg/ml)	ABSORBANCE OF SAMPLE AT 470nm	TOTAL ALKALOID CONTENT IN SAMPLE (µg/ml)
1	5	0.003	-1.5
2	50	0.002	-2
3	100	0.008	1
4	250	0.010	2
5	500	0.005	-0.5

Table 4:- Total alkaloid content present in *C. papaya*and *P.guajava* at different concentrations.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF PHENOLS FOR *C. PAPAYA* AND *P. GUAJAVA*

The quantitative approach for phenols determined the total phenol content in *C. papaya* and *P. guajava* and the standard - gallic acid results are given below.

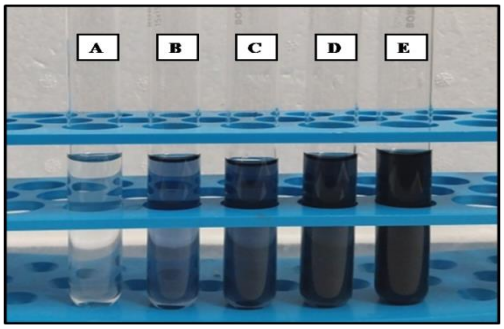
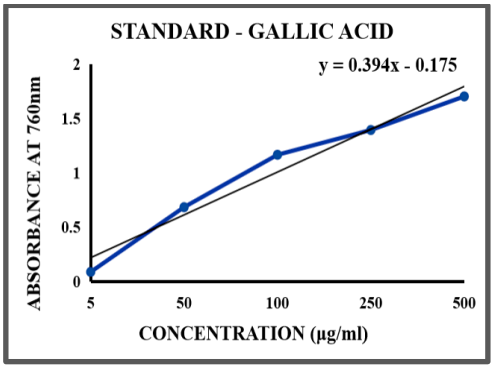


Fig 4:- Quantitative analysis of Gallic acid as standard for phenols at different concentrations (A-5µg/ml, B-50µg/ml, C-100µg/ml, D-250µg/ml and E-500µg/ml).

SL.NO.	CONCENTRATION OF STANDARD (µg/ml)	ABSORBANCE OF STANDARD AT 760nm
1	5	0.088
2	50	0.684
3	100	1.166
4	250	1.394
5	500	1.703

Table 5:- Absorbance of Gallic acid at different concentrations.



Graph 2:- Graph showing absorbance of standard -Gallic acid at different concentrations.

TOTAL PHENOL CONTENT IN *C. PAPAYA*AND *P. GUAJAVA*

The sample *C. papaya* contains the highest amount of phenols at a concentration of 500µg/ml and lowest at a concentration of 5µg/ml, indicating the content was below the quantifiable level at this specific concentration compared to standard - Gallic acid. The concentration of phenols in sample *C. papaya* was almost equivalent to that of standard gallic acid.The sample *P. guajava* contains the highest amount of phenols in the concentration 500µg/ml in comparison with the standard Gallic acid. The concentration of phenols in the sample *P. guajava* was higher compared to standard gallic acid.

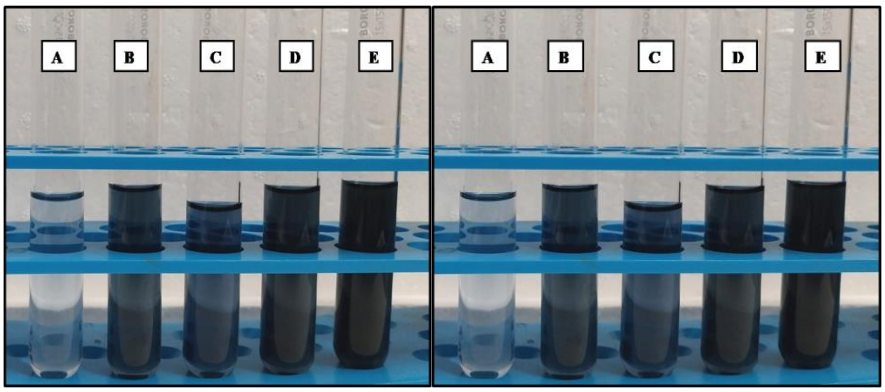


Fig 5:- Quantitative phytochemical analysis of *C. papaya*and *P. guajava*for phenols at different concentrations (A-5µg/ml, B-50µg/ml, C-100µg/ml, D-250µg/ml and E-500µg/ml).

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE OF SAMPLE AT 760nm	TOTAL PHENOL CONTENT (µg/ml)
1	5	0.022	-0.388
2	50	0.928	1.911
3	100	1.043	2.203
4	250	1.257	2.746
5	500	1.364	3.017

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE OF SAMPLE AT 760nm	TOTAL PHENOL CONTENT (µg/ml)
1	5	0.974	2.027
2	50	1.488	3.332
3	100	1.758	4.017
4	250	1.867	4.294
5	500	1.948	4.500

Table 6:- Total phenol content present in *C. papaya*and *P. guajava*at different concentrations.

ANTI-OXIDANT ACTIVITY OF *C. PAPAYA* AND *P. GUAJAVA* BY FERRIC REDUCING ANTIOXIDANT POWER ASSAY (FRAP)

The anti-oxidant activity of *C. papaya* and *P. guajava* was assessed using FRAP assay, with ascorbic acid as standard. The results demonstrated that both *C. papaya* and *P. guajava* have significant anti-oxidant potential. In FRAP assay, higher absorbance at a wavelength of 700nm indicated greater anti-oxidant capacity as it reflects a greater reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}).

