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Assessment of antimicrobial potential of polyphenol-rich *Ananas comosus* peel powder at different drying conditions

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The main objective of present study is to focus on recycling of pineapple waste by estimating its antimicrobial potential against selected pathogenic strains. Four drying techniques, hot air oven (S1), microwave (S2), sun (S3), and freeze (S4), were applied to dry the peels. Methanol, acetone, and water, were used as solvent for extract preparation. Then these extracts were evaluated for antimicrobial activity against 3 fungal, 6 Gram-negative and 2 Gram-positive strains through agar well diffusion and broth dilution methods. Further, HPLC and TLC bioautography method was applied to reveal phytochemicals responsible for antimicrobial activity. Methanolic extract of S1 sample has shown the best zone of inhibition (23±0.014 mm) against *Pseudomonas fluorescens* strain. In gram-positive strains maximum zone (25±0.014 mm) was noted against *Staphylococcus aureus* and in fungal strains, maximum zone (22 mm) was observed against *Fusarium sp.* and *Tilletia indica* with lowest MIC (12.24 mg/mL) and LD_{50} value were calculated against *Bacillus megaterium*. Quantitative analysis through HPLC shows highest concentration of Bromelain in pineapple peel, along with the presence of ellagic acid, ferulic acid, catechin, caffeic acid, and gallic acid. Results of this study show the highest antimicrobial potential of pineapple peel for use in the pharmaceutical or neutraceutical industry.

Keywords: *Ananas comosus*, Antibacterial, Antifungal, Neutraceuticals, Peel, Phytochemicals

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Introduction

India has diverse varieties of agricultural production, such as vegetables, fruits, ornamental plants, spices, aromatic plants, roots, tubers, medicinal herbs, and plantation crops. Hence, the large amount of agricultural biomass residues is widely produced through the agriculture sector[1]. The food processing industries are highly focused on processing the bio waste into valuable products like fuels, pharmaceuticals, and chemicals. According to the World Health Organization (WHO) report, 65% of Indians preferred it as a vital health-promoting medicinal plant. However, 40% of medicinal recipes are prepared from these plants. As per the reports, 85% of the medicinal components are obtained from the aerial region of the medicinal plants[2].

Ananas comosus (*A. comosus*) commonly known as pineapple[3], is the herbaceous perennial plants i.e., monocotyledonous which belongs to the family *Bromeliaceae*, which is named after one of the enzymes called bromelain. *A. comosus* is a short plant with extension of around 90-120 cm, and height of 75-150 cm with certain spiny leaves, fibrous parts including corpulent stump[3]. Around 75-85% of this fruit generates waste in the form of crown, core, and peel, whereas 30-35% waste contributes to the peel part alone[4]. *A. comosus* is grown in the tropical and subtropical regions and is the most essential marketable fruit globally. Also, it is utilized in preserved edible form worldwide and consumed in the form of jams, juices, and other essential concentrates. India is regarded as the world's fourth-largest producer of *A. comosus* fruit. Around 9% of the total fresh pineapple production is reported by India[5].

Currently, India is the second-highest producer of fruits globally. In contrast, the major leading producers of the *A. comosus* are Costa Rica, China, Brazil, Thailand, Philippines, and India, with a total cultivation area of around 909.84 thousand ha and 19412.91 thousand tons production per annum around the world. Moreover, in India, the 7% share of the total production rate of around 1,415.00 thousand tons, which were harvested in an 89 thousand ha area[6-8]. The leading pineapple producers in India are Kerala, Karnataka, West Bengal, Goa, and

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Maharashtra. On the other hand, in Bihar, Uttar Pradesh, Gujarat, Tamil Nadu, Orissa, and Andhra Pradesh states, it is grown on a small scale only. It is grown in hilly, northeastern, and coastal parts of the Indian peninsula because pineapples need a lot of rainfall[9].

As per the research, it is demonstrated that approx 75-85% of the production is discarded in the form of biowaste, such as peel, crown, and core, despite the fact that 30-35% of the waste belongs to the peel alone[10]. Studies have demonstrated that the demand for antimicrobial agents has increased due to the need to preserve foods. However, the antimicrobial agents had a tendency to inhibit the growth of the microbes. Researchers have focused on exploring the multiple antimicrobial agents from plants and their respective byproducts, especially for the treatment of diseases, preservation of foods, and other food safety. Thus, due to the presence of essential secondary metabolites, *A. comosus* peel exhibits inhibitory action against the pathogenic micro-organisms such as *Salmonella* species, *Escherichia coli*, *Shigella* species, *Enterococcus*, *Clostridium botulinum*, *Vibrio* species, and *Klebsiella pneumonia*[11]. A study showed that the gram-positive and gram-negative bacterial strains, including *Vibrio cholerae* (MCV09), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 29213), were effectively inhibited by the ethyl acetate extract of pineapple peel[12].

In addition, the presence of flavonoids, saponins, polyphenols, and other essential secondary metabolites present in the extract of the peel of *A. comosus*[13-15] has a vital antimicrobial action against the Gram-positive bacteria. The bromelain and saponin content act on the membrane of the bacteria. Moreover, the bromelain is a proteolytic enzyme that degrades the protein present in bacteria. Saponins enhance permeability, altering the structure and function by disrupting the cell wall, which further allows the anti-microbial agents to enter the cell and disrupt the cellular metabolism, resulting in cell lysis. Furthermore, the polyphenols help inhibit the bacterial DNA gyrase by attaching to the ATP binding site[16]. The Gallic acid has both anti-fungal and anti-bacterial activity. Moreover, the tannin content shows antimicrobial action by deprivation ofthe iron and hydrogen binding, mainly the interaction of vital proteins[17].

