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Navya Raj AR
Bioroot Exploration India Pvt. Ltd., Thiruvananthapuram, Kerala, India

Dr. Parvathy Prasad
Bioroot Exploration India Pvt. Ltd., Thiruvananthapuram, Kerala, India

Corresponding Author:
Dr. Parvathy Prasad
Bioroot Exploration India Pvt. Ltd., Thiruvananthapuram, Kerala, India

Exploring the potential of *Ananas comosus* and application of its bioactive compounds

Navya Raj AR and Parvathy Prasad

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Abstract
This study explores the therapeutic potential of aqueous extract of *Ananas comosus* peel. *A. comosus*, commonly known as pineapple, is a widely consumed tropical fruit, with its peel often discarded as waste despite being rich in bioactive compounds. The plant contains various phytochemicals, which account for its therapeutic properties. Qualitative and quantitative analysis confirmed the presence of key bioactive compounds, including phenols, saponins, flavonoids and alkaloids. Extracts of the peel exhibited significant pharmacological properties such as anti-oxidant, anti-inflammatory, anti-diabetic, anti-ulcer, anti-microbial, and anti-urolithiatic activities. The peel extract showed significant anti-oxidant, anti-inflammatory and anti-urolithiatic activity compared to standards. The anti-diabetic and anti-ulcer activities were maximum at the lowest concentration; however, anti-microbial activity was not observed in the extract. These findings suggest that *A. comosus* peel could be a valuable source for bioactive compounds with therapeutic potential.

Keywords: *Ananas comosus* peel, anti-inflammatory activity, anti-urolithiatic activity, anti-oxidant activity, bioactive compounds, phytochemical analysis

Introduction
Plants play an indispensable role in sustaining life on Earth, serving as primary producers that convert solar energy into biochemical compounds vital to all living organisms. In addition to producing oxygen through photosynthesis, they are the underlying basis of food chains and supply a wide range of medicinal compounds that are essential to human health. Through novel drug development and pharmacological research, medicinal plants are utilized as a natural source of bioactive substances that are used to cure and prevent a variety of illnesses as well as to develop new therapeutic agents. Bromeliads are a diversified group of medicinally relevant plants that are well-known for producing distinct compounds with promising pharmacological and therapeutic potential. Unlike other bromeliads, pineapple (*A. comosus*) peel showed strong therapeutic potential, including anti-diabetic and anti-microbial effects, making it a valuable, sustainable source for nutraceutical and pharmaceutical use. *Ananas comosus* (pineapple), a tropical fruit from the Bromeliaceae family, is valued for its nutritional and medicinal properties. Its peel, a major by-product of fruit processing, is rich in bioactive compounds such as flavonoids, phenolic acids (chlorogenic and ferulic acids), tannins, and bromelain (Abdullah & Hanafi, 2008) [1]. These phytochemicals contribute to the peel's strong anti-oxidant (Tochi *et al*., 2008) [2], anti-inflammatory (Devi *et al*., 2018) [3], anti-microbial (Bhattacharyya, 2008) [4], anti-diabetic (Shimabukuro *et al*., 2006) [5], and gastroprotective properties (Ajiboye & Oluwole, 2012) [6]. Anti-oxidants in the peel helps to neutralize free radicals, potentially preventing oxidative stress-related conditions such as kidney stone formation (Ahmad & Sharma, 2012) [7]. Bromelain, along with flavonoids and vitamin C, enhances anti-inflammatory responses and supports wound healing and digestion (Maurer, 2001 [8]; Yong *et al*., 2018) [9]. The peel extract also showed inhibitory effects on α-amylase and α-glucosidase enzymes, suggesting its role in blood glucose regulation (Ogihara*et al*., 2002) [10]. Furthermore, it offers a natural alternative to synthetic supplements and anti-biotics, which often have adverse effects (Carneiro *et al*., 2002) [11]. This study highlights the therapeutic potential of *A. comosus* peel extract as a sustainable source for natural health-promoting agents.

Materials and Methods
Sample collection
Pineapples were collected from the local market of Thiruvananthapuram. The peels were carefully separated from the fruit and chopped into smaller pieces to facilitate the drying process.

These pieces were then dried in a hot air oven at 60 degree Celsius (°C) for 8 hours (hrs) to completely remove any moisture. Once dried, the peels were grounded into a fine powder using grinder.

Extraction
Soxhlet extraction was conducted using distilled water (DW) as solvent. A measured amount of powdered pineapple peel was loaded into a soxhlet thimble, which was then placed in the soxhlet apparatus. DW was added to the round-bottom flask, and the extraction process was carried out for 6-8 hrs, allowing the solvent to circulate continuously over the sample. After the extraction was completed, the resulting water extract was collected. If necessary, the extract was concentrated by removing excess water using rotary evaporator.

Qualitative analysis phytochemicals
Phytochemical screening of the test sample was performed using standard methods to detect key bioactive compounds such as alkaloids, flavonoids, phenols, tannins, terpenoids, and saponins.

Test for phenols
A few drops of 5 percent (%) ferric chloride ($FeCl_3$) solution were added to 1 millilitre (mL) of *A. comosus* peel aqueous extract. The development of a dark green or bluish-black colour confirmed the presence of phenolic compounds.

Test for tannins
To 400 microliter (µL) of *A. comosus* peel aqueous extract, 4 mL of NaOH was added. The appearance of an emulsion confirmed the presence of tannins.

Test for terpenoids
To 1 mL of *A. comosus* peel aqueous extract, 2 mL of chloroform was added, followed by few drops of concentrated H_2SO_4. The formation of a reddish-brown interface indicated the presence of terpenoids.

Test for saponins
To 1 mL of *A. comosus* peel aqueous extract, 1 mL of DW was added and the mixture was shaken vigorously. The formation of foam on the surface indicated the presence of saponins

Test for flavonoids
To 1 mL of *A. comosus* peel aqueous extract, 2 mL of 2% NaOH was added, resulting the solution turning into an intense red colour. Upon the addition of few drops of diluted acid, the colour changed to colourless, indicating the presence of flavonoids.

Test for alkaloids
To 1 mL of *A. comosus* peel aqueous extract, a few drops of Wagner's reagent were added. The formation of a brown or reddish precipitate upon the addition of the reagent indicated the presence of alkaloids.

Quantitative analysis of phytochemicals
Saponins
Take 1 mL of *A. comosus* peel aqueous extract combine it with 0.2 mL of vanillin solution. Add 2.5 mL of concentrated H_2SO_4 to the mixture and mix gently. Place the mixture in a water bath at 60 °C for 15 minutes (mins). After incubation, allow the solution to cool to room temperature (RT), and absorbance was recorded at 544 nano meter (nm).