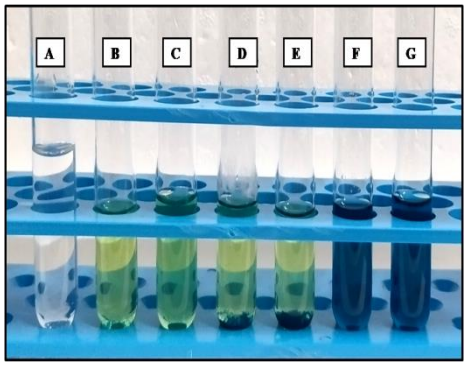


Fig 6:- Anti-oxidant assay for Ascorbic acid at different concentrations (A-Blank, B-Control, C-5µg/ml, D-50µg/ml, E-100µg/ml, F-250µg/ml and G-500µg/ml).

SL.NO	CONCENTRATION (µg/ml)	ABSORBANCE AT 700nm	PERCENTAGE INHIBITION
1	5	0.404	79.51
2	50	1.028	47.87
3	100	1.501	23.73
4	250	3.000	-52.12
5	500	3.000	-52.12
6	Control	1.972	-

Table 7:- Absorbance and percentage inhibition by FRAP method for ascorbic acid at different concentrations.

ANTI-OXIDANT ASSAY FOR *C. PAPAYA* AND *P. GUAJAVA*

The percentage inhibition of*C. papaya* decreases as the concentration increases, similar to the standard - ascorbic acid. This indicated that the sample exhibited anti-oxidant activity at lower concentrations 5µg/ml and 50µg/ml. Moreover, the *C. papaya* exhibited maximum absorbanceat concentrations 250µg/ml and 500µg/ml equivalent to standard Ascorbic acid.

The percentage inhibition of *P. guajava*decreases as the concentration increases, similar to the standard - Ascorbic acid. This indicated that the sample exhibited anti-oxidant activity at a very low concentration

5µg/ml. Additionally;*P.guajava* exhibited maximum absorbance at concentrations 250µg/ml and 500µg/ml equivalent to standard Ascorbic acid.

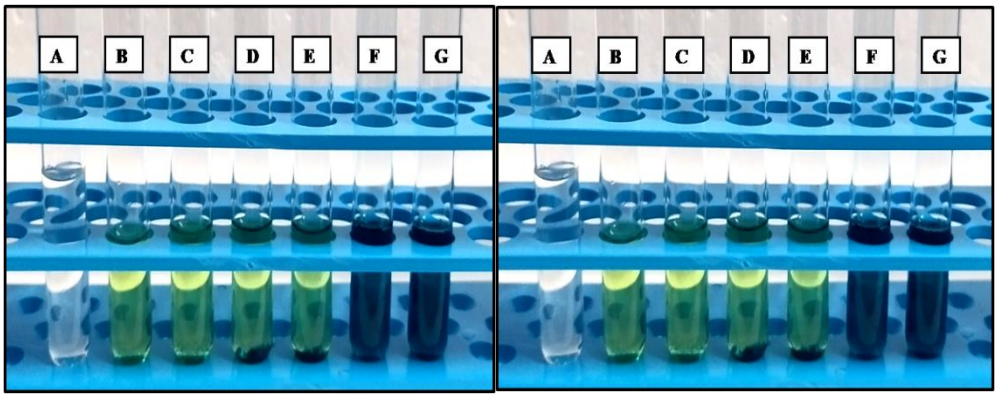


Fig 7:- Anti-oxidant assay for *C. papaya*and *P. guajava*at different concentrations (A-Blank, B-Control,C-5µg/ml, D-50µg/ml, E-100µg/ml, F-250µg/ml, G-500µg/ml).

SL.NO	CONCENTRATION (µg/ml)	ABSORBANCE AT 700nm	PERCENTAGE INHIBITION
1	5	0.335	83.01
2	50	0.946	52.02
3	100	1.403	28.85
4	250	3.000	-52.12
5	500	3.000	-52.12
6	Control	1.972	-

SL.NO	CONCENTRATION (µg/ml)	ABSORBANCE AT 700nm	PERCENTAGE INHIBITION
1	5	1.229	37.67
2	50	1.732	12.17
3	100	1.805	8.46
4	250	3.000	-52.12
5	500	3.000	-52.12
6	Control	1.972	-

Table 8:- Absorbance and percentage inhibition for *C. papaya*and *P. guajava*at different concentrations.

ANTI-DIABETIC ACTIVITY OF *C. PAPAYA* AND *P. GUAJAVA*

The samples *C. papaya* and *P. guajava* were investigated for their ability to inhibit the enzyme alpha-amylase. The findings demonstrated that both the samples have potential as anti-diabetic agents, compared to standard ascorbic acid.

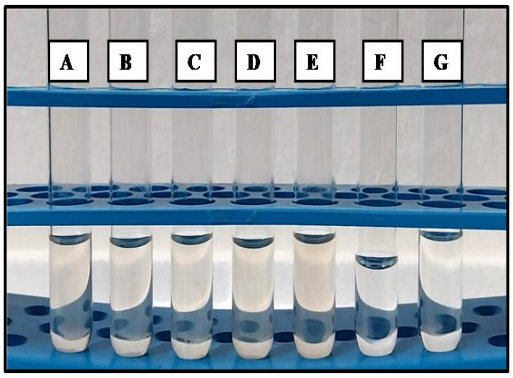
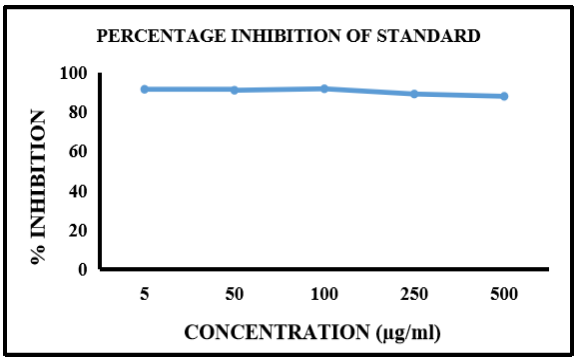


Fig 8:- Anti-diabetic assay by alpha amylase method for ascorbic acid at different concentrations (A-5µg/ml, B-50µg/ml, C-100µg/ml, D-250µg/ml, E-500µg/ml, F-Blank,G- Control).