The study examined the antibacterial action of the peel extract, which showed high inhibitory activity for *P. aeruginosa*, *Salmonella typhi*, and *S. aureus*. However, the data showed that the peel extract of *A. comosus* exhibits antimicrobial potential that is vital in developing pharmacological therapy against pathogenic bacteria[18]. Thus, the present study is focused on exploring the antimicrobial potential of pineapple peel.

Materials and Methods

Chemicals and Apparatus

Methanol, ethanol, acetone, dimethyl sulphoxide (DMSO), petroleum ether, ethyl acetate, formic acid, sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), sulfuric acid (H_2SO_4), chloroform, ammonium, acetic acid, n-hexane, acetonitrile (CAN), nutrient broth (NB), nutrient agar (NA), UV-Visible spectroscopy and, Shimadzu LC-20 HPLC system.All the chemicals and reagents used in this study were of analytical grade.

Sample preparation

The outer peel of *A. comosus* fruit was collected from local vendors of the urban Prayagraj area. Then, it was washed with distilled water to remove dust and dirt particles, and divided into four parts to be left for drying in four different drying conditions. First part was dried in hot air oven at 70°C for 72h (S1); second part was kept for microwave drying for 10-15 minutes (S2); third part kept for sun drying until complete moisture was removed (S3), and; the fourth last part was lyophilized in lyophilizer at -20°C ±2°C (S4).

Extract preparation

Dried peel samples were soaked overnight in three different solvents, namely methanol, acetone, and aqueous, in a 1:10 ratio (sample: solvent). Then, it was filtered using Whatman No. 1 filter paper, and the collected filtrate was left to evaporate in a hot water bath to get a crude extract. This crude extract was collected in a glass bottle and stored at 4°C till further use. Dimethyl Sulphoxide (DMSO) was used to make extracts from various concentrations.

Microorganisms

Tilletia indica, *Fusarium* sp., *Alternaria* sp., *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas fluorescens* (ATCC 13525), *Escherichia coli* (ATCC 8739), *Enterobacter aerogenes* (ATCC 13048), *Shigella flexneri* (ATCC 12022), *Salmonella enteric* (ATCC 35664), *Staphylococcus aureus* (ATCC 29213), and *Bacillus*

megaterium (ATCC 14581) were obtained from our lab stock.

Phytochemical screening test

The crude aqueous, ethanol, methanol, ether, chloroform, and acetone extracts of *A. comosus* peels were subjected to phytochemical screening tests to reveal the presence and absence of major secondary metabolites extracted in the particular solvent by using standard methods as given by Shakyawar *et al.*[19]. In the result section, these qualitative results are shown as [+] for the presence and [-] for the absence of phytochemicals.

Antimicrobial activity

The agar disc diffusion method was used to confirm the antimicrobial activity of pineapple peel extract against the eleven above-mentioned pathogenic strains according to the method given by Kumar *et al.*[20], with some modifications. In short, previously prepared crude extract was dissolved in 100% DMSO at a concentration of 25, 50, and 100 mg/mL. Approximately 20 mL of sterile nutrient agar medium was transferred into sterile petri dishes and left to solidify. On the solidified agar plate, wells of 6 mm size were made by a sterile cork borer. Then, the peel extract of different concentrations was loaded into the wells and incubated at 37°C for 24 hours with suitable positive and negative controls. For antibacterial activity, ampicillin, amoxicillin, imipenem, and ceftriaxone, and for antifungal activity, nystatin was used as a positive control in 10 mcg and 0.5 mg/mL concentration. For negative control, 100% DMSO was used. Measuring the diameter of the zone of inhibition in mm showed antibacterial and antifungal activity of pineapple peel extracts. All the assays were performed in triplicate.

Relative percentage (%) zone of inhibition

Relative percentage zone of inhibition of three different solvent extracts of pineapple peel against three fungal strains and eight bacterial strains was calculated by the formula given below:

$$\% \text{ Relative zone of inhibition (in mm)} = \frac{\text{Zone of inhibition of sample (Peel extract)} - \text{Zone of inhibition of negative control}}{\text{Zone of inhibition of positive control (Antibiotic drug)}} \times 100$$

Minimum inhibitory concentration (MIC)

The standard broth dilution method was applied for evaluating the antimicrobial efficacy of *A. comosus* peel by obtaining the optical density of agar broth with culture and samples of different concentrations and without samples. Serial two-fold dilutions of pineapple peel extract in concentrations ranging from 2 to 10 mg/mL with adjusted bacterial concentration (10^8 CFU/mL) were used to determine MIC in agar broth. Whereas, control contained only inoculated broth, then incubated for 24h at 37°C. The endpoint of MIC is the lowest concentration of extract, where the non-significant optical density is seen in comparison to the control optical density.