Phenols
Take 1 mL of *A. comosus* peel aqueous extract and transfer it into a clean test tube. Add 2 mL of Folin-Ciocalteu reagent to the sample and mix gently. Add 4 mL of 7.5% sodium carbonate (Na_2CO_3) solution to the mixture and mix thoroughly. Incubate the test tube in dark for 30 mins. After incubation, the absorbance was measured at 760 nm using spectrophotometer.

Flavonoids
To 1 mL of *A. comosus* peel aqueous extract, 2 mL of 5% sodium nitrate ($NaNO_3$) was added. After 5 mins, 3 mL of 10% aluminium chloride **(**$AlCl_3$) was added, followed by 2 mL of 1 molar (M) NaOH. The mixture was kept for 5 mins and then diluted to 10 mL with DW. Quercetin, with different concentrations ranging from 200-1000 µg/mL, served as standard. All reagents, except for the sample, were used as blank. Absorbance was recorded at 510 nm.

Alkaloids
To 1 mL of *A. comosus* peel aqueous extract, 5 mL of phosphate buffer (pH 4.7) and 5 mL of bromocresol green solution were added. The mixture was then transferred to a separating funnel and agitated. The complex was extracted with 1, 2, 3, and 4 mL of chloroform through vigorous shaking and collected in a test tube. Quercetin, with concentrations ranging from 0.2 to 1 mg/mL, was used as standard. All reagents, excluding the sample, were used as blank. Absorbance was measured at 470 nm.

Anti-oxidant activity
The anti-oxidant properties of *A. comosus* peel extract were examined using the FRAP (Ferric Reducing Anti-oxidant Power) assay, which evaluates the extract's effectiveness in reducing iron ions. To 1 mL of *A. comosus* peel aqueous extract at various concentrations (50, 75, 100, 250, and 500 µg/mL), incorporate 2.5 mL of sodium phosphate buffer and 2.5 mL of potassium ferricyanide ($C_6N_6FeK_3$). Thoroughly blend the solution and incubate at 50°C for 20 minutes. Following incubation, 2.5 mL of trichloroacetic acid (TCA) was added and mixed thoroughly. Gathered the supernatant and combined it with 2 mL of distilled water. Subsequently, added 0.5 mL of FeCl3 solution, mixed well and incubated at room temperature for 10 minutes. Absorbance was measured at 700 nm.

Anti-inflammatory activity
The anti-inflammatory activity of the sample was assessed by employing the protein denaturation method. To various concentrations (50, 75, 100, 250, and 500 µg/mL) of the aqueous extract from *A. comosus* peel and the reference medication diclofenac, 0.2 mL of 1% BSA (Bovine Serum Albumin) and 1.8 mL of PBS (Phosphate Buffered Saline, pH 6.4) were incorporated. The mixtures were stored at room temperature for 20 minutes, then incubated in a water bath at 70°C for 5 minutes. Once cooled to room temperature, the absorbance was measured at 660 nm. A control was made with all the reagents excluding the sample or standard, with methanol acting as the blank.

Anti-Urolithiatic activity

Semi-permeable membranes were prepared using farm eggs by decalcifying them in 2M hydrochloric acid (HCl) overnight to remove the eggshells completely. The contents were completely squeezed out from the decalcified eggs, thoroughly washed with DW, briefly
immersed in an ammonia solution, and then rinsed again with DW. For the experiment, 1 mg of calcium oxalate (CaC_2O_4) and 10mg of *A. comosus* peel extract were packed into semi-permeable membranes, which were suspended in 100mL of 0.1 M Tris buffer within conical flasks. Four groups were set up as blank group with 1 mg of CaC_2O_4 only, two positive controls with 1 mg of CaC_2O_4 and 10 mg of standard drugs (cystone or potassium citrate), and a test group with 1 mg of $CaC_2O_4$and 10 mg of *A. comosus* peel extract. The flasks were incubated at 37 °C for 2 hrs, after which the contents of the membranes were transferred to test tubes. Approximately 2 mL of 1N H_2SO_4 was added to each test tube and titrated with 0.9494 normal (N) $KMnO_4$ until a light pink endpoint was reached. The amount of undissolved CaC_2O_4 was subtracted from the initial amount to determine the calcium oxalate dissolved by *A. comosus* peel extract.

Anti-diabetic activity

The anti-diabetic property of *A. comosus* peel aqueous extract was evaluated using α-amylaseenzyme inhibition assay. Various concentrations of sample (50, 75, 100, 250, and 500 µg/ml) were prepared in five test tubes and diluted to 1 ml using the appropriate solvent. To each tube, 100µl of sodium phosphate buffer and 100 µl of α-amylase enzyme were added, followed by incubation at RT for 10 mins. After incubation, 1ml of starch solution was introduced into all tubes and incubated again at RT for another 10 mins**.** Subsequently, 1 mlofdinitrosalicylic acid (DNS) reagent was added, and the tubes were placed in a boiling water bath for 10 mins**.** After cooling, 100µl of DW was added to each tube, and the absorbance was measured at 540nmusing a visible spectrophotometer. Acarbose was used as standard, following the same concentration range for the sample.

Anti-microbial activity

The anti-microbial activity of aqueous extract of *A. comosus* peel was evaluated using agar well diffusion method. One bacterial species, *Staphylococcus aureus (S. aureus)*, and one fungal species, *Aspergillus niger (A.niger)* were utilized for the test. The bacterial species were inoculated in nutrient broth, while the fungal species were inoculated in potato dextrose agar (PDA). Both were incubated at 37°C for 24 hrs in an incubator. Nutrient agar and PDA were prepared as culture media, autoclaved, and then poured into sterilized petri plates. After incubation, the respective microbes were plated onto the prepared media. Autoclaved nutrient agar (25 ml) was poured into sterile petri plates. After solidification, 100 µl of 24 hrs old broth culture was swabbed onto plates using sterile cotton swabs. Wells were created with sterile micropipette tips, and 30µl of various sample concentrations were added. The plates were then incubated at 37°C for 24 hrs. Zones of inhibition were measured in millimetres and recorded. Gentamicin served as positive control (PC), while DW acted as negative control (NC).

Anti-ulcer assay

The acid neutralizing capacity method was used to determine the anti-ulcer activity of the aqueous extract of *A. comosus peel*. 5 mL of the sample at different concentrations (50, 75, 100, 250, and 500 µg/ml) were prepared in five conical flasks and filled to 70 ml with DW. The solution was stirred for 1 min and then for 15 mins after adding 15 ml of diluted HCl using a magnetic stirrer. 2 to 3 drops of phenolphthalein were added to the mixture, which was then titrated against 0.5N NaOH until a pink colour formed. 5 mL of Gelusil (2.5g) was used as the standard anti-ulcer agent.

