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Antibacterial Properties and Phytochemical Profile of *Amorphophallus paeoniifolius*: A Natural Approach to Synbiotic Therapy for PCOS

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Abstract: Polycystic Ovarian Syndrome (PCOS) is a prevalent endocrine disorder affecting 6% to 20% of women of reproductive age and is commonly associated with infertility, obesity, and insulin resistance. Emerging evidence implicates gut microbiota dysbiosis in the pathophysiology of PCOS, highlighting the potential of microbiome-targeted therapies using probiotics, prebiotics, or synbiotics. This study evaluates the phytochemical composition and antibacterial activity of *Amorphophallus paeoniifolius* (Elephant Foot Yam) extracts, traditionally used in medicinal applications, to explore their potential for gut microbiome modulation. Extracts prepared using ethanol, petroleum ether, and hexane were screened for key bioactive compounds, including alkaloids, phenols, flavonoids, and tannins. Antibacterial efficacy was assessed against gut-associated bacteria: *Escherichia coli*, *Enterococcus faecalis*, *Shigella dysenteriae*, and *Enterobacter aerogenes*. The ethanol extract demonstrated significant phytochemical richness and antibacterial activity comparable to ciprofloxacin. Importantly, selective antibacterial action was observed, with minimal inhibition of beneficial probiotic strains such as *Lactobacillus spp*. isolated from yogurt and curd, contrasting with the broad-spectrum inhibition by ciprofloxacin. These findings suggest that *A. paeoniifolius* ethanol extracts hold promise as natural agents for selective modulation of gut microbiota and the development of synbiotic formulations aimed at managing PCOS and improving gut-hormonal health.

Keywords: Polycystic Ovarian Syndrome, *Amorphophallus paeoniifolius*, Phytochemicals, Antibacterial activity, Probiotics, *Escherichia coli*, *Enterococcus faecalis*, *Shigella dysenteriae*, *Enterobacter aerogenes*

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Introduction

Polycystic ovarian syndrome (PCOS) is one of the most prevalent endocrine disorders affecting women of reproductive age worldwide. It is characterized by hormonal imbalances, ovarian dysfunction, and metabolic complications, including obesity, insulin resistance, and cardiovascular disease, in addition to infertility (Senthilkumar and Arumugam, 2025). Emerging

evidence highlights the strong association between gut microbiota dysbiosis and PCOS, particularly through its role in modulating insulin resistance and systemic inflammation (Martinez-Montoro *et al*., 2022).

Several studies have reported distinct alterations in gut microbial composition among women with PCOS. Zeng *et al*. (2019) demonstrated that PCOS patients with insulin resistance exhibited decreased levels of Prevotella and increased abundance of Bacteroides compared with healthy individuals. This microbial imbalance has been linked to disruptions in bile acid metabolism, which in turn aggravates insulin resistance (Qi *et al*., 2019, 2020). Opportunistic and pro-inflammatory bacteria such as *Escherichia*, *Shigella*, *Enterobacteriaceae*, *Streptococcus*, and *Enterococcus* are often enriched in PCOS patients, further contributing to chronic inflammation and hormonal dysregulation (Senthilkumar and Arumugam, 2025; Jiang *et al*., 2025). Conversely, a reduction in beneficial commensals weakens gut barrier function and exacerbates metabolic dysfunctions. These findings underscore the need for therapeutic approaches that can selectively suppress pathogenic microbes while supporting probiotic populations.

Conventional pharmacological treatments for PCOS, including hormonal therapies and insulin sensitizers, are often associated with adverse effects and variable outcomes. Hence, complementary and natural remedies, such as herbal medicine and acupuncture, are gaining recognition as safer and more sustainable alternatives (Li *et al*., 2022; Lee *et al*., 2025). Among promising plant-based candidates, *Amorphophallus paeoniifolius* (elephant foot yam), a tuberous plant of the Araceae family, holds considerable potential. Traditionally, its corm has been utilized as a restorative, carminative, and tonic, and in the management of piles, dysentery, and abdominal ailments (Kainthla *et al*., 2021). Scientific investigations have confirmed its diverse pharmacological activities, including anti-inflammatory, antioxidant, analgesic, anti-convulsant, anthelmintic, antitumor, and cytotoxic effects (Singh and Wadhwa, 2014). In addition, aqueous and ethanolic extracts of *A. paeoniifolius* have shown significant antibacterial activity against a range of pathogens (Dey *et al*., 2012).

The bioactivity of plant-derived compounds is influenced by extraction methods, solvent polarity, and physicochemical parameters such as solvent-to-solid ratio, temperature, and duration of extraction (Costa *et al*., 2016). Identifying the most suitable solvent system is therefore critical to maximizing the yield and effectiveness of bioactive constituents. Given its medicinal history and antibacterial potential, *A. paeoniifolius* represents an attractive candidate for developing natural therapeutics targeting gut dysbiosis in PCOS.