High-performance liquid chromatography (HPLC)

HPLC analysis was performed on a Shimadzu LC-20AT HPLC system, operated with a pump, UV-vis detector, injector, and C-18 column (250×4.6 mm). 80% Methanolic extract of S1, S2, S3, and S4 extracts of *A. comosus* peel in a 1:10 ratio (peel powder: solvent) and 6 reference compounds (100, 75, and 50 ppm) were filtered with a 0.45 µm micro filter, and 20 µL was injected into the system through the injector. Further, the mobile phase used for the analysis was 50% v/v water: 50% v/v acetonitrile: 0.5% v/v formic acid, and the flow rate was kept at 1mL/min at an ambient temperature (37°C) and monitored at 280 nm. Sample peaks were identified and quantified using LC lab solution software[21] by comparing the retention times of standard.

Determination of active ingredient using TLC plate

Five µL of 100 mg/mL concentration of S1 methanolic and S3 acetone extracts were spotted on a silica gel 60 F254 plate in a narrow band and eluted using different mobile phases. The mobile phase was optimized for developing the plates using 20 different combinations of mobile phases. As the mobile phase reached three-fourths, the plates were dried, and the developed plates were visualized under UV irradiation of 254 nm to detect the separation of the compounds and observe the Rf value[22]. Rf value was calculated by using the following equation:

$$R_f = \frac{Distance\ Travelled\ by\ Sample}{Distance\ Travelled\ by\ Solvent}$$

Bioautography

For bioautography, developed TLC plates were dried under air for 24 hours to remove the traces of mobile phase on the plates. An overnight-grown bacterial culture of *S. aureus* in nutrient broth was used, and a density of approximately 2×10^8 density was used. After that, the prepared TLC plates were sprayed with bacterial suspensions until wet in a

Laminar flow cabinet. These plates were incubated at 37°C overnight in an incubator, then sprayed with a 2 mg/mL solution of MTT and again incubated overnight. The colourless inhibition zones were visually determined[23-24].

Statistical evaluation

To estimate the accuracy of the experimental data, each experiment was performed in triplicate, and the result was expressed as the mean ± standard deviation of three replications. All the statistical analysis was done using MS Excel 2007 and Origin software, version 8.5, at 5% significance level (P <0.05).

Results and Discussion

Phytochemical screening

Results of phytochemical screening test in aqueous, methanolic, ethanolic, ether, chloroform, and acetone extracts of pineapple peel revealed the presence of cardiac glycosides, terpenoids[25], coumarins, phenols, steroids, betacyanins, tannin, saponins, quinonnes, flavonoids, and alkaloids[26-28], in all the solvents except ether and chloroform. In contrast, glycosides were absent in all the extracts. Higher concentrations of flavonoids, terpenoids, steroids, betacyanin, and quinones were observed in ethanolic, methanolic, aqueous, and acetone extracts[29]. Thus, methanol, acetone, and aqueous extracts were finalized for further antimicrobial tests. Table 1 below summarizes the presence and absence of phytoconstituents in different extracts.

Antifungal activity

In-vitro antifungal activity against selected pathogenic fungal strains revealed the efficiency of methanolic, acetone, and aqueous extracts of *A. comosus* peel in acting as an antifungal agent. Results of the antifungal test show that the highest zone of inhibition noted for *T. indica* was 22 mm for S1 acetone extract at a concentration of 100 mg/mL. In comparison, for *Fusarium* sp., it was 22 mm of S3 methanolic extract of 100 mg/mL concentration, and for *Alternaria* sp., it was 16 mm of S1 methanolic extract of 100 mg/mL concentration (Table 2). The analyzed results were compared with the positive control to estimate its relative percentage of zone of inhibition (Table 3). It was observed that as the concentration increased, the antifungal activity also increased against the selected strains, which indicates that the antifungal activity is concentration-dependent. The standard drug Nystatin was taken as a positive control, and a 25 to 30 mm zone was observed for different fungal strains. On the other hand, DMSO was taken as a negative control.

Antibacterial activity

To estimate the antibacterial potential of *A. comosus* peel extract, a zone of inhibition test was conducted against six selected gram-positive and two gram-negative pathogenic bacterial strains. Results of the study revealed that in the group of gram-negative strains, the highest zone of inhibition was observed against *P. fluorescens*, which was 23 mm for S1 methanolic extract with a concentration of 100 mg/mL. *P. aeruginosa* strain showed approximately negligible inhibition zone for all the extracts (Table 4). The analyzed results of gram-positive and gram-negative strains were compared with the positive control to estimate their relative

Table 1 — Qualitative phytochemical analysis of *A. comosus* peel extracts

Phyto-constituents	Test name	Response					
		Aqueous	Ethanol	Methanol	Ether	Chloroform	Acetone
Glycosides	Keller-Killiani test	-	-	-	-	-	-
Cardiac glycosides	Keller-Killiani test	+	+	+	-	-	+
Terpenoids	Chloroform test	++	++	++	-	-	+
Coumarins	Ferric chloride test	+	-	+	-	-	-
Phenols	Ferric chloride test	+	-	+	-	-	-
Steroids	Ferric chloride test	++	+	++	-	-	++
Betacyanin	Alkaline reagent test	+	-	++	-	-	+
Tannin	Ferric chloride test	+	-	+	-	-	+
Saponin	Foam test	++	+	+	-	-	+
Quinones	Ferric chloride test	++	++	++	-	-	++
Flavonoids	Alkaline reagent test	++	+	++	-	-	+
Alkaloids	Mayer's test	+	+	-	-	-	-

++ = shows higher concentration.