Results

Qualitative phytochemical analysis of aqueous extract of *A. Comosus* peel

The phytochemical analysis of aqueous extract of *A. comosus* revealed the presence of alkaloids, saponins, flavonoids, and phenols, while tannins and terpenoids were absent. The contents were analysed through various phytochemical tests respectively.

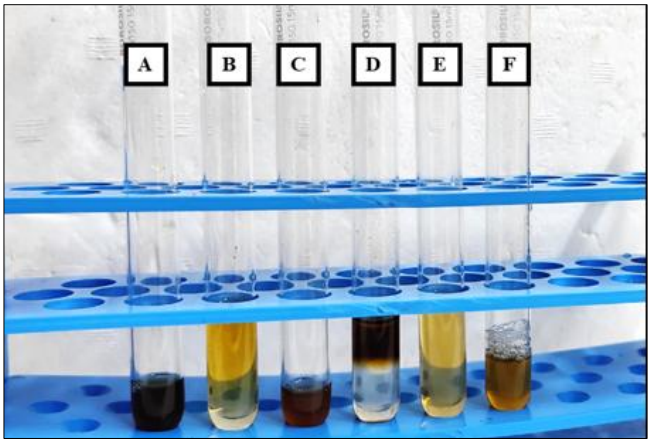


Fig 1: Qualitative Analysis of Phytochemicals in Aqueous Extract of *A. comosus* peel (A- Phenols, B- Tannins, C- Alkaloids, D- Terpenoids,E- Flavonoids, F- Saponins).

Table 1: Phytochemical analysis of different phytochemicals present in aqueous extract of *A. comosus peel*

Phytochemicals	Aqueous extract
Phenols	+
Tannins	–
Alkaloids	++
Terpenoids	–
Flavonoids	+
Saponins	+++

Quantitative phytochemical analysis of aqueous extract of *A. comosus* peel

Quantitative estimation of saponins

Diosgenin used as standard to quantify the saponin content in the aqueous extract of *A. comosus* peel. Using the standard curve equation y = 0.0059x - 0.0318, the saponin concentration was determined to be 100.47 µg/mL, indicating its significant presence in the sample.

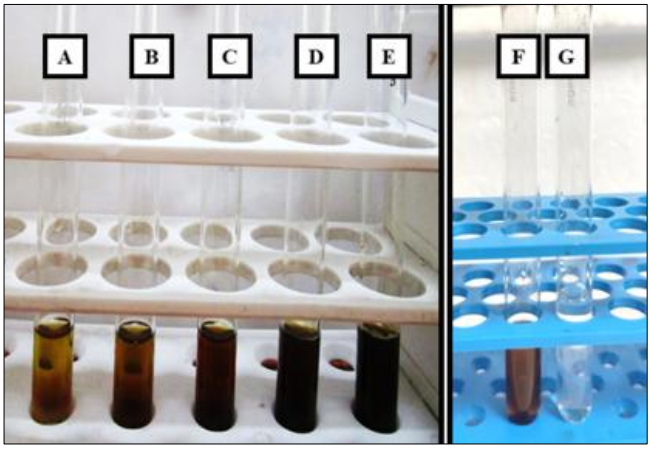
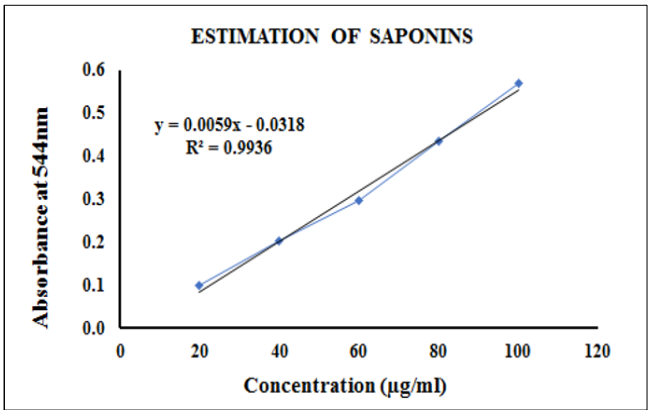


Fig 2: Quantitative estimation of saponins with A-E as Diosgenin-Standard (20,40,60,80,100µg/mL), F - aqueous extract of *A. comosus* peel and G - blank.

Table 2: Absorbance value of Diosgenin at different concentrations (20, 40, 60, 80,100µg/mL) and aqueous extract *of A. comosus peel*

Concentration (µg/mL)	Absorbance at 544 nm
20	0.098
40	0.202
60	0.297
80	0.434
100	0.568
A. comosus peel	0.561



Graph 1: Standard curve of Diosgenin

Table 3: Estimation of saponins in aqueous extract of *A. comosus* peel

Sample	Saponins quantity (µg/mL)
A. comosus peel	100.47

Quantitative estimation of phenols

Gallic acid was used as standard to determine phenol content in the aqueous extract of *A. comosus* peel. Using the standard curve equation y = 0.3155x - 0.2623 the phenol concentration was calculated as 174.27 µg/mL, indicating its significant presence in the sample.

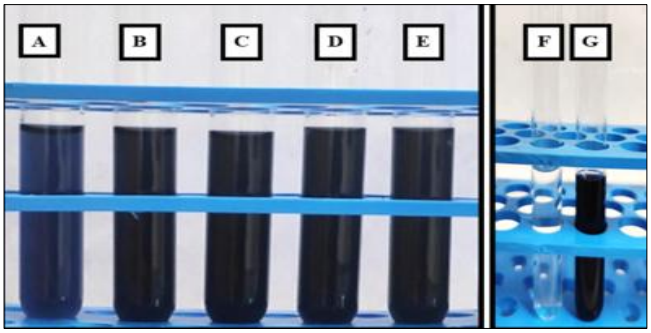


Fig 3: Quantitative estimation of phenols with (A - E) Gallic acid at different concentrations (10,40,50,75,100 µg/mL), F - blank, and G-aqueous extract of *A. comosus* peel

Table 4: Absorbance value of Gallic acid at different concentrations (10, 40, 50, 75,100 µg/mL) and aqueous extract of *A. comosus peel.*

Concentration (µg/mL)	Absorbance at 720nm
10	0.016
40	0.393
50	0.692
75	1.060
100	1.260
A. comosus Peel	2.400