SL.NO	CONCENTRATION (µg/ml)	ABSORBANCE AT 540nm	PERCENTAGE INHIBITION
1	5	0.103	91.40
2	50	0.109	90.90
3	100	0.100	91.65
4	250	0.132	88.99
5	500	0.146	87.82
6	Control	1.199	-

Table 9:- Absorbance and percentage inhibition of ascorbic acid at different concentrations.



Graph 3:- Graph representing the percentage inhibition of ascorbic acid at different concentrations.

ANTI-DIABETIC ASSAY FOR *C. PAPAYA* AND *P. GUAJAVA*

The results showed that *C. papaya* exhibited significantly higher inhibition at concentrations 5, 50 and 100µg/ml, indicating a potentially higher anti-diabetic activity compared to standard ascorbic acid. The percentage inhibition of *C. papaya* sample was almost equivalent to that of standard ascorbic acid, confirming them as potential anti-diabetic agent.

The result demonstrated that *P. guajava* exhibited a significant percentage inhibition at lower concentration 5µg/ml compared to standard ascorbic acid, indicating notable anti-diabetic potential at lower concentrations. The extract of *P. guajava* exhibited maximum absorbance at concentrations 250µg/ml and 500µg/ml in comparison with standard.

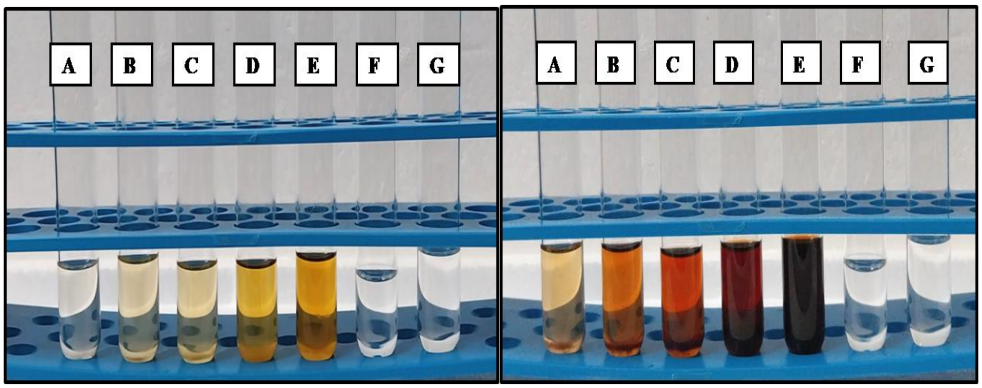
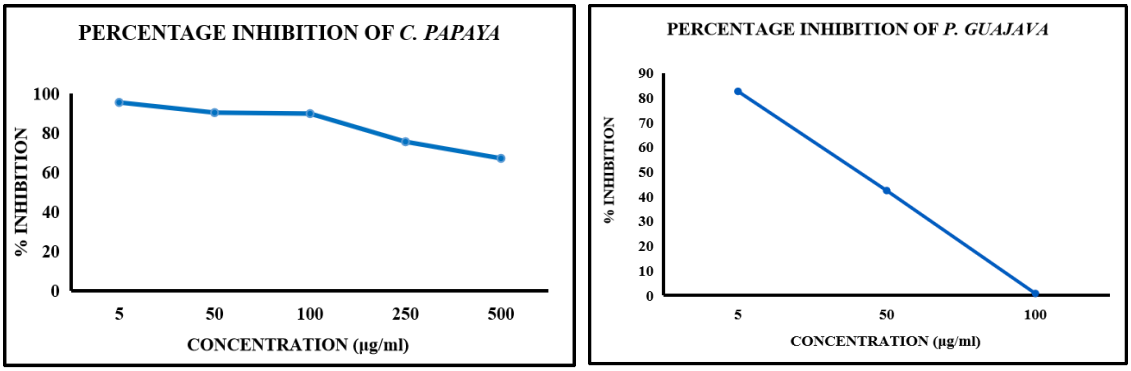


Fig 9:- Anti-diabetic assay for *C. papaya*and *P. guajava* at different concentrations (A- 5 µg/ml, B-50µg/ml, C-100µg/ml,D-250µg/ml, E-500µg/ml, F-Blank, G- Control).

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE AT 540nm	PERCENTAGE INHIBITION
1	5	0.054	95.49
2	50	0.116	90.32
3	100	0.122	89.82
4	250	0.293	75.56
5	500	0.394	67.13
6	Control	1.199	-

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE AT 540nm	PERCENTAGE INHIBITION
1	5	0.208	82.65
2	50	0.690	42.45
3	100	1.190	0.75
4	250	3.000	-150.20
5	500	3.000	-150.20
6	Control	1.199	-

Table 10:- Absorbance and percentage inhibition of *C. papaya*and *P. guajava*at different concentrations.