+ = indicates presence of phytochemicals and - = indicates absence of phytochemicals

Table 2 — Antifungal activity of *A. comosus* peels extract against *Tilletia indica*, *Fusarium* sp. and *Alternaria* sp.

Sample	Concentration (mg/mL)	Zone of inhibition (in mm)								
		Tilletia indica			*Fusarium* sp.			*Alternaria* sp.		
		M.E	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E	A.E.	Aq.E
S1	25	13	16	13	12	13	11	13	13	13
	50	18	20	15	13	14	12	14	13	14
	100	20	22	19	17	16	15	16	16	15
S2	25	NV	11	NV	NV	13	NV	13	11	NV
	50	10	12	NV	NV	15	NV	14	12	NV
	100	11	15	NV	17	17	14	15	14	11
S3	25	12	13	14	NV	12	NV	NV	NV	NV
	50	13	15	17	13	12	9	NV	9	NV
	100	16	18	17	22	13	11	NV	10	10
S4	25	12	11	12	11	12	NV	11	10	NV
	50	12	12	12	20	11	NV	12	10	9
	100	16	13	14	20	18	NV	15	11	10
Nystatin	0.5		25			30			27	
DMSO		0	0	0	0	0	0	0	0	0

*NV: No visible zone of inhibition.

Table 3 — Relative % zone of inhibition of *A. comosus* peel extract against *Tilletia indica, Fusarium* sp. and *Alternaria* sp.

Sample	Concentration (mg/mL)	% Relative zone of inhibition								
		Tilletia indica			*Fusarium* sp.			*Alternaria* sp.		
		M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.
S1	25	52	64	52	40	43.3	36.6	48.1	48.1	48.1
	50	72	80	60	43.3	46.6	40	51.8	48.1	51.8
	100	80	88	76	56.6	53.3	50	59.2	59.2	55.5
S2	25	-	44	-	-	43.3	-	48.1	40.7	-
	50	40	48	-	-	50	-	51.8	44.4	-
	100	44	60	-	56.6	56.6	46.6	55.5	51.8	40.7
S3	25	48	52	56	-	40	-	-	-	-
	50	52	60	68	43.3	40	30	-	33.3	-
	100	64	72	68	73.3	43.3	36.6	-	37	37
S4	25	48	44	48	36.6	40	-	40.7	37	-
	50	48	48	48	66.6	36.6	-	44.4	37	33.3
	100	64	52	56	66.6	60	-	55.5	40.7	37

percentage of zone of inhibition (Tables 5 and 6). In the gram-positive group, a maximum zone of inhibition of 25 mm was observed for *S. aureus* ATCC 29213 in S1 acetone extract at 100 mg/mL concentration (Table 7). Fig. 1, given below, shows some of the results of the zone of inhibition against several pathogens at different levels of concentration. Similar results were reported by Lubaina *et al.*[12]; Jaisinghani and Patil[30]; Jatav*et al*[31]. The standard drugs ampicillin, ceftriaxone, imipenem, and amoxicillin were taken as positive controls, and 18 to 37 mm of zone was observed for different bacterial strains. On the other hand, DMSO was taken as a negative control.

Minimum inhibitory concentration (MIC)

For the broth dilution assay, 2 to 10 mg/mL concentrations of pineapple peel extracts were used to observe the lethal dose 50 (LD50) and MIC of the extracts,which shows promising results in the agar disc diffusion method. It looks like Gram-positive bacteria were more vulnerable to the S1 methanolic extract than the Gram-negative bacteria, represented by the lowest MIC of 3.06 mg/mL, and 50% of cell death was observed at 12.24 mg/mL, which was considered as LD_{50} (Table 8). Almost similar results were shown by Gopalraaj and Velayudhannair[32]. This vulnerability might be seen due to several bioactive compounds in phenolic and flavonoid forms, which change the permeability of cells, permitting the cells to enter bioactive compounds and leakage of some intracellular cell contents, resulting in cell death.

High-performance liquid chromatography (HPLC)

Based on the results obtained from the phytochemical screening test of *A. comosus* peel, it

Table 4 — Antibacterial activity of *A. comosus* peel extract against gram negative bacteria