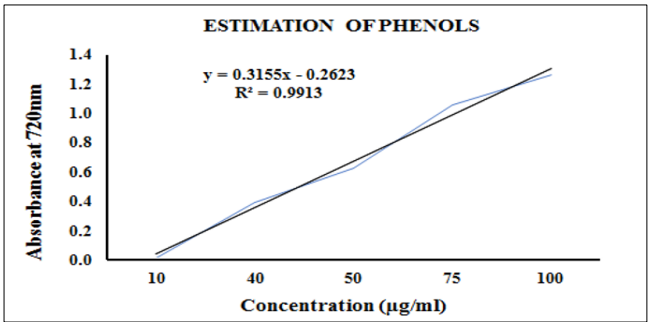


Table 5: Estimation of phenols in aqueous extract of *A. comosus* peel

Sample	Phenol quantity
A. comosus peel	$174.27 \mu\text{g/ml}$

Quantitative estimation of flavonoids

Quercetin was used as standard to determine flavonoid content in the aqueous extract of *A. comosus* peel. Using the standard curve equation y=0.45x+0.226, the flavonoid concentration was calculated as 0.068 µg/mL, indicating its presence in the sample.

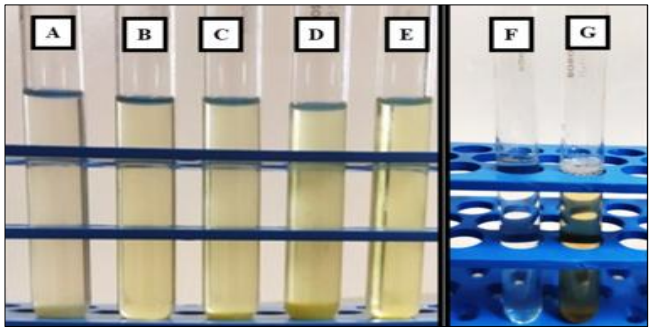
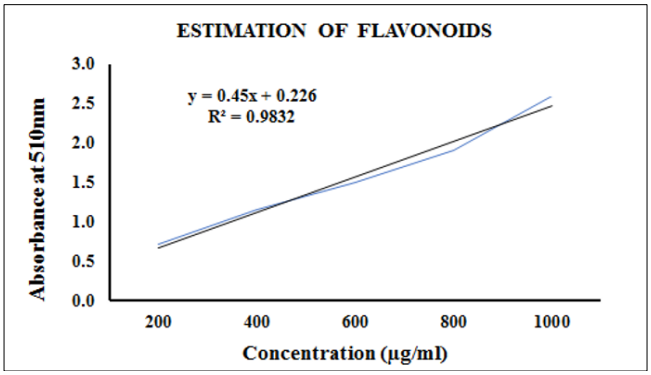


Fig 4: Quantitative estimation of flavonoids- (A - E) Quercetin at different concentrations (200, 400, 600, 800, and 1000 µg/mL), (F)Blank, (G) *A. comosus* peel aqueous extract.

Table 6: Absorbance value of Quercetin at different concentrations (200, 400, 600, 800, 1000µg/mL) and aqueous extract of *A. comosus peel*.

Concentration (µg/mL)	Absorbance at 510nm
200	0.718
400	1.158
600	1.501
800	1.912
1000	2.591
***A. comosus* Peel**	**0.257**



Graph 3: Standard curve of Quercetin

Table 7: Estimation of flavonoids in aqueous extract of *A. comosus peel.*

Sample	Flavonoid quantity
A. comosus peel	0.068 μg/mL

Quantitative estimation of alkaloids

Quercetin was used as standard to determine alkaloid content in the aqueous extract of *A. comosus* peel, calculated using the standard curve equation y = 0.01x + 0.0266. The alkaloid concentration was found to be 2.54 mg/mL, indicating its presence in the sample.

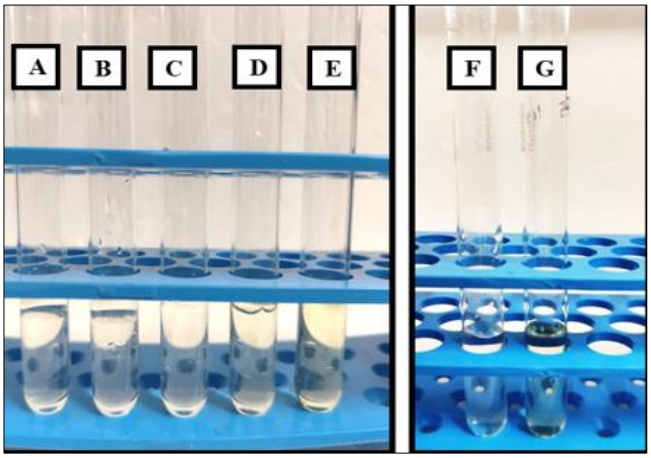
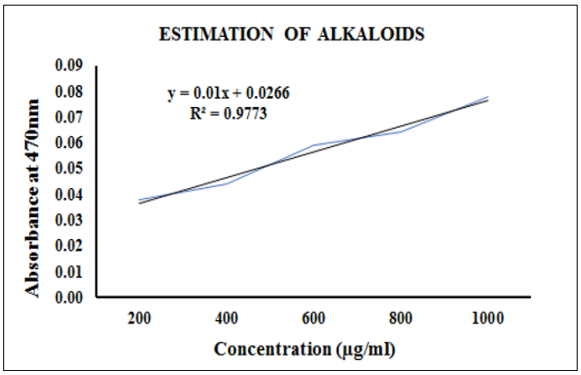


Fig 5: Quantitative estimation of alkaloids (A - E) Atropine at different concentrations (200-1000 µg/mL), (F) Blank, (G) *A. comosus* peel aqueous extract.

Table 8: Absorbance value of atropine at different concentrations (200-1000 µg/mL) andaqueous extract of *A. comosus peel.*

Concentration (mg/mL)	Absorbance at 470nm
200	0.038
400	0.044
600	0.059
800	0.064
1000	0.078
A. comosus Peel	0.052



Graph 4: Standard curve of Quercetin

Table 9: Estimation of alkaloids in aqueous extract of *A. comosus peel*

Sample	Alkaloid quantity
A. comosus peel	2.54 mg/mL

Anti-oxidant activity

The Ferric reducing anti-oxidant Power (frap) assay was performed to assess the anti-oxidant activity of the aqueous extract of *A. comosus* peel. Results showed that absorbance increased with concentration, indicating greater anti-oxidant activity at higher concentrations. Thus 500µg/ml concentration exhibited the highest absorbance as 1.087, while 50µg/ml concentration showed the lowest absorbance as 0.752.