Graph 4:- Graph representing the percentage inhibition of *C. papaya*and *P. guajava*at different concentrations.

ANTI-ARTHRITIC ACTIVITY OF *C. PAPAYA* AND*P.GUAJAVA* BY EGG ALBUMIN PROTEIN DENATURATION ASSAY

The anti-arthritic activity of *C. papaya* and *P. guajava* was evaluated using egg albumin protein denaturation assay, with Diclofenac as standard. The results demonstrated that both *C. papaya* and *P. guajava* exhibited significant anti-arthriticactivity, particularly at lower concentrations.

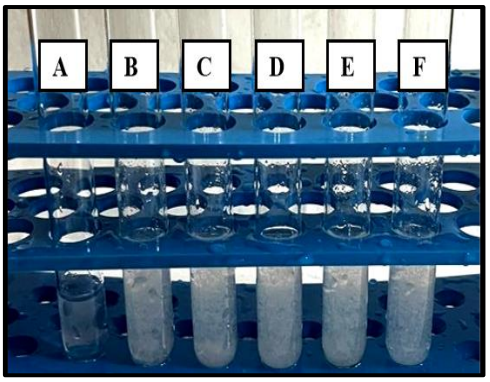


Fig 10:- Anti-arthritic activity by egg albumin protein denaturation assay for diclofenac at different concentrations (A-Control, B-5µg/ml, C-50µg/ml, D-100µg/ml, E-250µg/ml andF-500µg/ml).

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE at 660nm	PERCENTAGE INHIBITION
1	5	0.325	65.31
2	50	0.407	56.56
3	100	0.539	42.47
4	250	0.595	36.49
5	500	0.638	31.91
6	Control	0.937	-

Table 11:- Absorbance and percentageinhibition for diclofenac at different concentrations.

ANTI-ARTHRITIC ASSAY FOR *C. PAPAYA*AND *P. GUAJAVA*

The *C. papaya*leaf extract demonstrated higher inhibition at lower concentrations 5µg/ml, 50µg/ml and 100µg/ml compared to standard Diclofenac.However, at higher concentrations 250µg/ml and 500µg/ml, they demonstrated a lower inhibition compared to standard diclofenac.The *P. guajava* leaf extract demonstrated higher inhibition at lower concentrations 5, 50, 100 and 250µg/ml compared to standard Diclofenac.The *C. papaya* showed a lower inhibition at higher concentration 500µg/ml compared to standard Diclofenac.

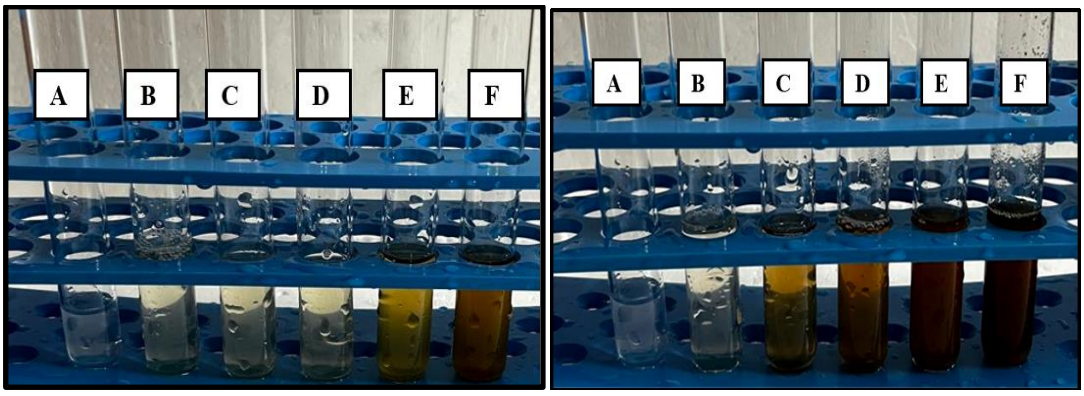
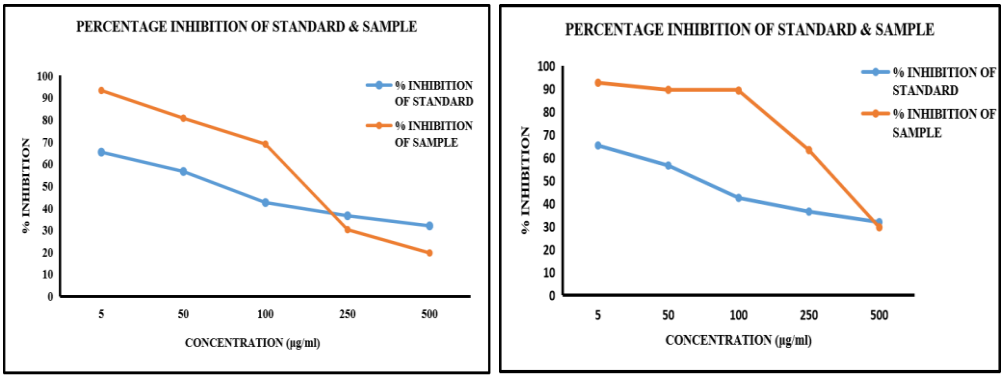


Fig 11:- Anti-arthritic assay for *C. papaya*and *P. guajava*at different concentrations (A- Control, B- 5µg/ml, C- 50µg/ml, D- 100µg/ml, E- 250µg/ml and F-500µg/ml).