Sample	Concentration (mg/mL)	Zone of inhibition (in mm) Gram negative bacteria																	
		Pseudomonas aeruginosa			*Pseudomonas fluorescens*			*Escherichia coli*			*Enterobacter aerogenes*			*Shigella flexneri*			*Salmonella enterica*		
		M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.
S1	25	NV	NV	NV	16	13	11	11	11	13	12	11	NV	14	12	14	9	12	13
	50	NV	NV	NV	18	16	12	12	12	15	14	12	NV	15	13	15	10	12	14
	100	NV	NV	NV	23	18	17	12	12	16	17	13	NV	17	13	16	11	14	15
S2	25	NV	NV	NV	11	11	10	14	12	12	NV	9	NV	14	11	12	NV	12	12
	50	NV	9	NV	13	13	13	16	12	14	NV	11	9	14	12	14	NV	13	13
	100	12	11	10	14	13	14	19	13	16	NV	12	12	17	13	16	NV	15	13
S3	25	NV	NV	NV	NV	NV	NV	13	12	14	NV	NV	NV	13	14	13	15	12	13
	50	NV	NV	NV	NV	NV	NV	15	12	15	NV	NV	12	13	14	14	17	13	13
	100	NV	NV	NV	11	NV	12	18	14	16	NV	NV	15	14	15	14	18	15	14
S4	25	NV	NV	NV	NV	NV	NV	13	NV	11	NV	NV	NV	12	11	12	13	12	12
	50	NV	NV	NV	NV	NV	NV	14	NV	12	NV	9	NV	12	11	12	14	12	13
	100	NV	NV	NV	NV	NV	NV	14	NV	13	NV	9	NV	13	12	14	15	13	14
Ampicillin	10 mcg	-	-	-	-	-	-	-	-	-	-	-	-		14		-	-	-
Ceftriaxone	10 mcg	-	-	-	-	-	-		37			22		-	-	-	-	-	-
Imipenem	10 mcg		34		-	-	-	-	-	-	-	-	-	-	-	-		28	
Amoxicillin	10 mcg	-	-	-		18		-	-	-	-	-	-	-	-	-	-	-	-
DMSO		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*NV: No visible zone of inhibition.

Table 5 — Relative % zone of inhibition of *A. comosus* peel extract for gram negative strains

Sample	Concentration (mg/mL)	Relative % zone of inhibition of Gram negative bacteria																	
		Pseudomonas aeruginosa			*Pseudomonas fluorescens*			*Escherichia coli*			*Enterobacter aerogenes*			*Shigella flexneri*			*Salmonella enterica*		
		M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.
S1	25	-	-	-	88.8	72.2	61.1	29.7	29.7	35.1	54.5	50	-	100	85.7	100	32.1	42.8	46.4
	50	-	-	-	100	88.8	66.6	32.4	32.4	40.5	63.6	54.5	-	107.1	92.8	107.1	35.7	42.8	50
	100	-	-	-	127.7	100	94.4	32.4	32.4	43.2	77..2	59	-	121.4	92.8	114.2	39.2	50	53.5
S2	25	-	-	-	61.1	61.1	55.5	37.8	32.4	32.4	-	40.9	-	100	78.5	85.7	-	42.8	42.8
	50	-	26.4	-	72.2	72.2	72.2	43.2	32.4	37.8	-	50	40.9	100	85.7	100	-	46.4	46.4
	100	35.2	32.3	29.4	77.7	72.2	77.7	51.3	35.1	43.2	-	54.5	54.5	121.4	92.8	114.2	-	53.5	46.4
S3	25	-	-	-	-	-	-	35.1	32.4	37.8	-	-	-	92.8	100	92.8	53.5	42.8	46.4
	50	-	-	-	-	-	-	40.5	32.4	40.5	-	-	54.5	92.8	100	100	60.7	46.4	46.4
	100	-	-	-	61.1	-	66.6	48.6	37.8	43.2	-	-	68.1	100	107.1	100	64.2	53.5	50
S4	25	-	-	-	-	-	-	35.1	-	29.7	-	-	-	85.7	78.5	85.7	46.4	42.8	42.8
	50	-	-	-	-	-	-	37.8	-	32.4	-	40.9	-	85.7	78.5	85.7	50	42.8	46.4
	100	-	-	-	-	-	-	37.8	-	35.1	-	40.9	-	92.8	85.7	100	53.5	46.4	50

was clear that the presence of polyphenolic compounds is the reason behind the potential antimicrobial activity against different pathogenic cultures. Polyphenolic acids and flavonoids were determined through the HPLC method to identify and quantify these polyphenolic compounds present in the pineapple peel, and the results are demonstrated here in Table 9. By comparing the retention time of standards and samples in chromatogram it was noted that hot air oven dried sample extract (S1), sun dried sample extract (S3) and freeze dried sample extract (S4) exhibited all the six tested phytochemicals in varied concentrations while microwave dried sample (S2) showed the presence of only four phytochemicals, it may be due to the heat labile property.

The highest concentration of all the compounds was noted in S1, followed by the S3 sample due to a low temperature for a slightly longer time.

Table 6 — Relative % zone of inhibition of *A. comosus* peel extract for gram positive strains

Sample	Concentration (mg/mL)	Relative % zone of inhibition of Gram positive bacteria					
		Staphylococcus aureus			*Bacillus megaterium*		
		M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.
S1	25	45.4	77.2	-	60	75	-
	50	59	86.3	45.4	70	80	60
	100	68.1	113.6	68.1	75	85	65
S2	25	54.5	50	-	-	55	65
	50	59	63.6	50	60	60	70
	100	63.6	72.7	68.1	80	65	75
S3	25	-	-	-	-	-	-
	50	54.5	40.9	-	65	-	-
	100	68.1	45.4	-	85	-	-
S4	25	54.5	-	-	55	-	-
	50	59	-	-	60	-	-
	100	63.6	50	50	65	-	-

Table 7 — Antibacterial activity of *A. comosus* peel extract against gram positive bacteria