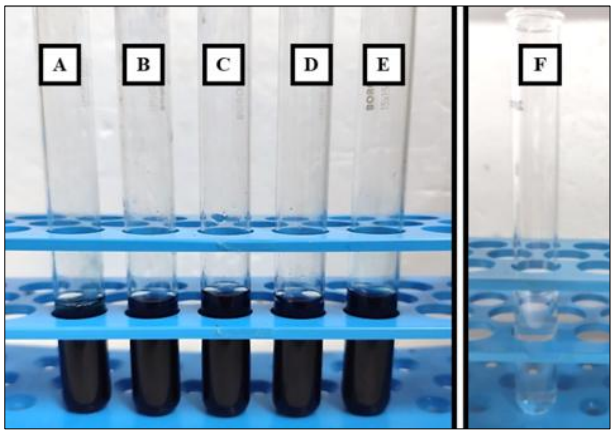


Fig 6: FRAP Assay - Ascorbic acid at different concentrations (50, 75, 100, 250, and 500 µg/mL) (A - E), with blank (F).

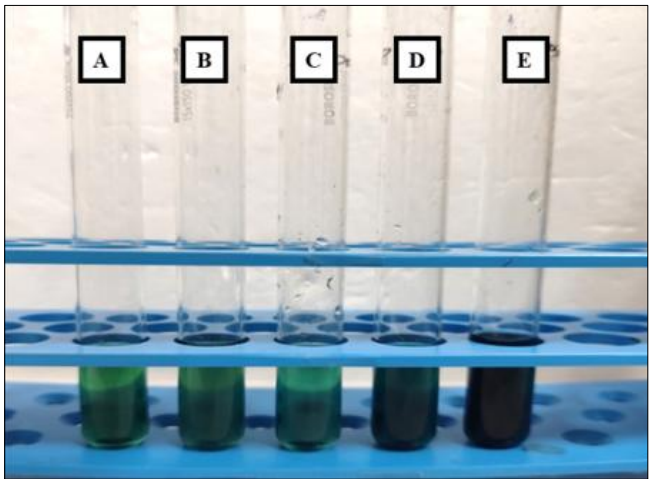
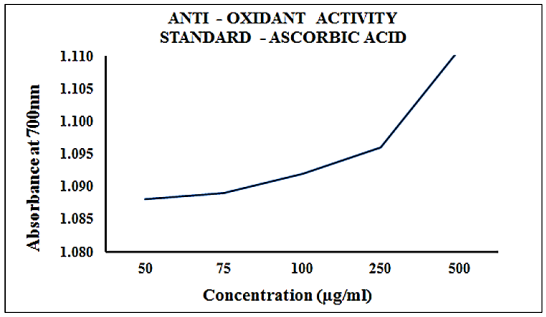


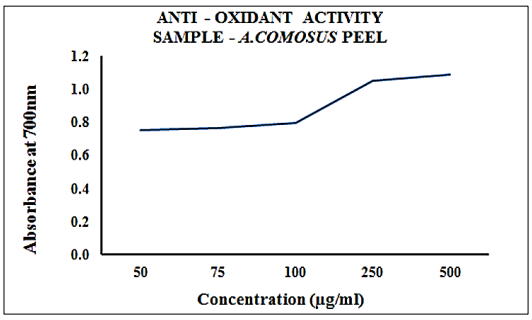
Fig 7: FRAP Assay (A - E) Aqueous extract of *A. comosus* peel at different concentrations (50, 75, 100, 250, and 500µg/mL)

Table 10: Absorbance of Ascorbic acid, sample - aqueous extract of *A. comosus* peel

Concentrations (µg/mL)	Ascorbic acid	A. comosus peel
50	1.088	0.752
75	1.089	0.768
100	1.092	0.796
250	1.096	1.049
500	1.111	1.087



Graph 5: Absorbance of standard - Ascorbic acid



Graph 6: Absorbance of *A. comosus* peel

Anti-inflammatory activity

The anti-inflammatory property was evaluated using protein denaturation assay, with diclofenac as standard. Diclofenac and *A. comosus* peel extract was tested at concentrations of 50, 75, 100, 250, and 500µg/mL. Results indicated higher inhibition at greater concentrations, with 19.85% inhibition at 50 µg/mL and 38.38% at 500 µg/mL.

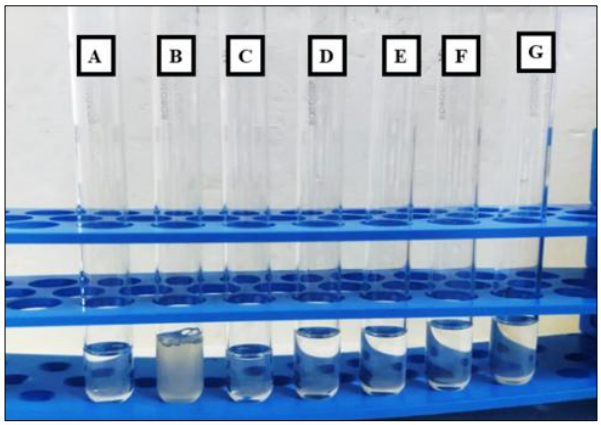


Fig 8: Protein denaturation assay with (A) - blank, (B) – control and (C-G) - diclofenac at different concentrations (50, 75, 100, 250 and 500 µg/mL).

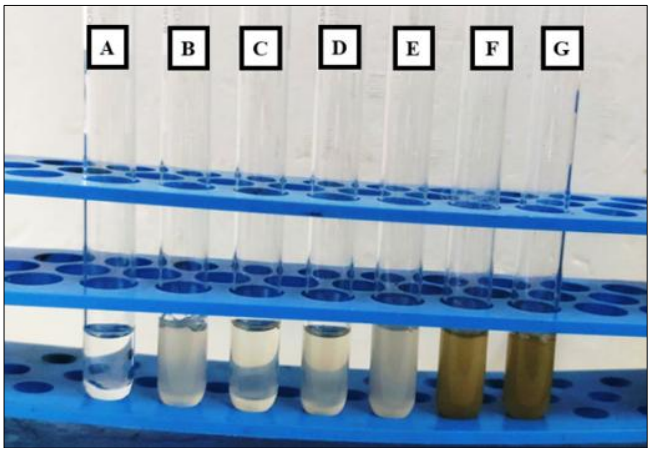
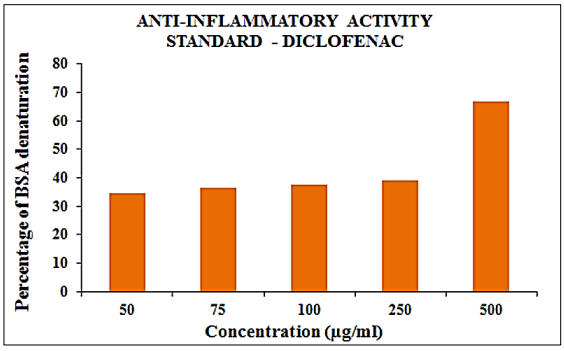