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE AT 660nm	PERCENTAGE INHIBITION
1	5	0.063	93.27
2	50	0.181	80.68
3	100	0.291	68.94
4	250	0.654	30.2
5	500	0.753	19.63
6	Control	0.937	-

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE AT 660nm	PERCENTAGE INHIBITION
1	5	0.063	92.63
2	50	0.181	89.54
3	100	0.291	89.22
4	250	0.654	63.28
5	500	0.753	29.66
6	Control	0.937	-

Table 12:- Absorbance and percentage inhibition for *C. papaya*and*P. guajava*at different concentrations.



Graph 5:- Percentage inhibition of *C. papaya*and *P. guajava*against diclofenac.

ANTI-MICROBIAL ACTIVITY OF *C. PAPAYA* AND *P. GUAJAVA*

The anti-microbial activity of *C. papaya* and *P. guajava* at different concentrations was studied by measuring the diameter of zone of inhibition against the bacteria *S. aureus* and *E. coli* and the fungus *C. albicans* using the agar well diffusion method.

ANTI-BACTERIAL ACTIVITY OF *C. PAPAYA*

The results revealed that there was no zone of inhibition for the aqueous extract of *C. papaya* against the test organisms *S. aureus* and *E. coli*.

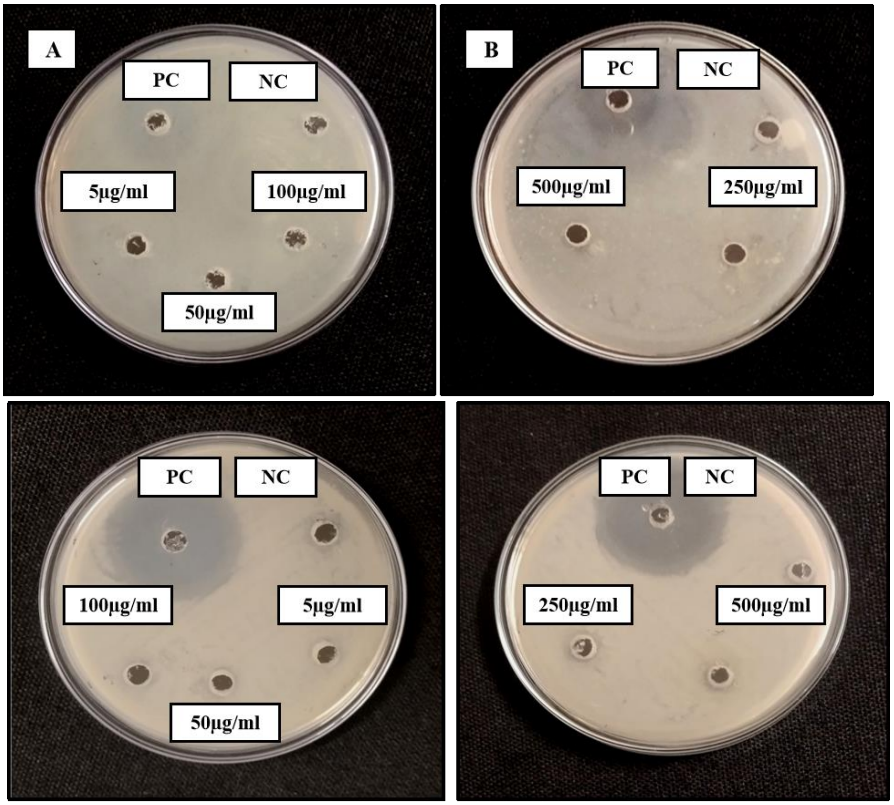


Fig 12:- Anti-bacterial screening of aqueous extract of *C. papaya* against *E. coli*and *S. aureus* at different concentrations (A- PC, NC, 5µg/ml, 50µg/ml and 100µg/ml and B- PC, NC, 250µg/ml and 500µg/ml).

ANTI-BACTERIAL ACTIVITY OF *P. GUAJAVA*

The results showed no zone of inhibition for aqueous extract of *P. guajava* against the test organisms *E. coli* and *S. aureus.*