Sample	Concentration (mg/mL)	Zone of inhibition (in mm) Gram positive bacteria					
		Staphylococcus aureus			*Bacillus megaterium*		
		M.E.	A.E.	Aq.E.	M.E.	A.E.	Aq.E.
S1	25	10	17	NV	12	15	NV
	50	13	19	10	14	16	12
	100	15	25	15	15	17	13
S2	25	12	11	NV	NV	11	13
	50	13	14	11	12	12	14
	100	14	16	15	16	13	15
S3	25	NV	NV	NV	NV	NV	NV
	50	12	9	NV	13	NV	NV
	100	15	10	NV	17	NV	NV
S4	25	12	NV	NV	11	NV	NV
	50	13	NV	NV	12	NV	NV
	100	14	11	11	13	NV	NV
Ampicillin	10 mcg		22			20	
DMSO		0	0	0	0	0	0

*NV: No visible zone of inhibition

Bromelain[33] (47.795 mg/g dry weight of peel) was highly quantified among all the compounds in S1 samples followed by catechin (2.286 mg/g DW), ferulic acid (2.213 mg/g DW), ellagic acid (0.64 mg/g DW), caffeic acid (0.429 mg/g DW) and gallic acid (0.377 mg/g DW). In the S2 sample, only bromelain (44.43 mg/g DW), catechin (19.187 mg/g DW), caffeic acid (0.761 mg/g DW), and ellagic acid (0.835 mg/g DW) were detected. On the other hand, S3 and S4 samples contained the highest amount of bromelain, at 13.763 and 6.641 mg/g DW, respectively. The lowest content of ferulic acid was

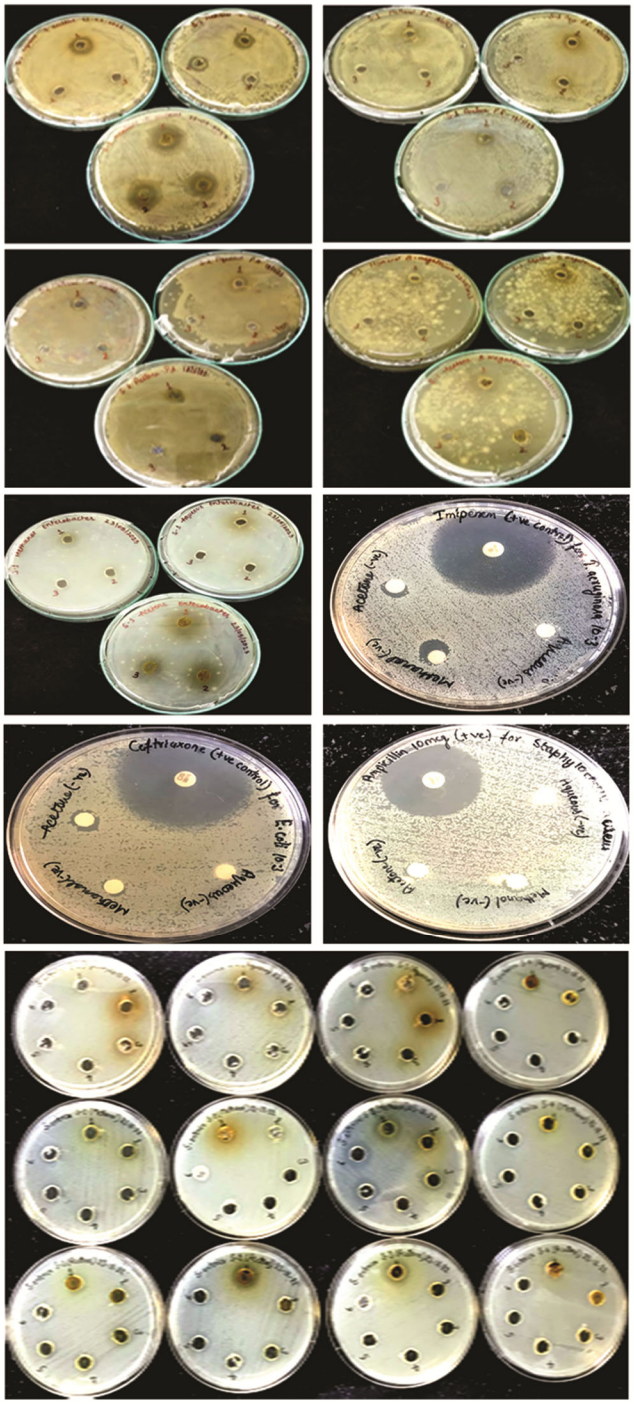

Fig. 1 — Zone of inhibition of *A. comosus* peel extract against pathogenic strains.

estimated in both S3 and S4 samples as 0.102 and 0.09 mg/g DW, respectively[34]. HPLC chromatogram of the pineapple peel extract is shown below in Fig. 2.

Thin layer chromatography (TLC)

As it was observed from the results of HPLC that S1 methanolic extract gave the best results, a TLC

plate bioautography test was performed on the S1 sample to extract the specific compound that was responsible for the antimicrobial activity. First, the mobile phase was optimized to get the maximum extraction of compounds. From 20 different combinations of mobile phases, two mobile phases obtained a maximum of 4 active bands, but the first best combination, consisting of methanol: n-hexane: petroleum ether: ethyl acetate: formic acid (MHPEF), shows partial extraction of compounds (Table 10). On further analysis, the last combination containing

Table 8 — MIC and LD_{50} value of *A. comosus* peel extract for gram negative and gram positive bacteria