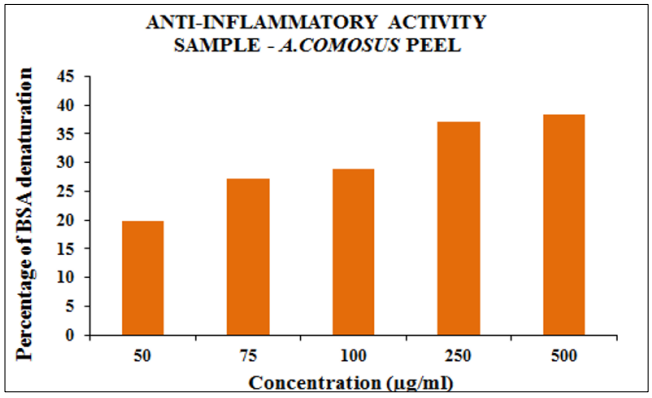
Fig 9: Protein denaturation assay with (A) -blank, (B) -control and (C-G) -aqueous *A.comosus*peel extract at different concentrations (50, 75, 100, 250, and 500 µg/mL).

Table 11: Absorbance at 700 nm and % inhibition of BSA denaturation by Diclofenac (standard) and aqueous extract of *A. comosus* peel at various concentrations (50–500 µg/mL). The absorbance value of control at 660 nm is 1.657

Concentration (µg/mL)	Antioxidant (Absorbance at 700nm)	Anti-inflammatory (% Inhibition of Denaturation)
50	0.752	19.85%
75	0.768	27.21%
100	0.796	28.90%
250	1.049	37.17%
500	1.087	38.38%



Graph 7: Anti- inflammatory activity of Diclofenac



Graph 8: Anti- inflammatory activity of aqueous extract of *A. comosus* peel

Anti- urolithiatic activity

Potassium citrate and cystone were used as positive control and standard demonstrating excellent anti-urolithiatic activity with the highest potential for dissolving calcium oxalate. The aqueous extract of *A.comosus* peel, though slightly less potent than the standard, showed significant activity in preventing or reducing calcium oxalate formation. This underscores the anti-urolithiatic potential of aqueous extract of *A. comosus* peel, highlighting its ability to inhibit kidney stone formation.

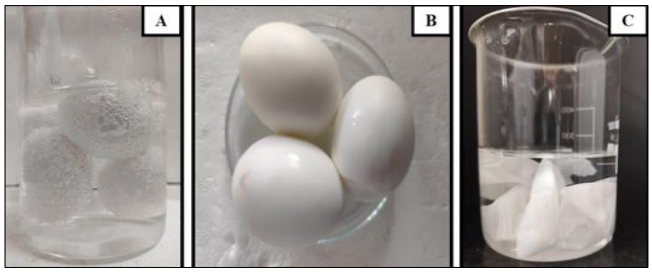


Fig 10: (A) Eggshell decalcification, (B) Decalcified eggs, (C) Egg membrane after decalcification.

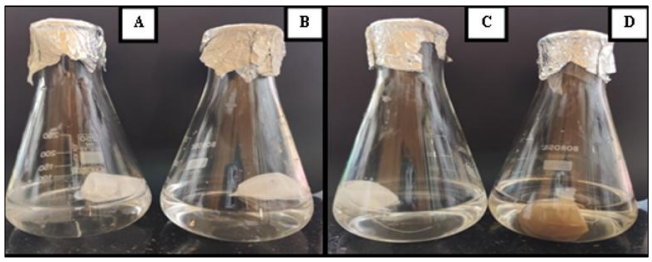


Fig 11: Egg membrane with contents suspended in Tris buffer. (A) Blank with CaC_2O_4, (B) Potassium citrate with CaC_2O_4, (C) Cystone with CaC_2O_4, and (D) Aqueous extract of *A. comosus* peel.

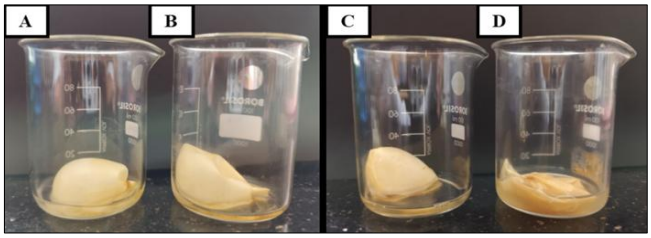


Fig 12: Membrane contents after titration with 0.9494 N $KMnO_4$, showing the light pink endpoint.

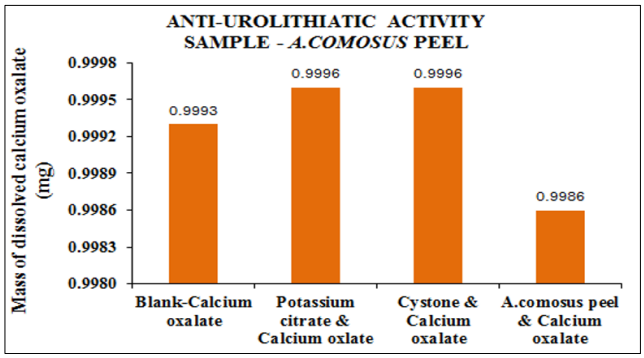
Calculation

Blank

Initial mass of CaC_2O_2 (Winitial)	= 1 mg
Volume of $KMnO_4$ taken	= 55mL
Volume of $KMnO_4$ used	= 1.0 mL
Normality of $KMnO_4$	= 0.9494N
Molar mass of $CaC2O_2$	= 146.12g/mol
Moles of $KMnO_4$	= (Normality of $KMnO_4$ × Volume of $KMnO_4$ used) ÷ 1000
	= (0.9494 × 1.0) ÷ 1000
	= 0.00094 mol
Moles of $CaC2O_2$	= 5 × moles of $KMnO_4$
	= 5 × 0.00094
	= 0.0047 mol
Mass of undissolved CaC_2O_4 (Weight of undissolved)	= Moles of CaC_2O_4 × Molar mass of CaC_2O_4 ÷ 1000
	= 0.0047 × 146.12 ÷ 1000
	= 0.00068 mg
Mass of dissolved (Weight of dissolved)	= Winitial - Wundissolved
	= 1- 0.00068
	= 0.9993 mg