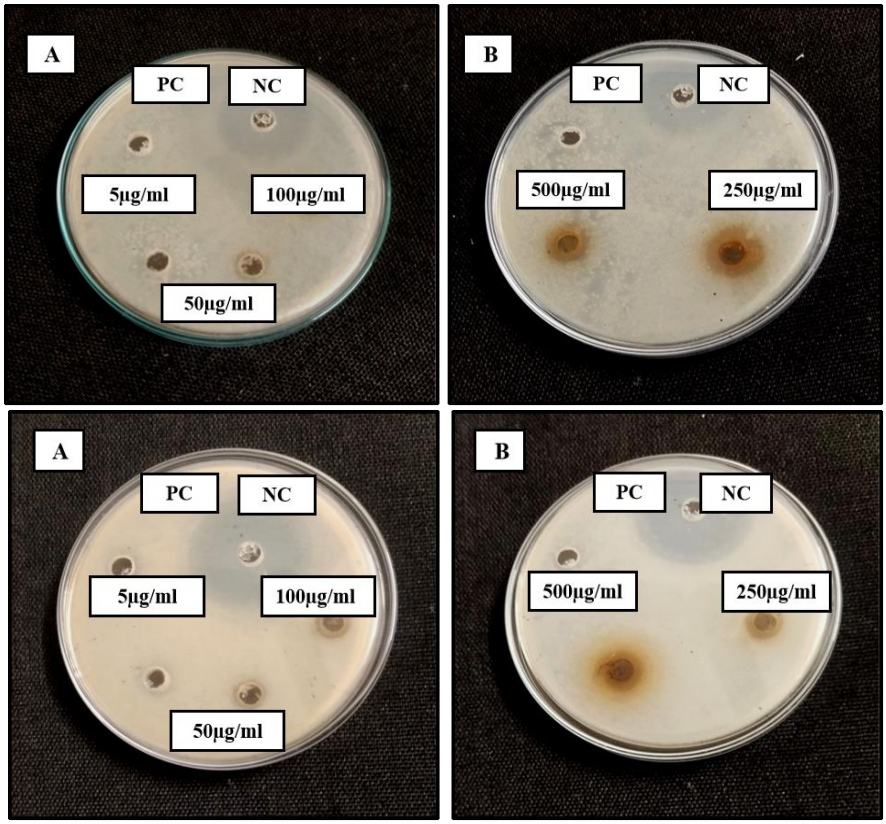


Fig 13:- Anti-bacterial screening of aqueous extract of *P. guajava* against *E. coli*and*S. aureus* at different concentrations (A- PC, NC, 5µg/ml, 50µg/ml and 100µg/ml and B- PC, NC, 250µg/ml and 500µg/ml).

ANTI-FUNGAL ACTIVITY OF *C. PAPAYA* ANDP. *GUAJAVA*

The results showed no zone of inhibition for the aqueous extract of *C. papaya* and *P. guajava* against the test organism *C. albicans*, suggests negative result for anti-fungal screening.

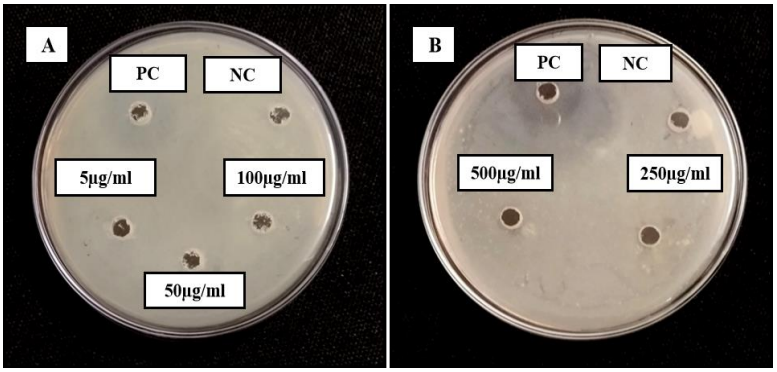


Fig 14:- Anti-fungal screening of aqueous extract of*C. papaya* against *C. albicans* at different concentrations (A- PC, NC, 5µg/ml, 50µg/ml and 100µg/ml and B- PC, NC, 250µg/ml and 500µg/ml).

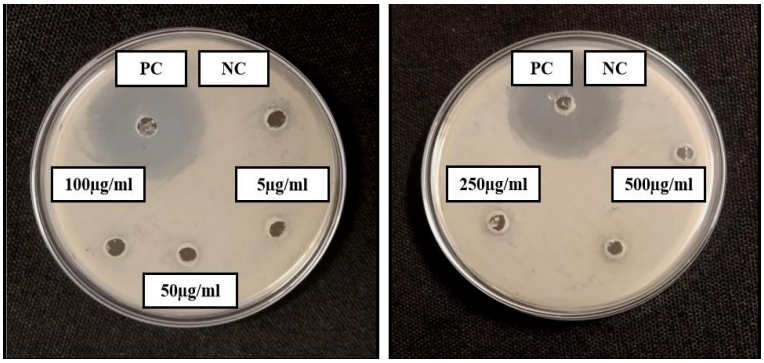


Fig 15:- Anti-fungal screening of aqueous extract of *P. guajava* against *C. albicans* at different concentrations (A- PC, NC, 5µg/ml, 50µg/ml and 100µg/ml and B- PC, NC, 250µg/ml and 500µg/ml).

WOUND HEALING ACTIVITY OF *C. PAPAYA*

The results showed that the sample *C. papaya* significantly stimulated the migration and proliferation rate of L929 cells in the wounded area at very lower concentrations of 5µg/ml compared to untreated control.