Name of pathogens	Sample	MIC (mg/mL)	LD_{50} (mg/mL)
Gram negative bacteria			
Pseudomonas fluorescens	S1 (M.E.)	12.8	3.2
Enterobacter aerogenes	S1 (M.E.)	17.96	4.49
Shigella flexneri	S1 (M.E.)	15.6	3.9
Gram positive bacteria			
Bacillus megaterium	S1 (M.E.)	12.24	3.06
Staphylococcus aureus	S1 (A.E.)	67.32	16.38

Table 9 — Quantitative result of phytochemicals present in *A. comosus* peel through HPLC analysis

Phytochemicals	Retention time (in min)	S1	S2	S3	S4
Bromelain (mg/g DW)	2.041	47.795	44.43	13.763	6.641
Ferulic acid (mg/g DW)	3.383	2.213	ND	0.102	0.09
Catechin (mg/g DW)	3.420	2.286	19.187	0.824	0.11
Caffeic acid (mg/g DW)	3.533	0.429	0.761	0.336	0.332
Gallic acid (mg/g DW)	3.791	0.377	ND	0.291	0.347
Ellagic acid (mg/g DW)	4.858	0.64	0.835	0.506	0.502

*ND: Not detected

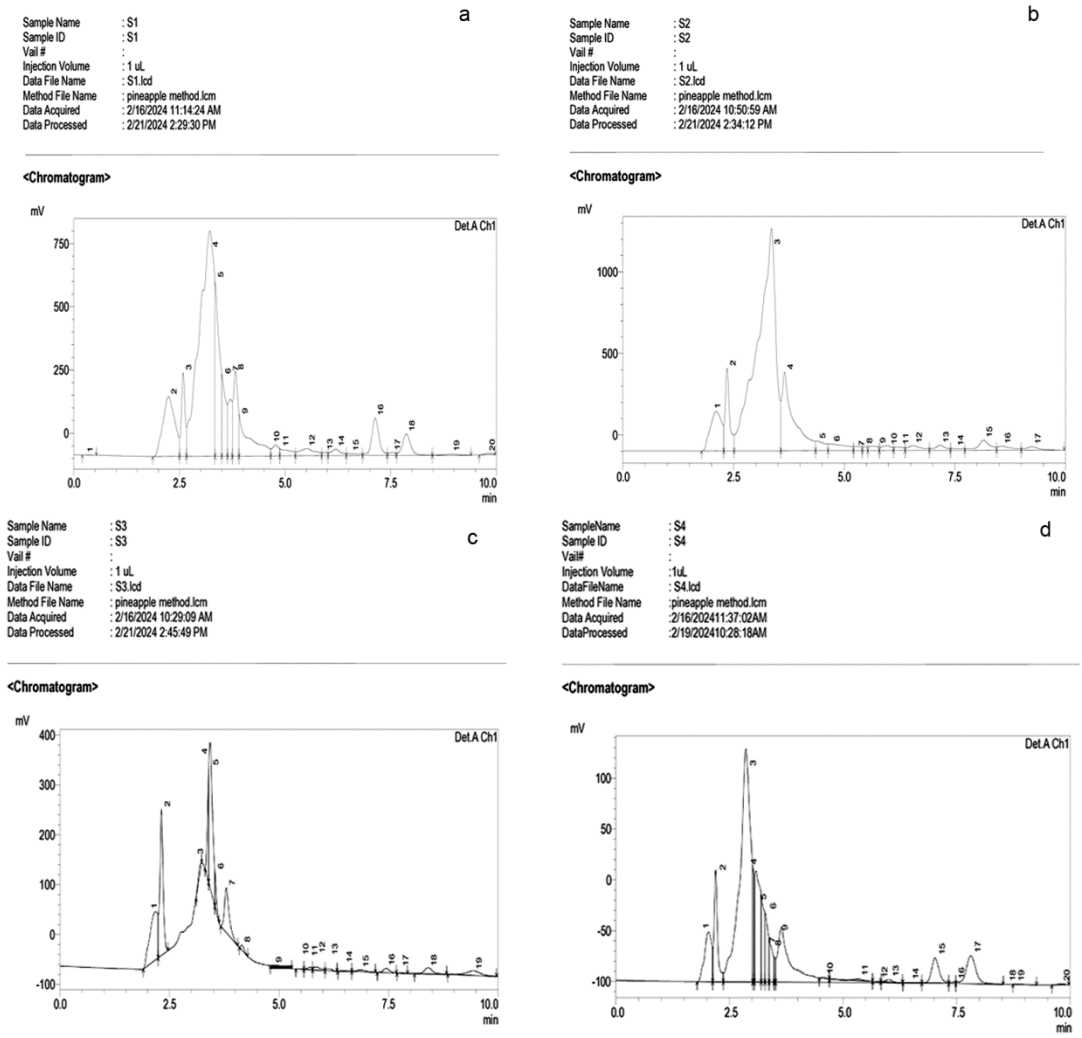

Fig. 2 — HPLC chromatogram of *A. comosus* peel methanolic extract a) Hot-air oven dried peel (S1), b) Microwave-dried peel (S2), c) Sun dried peel (S3), and d) Freeze dried peel (S4).