Table 12: Anti-urolithiatic activity of blank, positive control (potassium citrate and cystone) and sample (aqueous extract of *A. comosus* peel)

Biological Assay	Result for A. comosus Peel	Standard Control Result
Antioxidant (Absorbance at 700nm)	1.087	1.111 (Ascorbic Acid)
Anti-diabetic (% Inhibition)	53.03%	53.55% (Acarbose)
Anti-inflammatory (% Inhibition)	38.38%	66.81% (Diclofenac)
Antacid Capacity (ANC per gram)	17.3 (at 500 mg/mL)	14.7 (Standard 500)
Antimicrobial (Zone of Inhibition)	0 mm (against *S. aureus* & *A. niger*)	25mm / 18mm (Gentamycin)



Graph 9: Anti-urolithiatic activity comparison of blank (CaC_2O_4), potassium citrate with CaC_2O_4, Cystone with CaC_2O_4, and aqueous extract of *A. comosus* peel with CaC_2O_4

Anti-diabetic activity

The anti-diabetic activity of *A. comosus* peel extract was assessed using α-amylase inhibition, with acarbose as standard. Both showed increased inhibition with rising concentrations, with the extract exhibiting higher inhibition at higher doses.

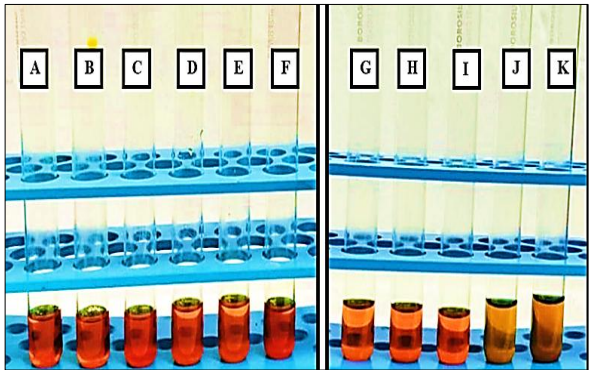
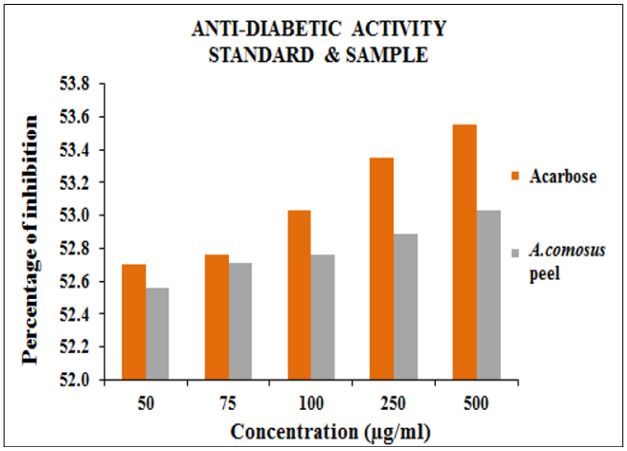


Fig 13: Anti-diabetic activity of *A. comosus* peel. (A) - Control, (B -F) - Acarbose at different concentrations (50, 75, 100, 250, and 500 µg/mL), and (G -K) - *A. comosus* peel extract at same concentrations.

Table 13: Absorbance and percentage inhibition of acarbose and *A. comosus* peel extract against α-amylase enzyme (Control – 1.518)

Conc. (µg/mL)	Antioxidant (Abs. 700nm)	Anti-inflammatory (% Inhibition)	Anti-diabetic (% Inhibition)
-	*A. comosus*	*A. comosus*	*A. comosus*
50	0.752	19.85%	52.56%
75	0.768	27.21%	52.71%
100	0.796	28.90%	52.76%
250	1.049	37.17%	52.89%
500	1.087	38.38%	53.03%



Graph 10: Percentage inhibition of standard and sample

Anti-microbial activity

Anti-microbial activity of aqueous extract of *A. comosus* peel was determined using agar well diffusion method. The results indicated that the extract exhibited no anti-microbial activity against *S. aureus*and *A. niger* when compared to positive control.

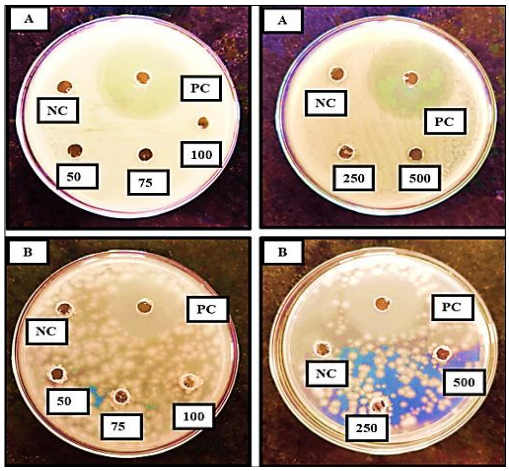


Fig 14: Anti-microbial activity of aqueous extract of *A. Comosus* peel against (A) *S. aureus*, (B) *A.niger*, PC- positive control, NC- Negative control

Table 14: Zone of inhibition of aqueous extract of *A. Comosus* peel against *S. aureus*, (B) *A. niger*

Category	Parameter / Assay	Concentration	Results (Extract)	Standard Control
Phyto-chemical	Alkaloid Quantity	-	2.54 mg/mL	-
Antioxidant	Reducing Power (Absorbance at 700nm)	500 μg/mL	1.087	1.111 (Ascorbic acid)
Anti-diabetic	α-amylase Inhibition	500 μg/mL	53.03%	53.55% (Acarbose)
Anti-inflammatory	Protein Denaturation Inhibition	500 μg/mL	38.38%	66.81% (Diclofenac)
Antimicrobial	Zone of Inhibition (*S. aureus* & *A. niger*)	50-500 μg/mL	0 mm	25mm / 18mm (Gentamycin)

Anti-ulcer activity

Anti-ulcer activity of aqueous peel extract of *A. comosus* was evaluated using acid neutralizing capacity method. The result showed significant anti-ulcer property of the sample. The anti-ulcer activity of the extract decreases steadily with increase in its concentration. This indicates the dose depending acid neutralizing potency of *A. comosus* peel extract.