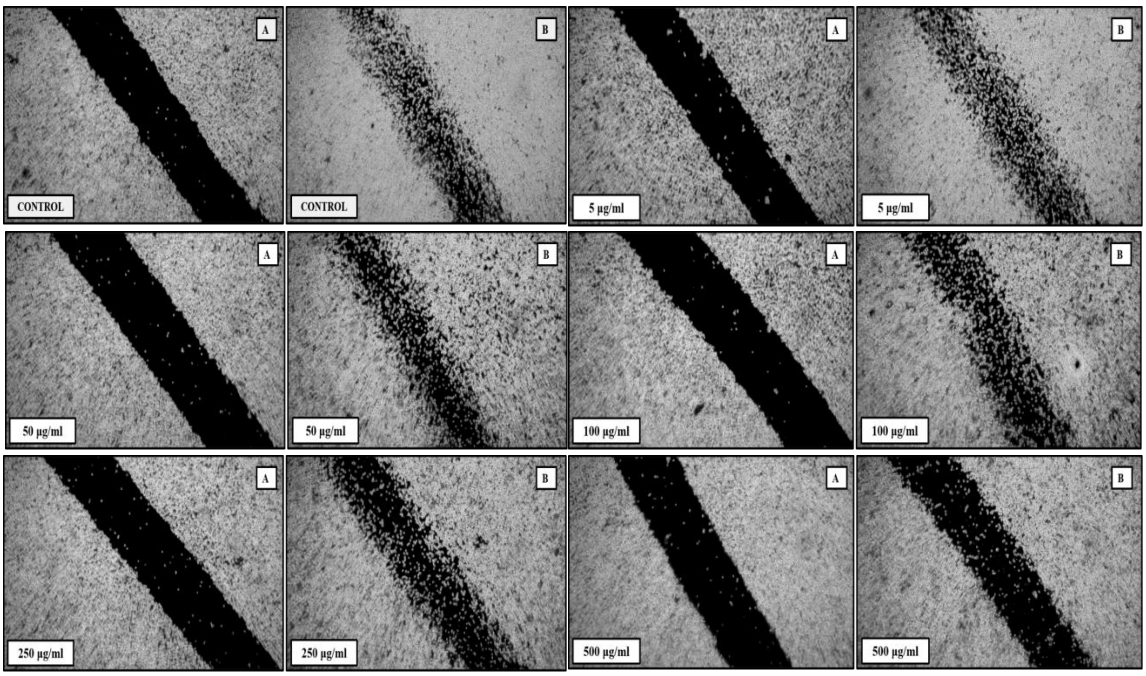
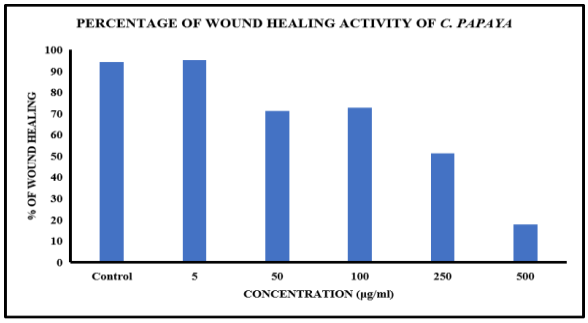


Fig 16:- *In-vitro* wound healing activity of *C. papaya* on L929 cells - control, 5µg/ml, 50µg/ml and 100µg/ml and B- PC, NC, 250µg/ml and 500µg/ml (A- Immediately after scratch and B - after 24 hours)

SL.NO	CONCENTRATION (µg/ml)	AREA % BEFORE	AREA % AFTER 24 hrs	% WOUND HEALING
1	Control	21.23	1.18	94.41
2	5	21.04	1.03	95.10
3	50	24.04	6.87	71.41
4	100	25.00	6.81	72.74
5	250	23.71	11.53	51.34
6	500	21.03	17.28	17.83

Table 13:- *In-vitro*percentage of wound healing activity of *C. papaya* on L929 cells at different concentrations



Graph 6:- Graph showing *in-vitro*percentage of wound healing activity of *C. papaya* on L929 cells

WOUND HEALING ACTIVITY OF*P. GUAJAVA*

The aqueous extract of *P. guajava* in the wounded area of L929 cells showed a decrease in both cell migration and cell proliferation rate compared to the untreated control.

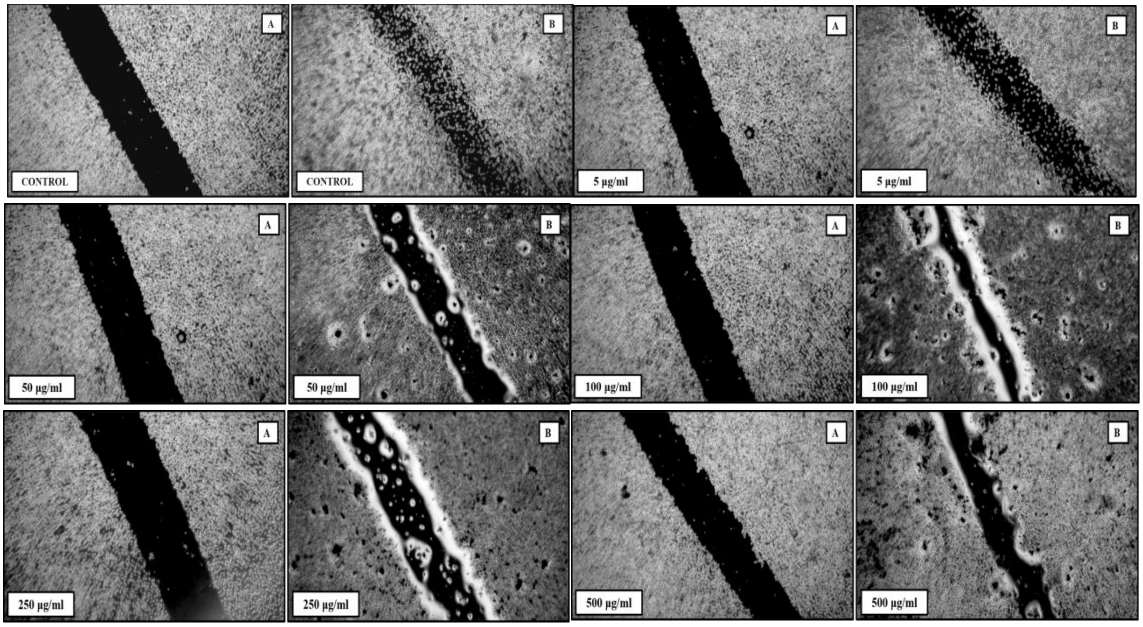
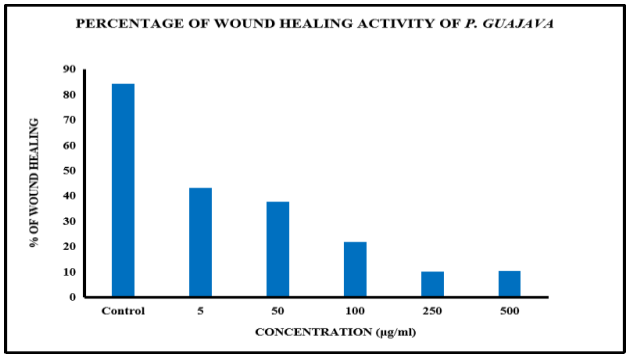