Table 10 — Active bands of polyphenolic and flavonoid components noted in *A. comosus* peel through TLC

Mobile phases	Ratio (v/v)	R_f value	Active bands
Methanol: Water: Formic acid (MWF)	70:29:1	0.746	1
Methanol: Chloroform: Formic acid (MCF)	80:19:1	0.944, 0.8	2
Methanol: n-hexane: Chloroform: Ethyl acetate: Formic acid (MHCEF)	40:40:10:9:1	0.912, 0.626	2
Methanol: n-hexane: Petroleum ether: Ethyl acetate: Formic acid (MHPEF)	40:40:10:9:1	0.765, 0.643, 0.513, 0.186	4
Methanol: Petroleum ether: Water: Formic acid (MPWF)	40:30:29:1	ND	0
Methanol: Ethyl acetate: Water: Formic acid (MEWF)	40:30:29:1	0.560, 0.66, 0.818	3
Methanol: Acetone: Water: Formic acid (MAWF)	40:30:29:1	0.890, 0.781	2
Methanol: Ethyl acetate: Formic acid (MEF)	70:29:1	0.948	1
Acetone: Ethyl acetate: Formic acid (AEF)	70:29:1	0.979, 0.912, 0.489	3
Acetone: Petroleum ether: Formic acid (APF)	70:29:1	0.292	1
Acetone: Petroleum ether: Ethyl acetate: Formic acid (APEF)	70:15:14:1	1, 0.874	2
Acetone: Ethyl acetate: Water: Formic acid (AEWF)	60:29:10:1	1, 0.732, 0.305	3
Acetone: Petroleum ether: Water: Formic acid (APWF)	60:29:10:1	ND	0
Acetone: Petroleum ether: Ethyl acetate: Formic acid (APEF1)	70:5:25:1	0.481	1
Acetone: Petroleum ether: Ethyl acetate: Formic acid (APEF2)	70:8:22:1	0.610, 0.175	2
Acetone: Petroleum ether: Ethyl acetate: Water: Formic acid (APEWF1)	60:8:22:10:1	0.851, 0.39	2
Acetone: Petroleum ether: Ethyl acetate: Water: Formic acid (APEWF2)	50:8:22:20:1	0.905, 0.508	2
Acetone: Petroleum ether: Ethyl acetate: Water: Formic acid (APEWF3)	40:8:22:30:1	0.976, 0.361	2
Acetone: Petroleum ether: Ethyl acetate: Water: Formic acid (APEWF4)	45:8:22:25:1	0.953, 0.453	2
Acetone: Petroleum ether: Ethyl acetate: Water: Formic acid (APEWF5)	35:8:22:35:1	0.325, 0.410, 0.751, 0.937	4

*ND: Not detected

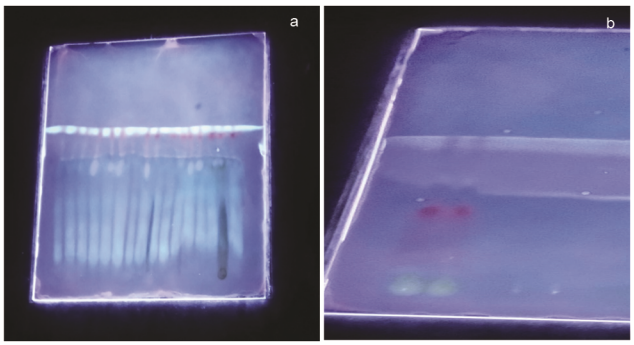

Fig. 3 — Thin-layer chromatography (TLC) results of *A. comosus* peel extract.

acetone, petroleum ether, ethyl acetate, water, and formic acid (APEWF) shows the maximum and clear spots of compounds on the TLC plate (Fig. 3). Thus, these active bands were responsible for giving the zone of inhibition against the tested pathogenic strains. As some of the bands were noted with high Rf values, this means their polarities were similar; hence, the solute was attracted by the solvent and moved at the same pace. According to Ilyas *et al.*[35] and Naz *et al.*[36], R_f value noted at 0.52, 0.55, 0.53, 0.48, 0.85, 0.97, 0.74, 0.6, and 0.38 cm corresponds to catechin, gallic acid, ellagic acid, ferulic acid, caffeic acid, rutin, trans-cinnamic acid, salicylic acid and quercetin, respectively. Thus, there is the possibility of the presence of these compounds in *A. comosus* peel, which are known for their free radical scavenging properties and giving a positive impact on human health[37].

Conclusion

The present study illustrates the potential of *A. comosus* peel as an antibacterial and antifungal agent. Qualitative and quantitative analysis of phytochemicals shows the presence of a number of polyphenolic and flavonoid components like terpenoids, coumarins, and phenols, which are the ultimate reason for antimicrobial activity. Results of agar well diffusion and broth dilution method demonstrate the highest antimicrobial activity in the minimum concentration of extract, i.e., in the group of gram-negative strains, the highest zone of inhibition was observed against *P. fluorescens* (23 mm) at 100 mg/mL concentration of S1 methanolic extract. In comparison, a group of Gram-positive strains shows a maximum 25 mm of zone against *S. aureus* ATCC 29213 in 100 mg/mL of S1 acetone extract. This study focused on the effect of different drying techniques on the antimicrobial potential of pineapple peel. It shows that hot air oven drying was the best technique to be applied, as it does not destroy the major secondary metabolites present in the peel part and gives the best results. Thus, the peel of pineapple can be utilized in the formulation of value-added food

products along with drug formulation to combat the problem of environmental pollution caused by this by-product.

Conflicts of interest

The authors declare that they have no conflict of interest.

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