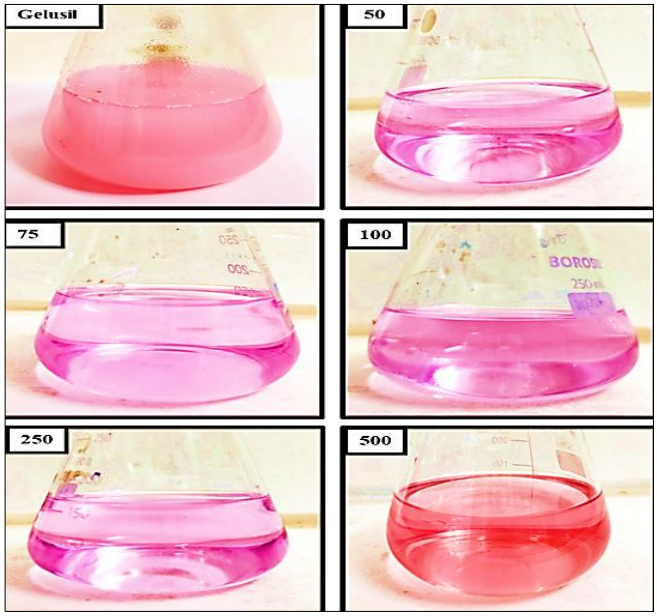
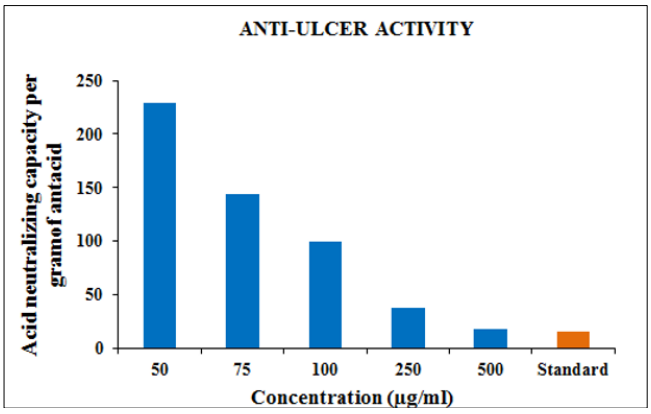


Fig 15: Acid neutralizing capacity of different concentrations (50,75,100,250,500 µg/mL) of Standard (Gelusil) and aqueous extract of *A. comosus* peel

Table 15: Acid Neutralizing Capacity (ANC) of Antacid at different concentrations

Assay Type	Parameter	Concentration	Extract Result	Standard Control
Phytochemical	Alkaloid Quantity	-	2.54 mg/mL	-
Antioxidant	Absorbance (700nm)	500 μg/mL	1.087	1.111 (Ascorbic acid)
Anti-diabetic	% Inhibition	500 μg/mL	53.03%	53.55% (Acarbose)
Anti-inflammatory	% Inhibition	500 μg/mL	38.38%	66.81% (Diclofenac)
Antimicrobial	Zone of Inhibition	50-500 μg/mL	0 mm	25mm / 18mm (Gentamycin)



Graph 11: Acid neutralizing capacity of the sample

Discussion

The present investigation focuses on the exploration of phytochemical compounds, therapeutic potential, and applications of bioactive compounds derived from the peel of *A. comosus* (pineapple), an underutilized by-product of the fruit processing industry that represents a sustainable source of health-promoting constituents. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins, and tannins, all of which are known for their significant pharmacological relevance. Quantitative estimations confirmed appreciable concentrations of these secondary metabolites, with phenols and flavonoids contributing substantially to the extract's anti-oxidant potential. The antioxidant activity, evaluated through the Ferric Reducing Antioxidant Power (FRAP) assay, demonstrated a concentration-dependent increase in reducing power, indicating the extract's strong capacity to donate electrons and neutralize free radicals; these results are consistent with earlier findings by (Saraswaty *et al*.,2017) [12] linking phenolic content to anti-oxidant efficacy. Anti-inflammatory potential was assessed using the protein denaturation method, where the extract exhibited marked inhibition of heat-induced protein denaturation, suggesting its capacity to modulate inflammatory processes through the stabilization of proteins and prevention of denaturation, in line with studies by (Putri *et al*.,2018) [13] that associate phenolic and flavonoid compounds with anti-inflammatory action. The anti-urolithiatic activity was evaluated by assessing the inhibition of calcium oxalate crystal nucleation and aggregation, revealing significant suppression of crystal growth, although slightly lower than that observed for standard agents such as potassium citrate and Cystone; this finding corroborates (Rahim *et al*., 2021) [14], who reported similar inhibitory effects from plant-derived phenolics on lithiasis formation. The α-amylase inhibitory assay demonstrated that the peel extract could retard starch

breakdown, indicating its potential role in postprandial blood glucose regulation and supporting its anti-diabetic promise, a result consistent with the observations of (Pendong*et al*., 2024) [15] on bromelain-rich pineapple extracts. Anti-microbial studies revealed that the aqueous extract of *A. comosus* peel exhibited no inhibitory effect against *S. aureus* and *A. niger* when compared to positive control, consistent with the observations of (Zharfan *et al*., 2017) [16] and (Syahidah *et al*.,2019) [17], who attributed such outcomes to the synergistic interactions among phenolics, flavonoids, and bromelain. The extract's anti-ulcer potential, evidenced by its ability to reduce gastric lesion formation in experimental models, is likely attributed to its anti-oxidant and anti-inflammatory activities. This aligns with the findings of Ajiboye and Oluwole (2012) [6] and Maurer (2001) [8], who reported the gastroprotective effects of pineapple-derived bromelain and phenolic compounds. Collectively, these findings emphasize that *A. comosus* peel, often discarded as waste, serves as a valuable, low-cost, and sustainable source of bioactive compounds with multifaceted therapeutic applications in nutraceutical, pharmaceutical, and functional food formulations. By valorising this agricultural residue, the study not only supports environmental sustainability and waste management but also opens avenues for the development of natural product-based interventions for oxidative stress, inflammation, microbial infections, urinary calculi, metabolic disorders, and gastrointestinal ailments, thereby contributing to the advancement of green chemistry, circular economy practices and integrative medicine.

Conclusion

The present study explores the therapeutic potential of *A. comosus* peel extract and its phytochemical compounds. Qualitative analysis confirms the presence of bioactive compounds such as phenols, flavonoids, saponins, and alkaloids. The extract exhibited significant anti-oxidant, anti-inflammatory, anti-diabetic, anti-microbial, anti-ulcer, and anti-urolithiatic activities, demonstrating its potential in managing various health conditions. Notably, its ability to inhibit calcium oxalate formation suggests a role in kidney stone prevention, while its acid-neutralizing capacity supports its use as a natural anti-ulcer agent. These findings provide strong *in-vitro* evidence of its therapeutic efficacy. Further *in-vivo* studies and clinical trials are required to fully understand its mechanisms, and to enhance its formulation, explore its applications in modern medicine.

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