Fig 17:- *In-vitro* wound healing activityof *P. guajava* on L929 cells - control, 5µg/ml, 50µg/ml and 100µg/ml and B- PC, NC, 250µg/ml and 500µg/ml (A- Immediately after scratchandB- after 24 hours)

SL.NO	CONCENTRATION (µg/ml)	AREA % BEFORE	AREA % AFTER 24 hrs	% OF WOUND HEALING
1	Control	18.318	2.899	84.174
2	5	17.433	9.937	42.998
3	50	17.422	10.872	37.596
4	100	15.948	12.489	21.689
5	250	20.714	18.622	10.099
6	500	15.817	14.170	10.412

Table 14:- *In-vitro*percentage of wound healing activity of *P. guajava* on L929 cells at different concentrations



Graph 7:- Graph showing *in-vitro*percentage of wound healing activity of*P. guajava* on L929 cells

DISCUSSION

The present study provide valuable insights into phytochemical, anti-oxidant, anti-diabetic, anti-arthritic, anti-microbial and wound healing properties of aqueous extract of *C. papaya* and *P. guajava*. Phytochemical screening confirmed the presence of alkaloids and phenols. Quantitative assessment revealed a significant alkaloid content in *C. papaya* at higher concentrations (50µg/ml and 500µg/ml), while at low concentration (5µg/ml), the content was below the quantifiable level. For *P. guajava*, notable alkaloid content was observed at concentrations of 100µg/ml and 250µg/ml, but it was below the quantifiable level at 5, 50 and 500µg/ml. The phenolic content in *C. papaya* was below the quantifiable level at lower concentration 5µg/ml and was high across all other concentrations for both *C. papaya*and *P. guajava*. (Smith & Brown, 2023) also investigated the phytochemical composition of*C. papaya* and *P. guajava* leaf extracts using quantitative as well as qualitative analysis methods, confirming high levels of phenolic compounds and alkaloids, indicative of their potential anti-oxidant and medicinal properties.

The anti-oxidant activity evaluated using the FRAP assay revealed maximum reducing power at concentrations 250 and 500µg/ml for both the extracts. As concentration increases, more anti-oxidants are available to participate in the reduction reaction, leading to higher absorbance values. (Zhao *et al*., 2018, Rajabzadeh*et al*., 2019) found similar results, for concentrations ranging from 200 - 500µg/ml exhibited highest anti-oxidant activity due to the presence of phenolic compounds. In terms of anti-diabetic activity, the inhibition of alpha amylase by *C. papaya* and *P. guajava* leaf extracts was significant at lower concentrations (5, 50, and 100 µg/ml), particularly for *C. papaya*, indicating notable anti-diabetic potential. (Juárez-Rojop*et al*., 2012) explored the hypoglycemic and anti-oxidant activities of *C. papaya* finding effective reduction in blood glucose levels. Additionally, (Thaipong*et al*., 2006) conducted a comparative study on the anti-diabetic activity of *P. guajava* extracts against standards like ascorbic acid and butylated hydroxytoluene and found strong anti-diabetic properties.

The anti-arthritic activity of *C. papaya* and *P. guajava* extracts was evaluated and the results demonstrated that they have a significant inhibitory effect on protein denaturation. (Owoyele*et a*l., 2008) demonstrated that ethanolic extracts of *C. papaya* leaves has significant analgesic and anti-inflammatory properties. The potent inhibitory effect of *P. guajava* leaf extract on protein denaturation was analysed *in-vitro* by (Dutta & Das, 2017).

However, anti-microbial activity assessed against the organisms *E. coli*, *S. aureus* and *C. albicans* revealed no significant inhibition by both the extracts *C. papaya* and *P. guajava*. This findings aligns with (Aiyegoro&Okoh, 2009), who reported that *C. papaya* and *P. guajava* extracts showed no anti-bacterial activity against the microbial strains of *S. aureus* and *A. niger*. In wound healing assay on L929 cells, it was demonstrated that the sample *C. papaya* showed significant activity at very low concentration of 5µg/ml. However,*P. guajava* exhibited a concentration dependent reduction in cell migration rate. (Santos *et al*., 2016)

suggested that *C. papaya* extract exhibited significant wound healing activity through its anti-oxidant and anti-inflammatory properties, by evaluating its wound closure rate and *P. guajava* reduced the migration rate of cells.

CONCLUSION

The study examined the biological applications of aqueous leaf extract of *C. papaya* and *P. guajava,* focusing on phytochemical evaluation, anti-oxidant, anti-diabetic, anti-arthritic, anti-microbial and wound healing properties. The findings revealed that both the extracts contain phytochemicals such as alkaloids and phenols, which contribute to anti-oxidant and anti-diabetic activities. The plant extracts *C. papaya* and *P. guajava* possess significant anti-oxidant and anti-arthritic potential.Moreover, the plant extracts effectively inhibitedthe alpha-amylase enzyme, indicating their potential in managing diabetes. However, the anti-microbial screening showed no significant activity against *E. coli*,*S. aureus*, and *C. albicans*. The *C. papaya* potentially increased the wound healing properties in L929 cells, while *P. guajava* showed a reduction in cell migration rate.

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