Recent advances in microbiome research suggest that modulating gut microbiota could serve as a novel therapeutic avenue for PCOS. Probiotic supplementation with strains such as *Lactobacillus acidophilus*, *Bifidobacterium bifidum,* and *Akkermansia muciniphila* has shown promising results in improving insulin sensitivity, androgen levels, and lipid metabolism (He *et al*., 2020). Likewise, prebiotics and synbiotics enhance these effects by fostering the growth of beneficial microbes, improving gut barrier integrity, and restoring microbial equilibrium (Thackray *et al*., 2018; Korpela *et al*., 2020). Integrating selective plant extracts with probiotics in synbiotic formulations has emerged as a promising strategy, though careful evaluation is required to ensure compatibility and functional synergy (Markowiak and Śliżewska, 2018; Zhang *et al*., 2021).

In the present study, multiple solvents were employed to identify the most effective extract of *A. paeoniifolius* against bacterial pathogens, which are often elevated in polycystic ovary syndrome (PCOS) and generally regarded as pro-inflammatory or opportunistic pathogens. Further, the study was designed to evaluate the antibacterial activity of these solvent extracts against food-borne bacterial isolates, while

simultaneously assessing their effects on beneficial probiotic strains.

Materials and Methods

Sample Collection

Fresh tubers of *Amorphophallus paeoniifolius* were collected from Mullurkkara, Thrissur District, Kerala, India (latitude: 10.7847°; longitude: 76.2581°). Healthy, undamaged tubers were selected for the study. The plant was identified and authenticated by experts at the Botanical Survey of India (BSI), Tamil Nadu. A voucher specimen (BSI/SRC/5/23/2016/Tech/230) was deposited and preserved for future reference.

Sample Preparation and Extraction

The tubers were washed, cut into small pieces, shade-dried, and ground into fine powder using a laboratory mill. The powdered material was stored in a desiccator until use. Extraction was carried out using a Soxhlet apparatus with ethanol, petroleum ether, and hexane through hot percolation. The resulting extracts were filtered through Whatman No. 1 filter paper and concentrated by evaporation at room temperature until dryness. Dried extracts were stored in airtight containers at 4 °C for further analysis.

Phytochemical Screening

The preliminary phytochemical screening of the ethanol, petroleum ether, and hexane extracts of *A. paeoniifolius* tuber was carried out to detect major classes of bioactive compounds, including alkaloids, flavonoids, terpenoids, phenols, tannins, steroids, saponins, and carbohydrates. Standard procedures described by Oloyede *et al*. (2011) were followed for the qualitative analysis.

Quantitative Analysis of Phytochemicals

The phytochemical constituents of different extracts of *A. paeoniifolius* were quantified using standard methods with slight modifications.

Alkaloids: Total alkaloid content was estimated according to Bhuvaneshwari and Sivasubramanian (2023). Briefly, 5 g of the powdered sample was extracted with 10% acetic acid, concentrated, and precipitated using concentrated ammonium hydroxide. The precipitate was collected, dried at 60 °C, and quantified gravimetrically.

Flavonoids: Total flavonoids were determined following the method of Bhuvaneshwari and Sivasubramanian (2023). Five grams of the sample was hydrolyzed with dilute HCl, filtered, and the residue was dried and weighed. The results were expressed as percentage flavonoid content relative to sample weight.

Terpenoids: Terpenoid estimation was performed as described by Indumathi *et al*. (2014). Ten grams of dried extract was treated with ethanol and partitioned with petroleum ether and hexane. The terpenoid content was calculated gravimetrically based on weight loss after solvent evaporation.

Phenols: Total phenolic content was quantified using the Folin–Ciocalteu method. Different extracts of the sample were reacted with Folin's reagent and sodium bicarbonate, and absorbance was measured at 650 nm. The concentration was determined from a standard calibration curve of gallic acid.

Tannins: Tannin content was estimated by the Folin–Denis method. Extracts were reacted with sodium carbonate and Folin–Denis reagent, and absorbance was recorded at 700 nm. Results were expressed as tannic acid equivalents (mg/g extract).

Antibacterial Activity

Preparation of inocula

Bacterial cultures of *E. coli*, *E. faecalis*, *S. dysenteriae*, and *E. aerogenes* were used as test organisms for evaluating the antibacterial potential of ethanol, petroleum ether, and hexane tuber extracts of *A. paeonifolius*. A loop full of each culture was suspended in 500 µl of sterilized media broth, respectively taken in an Eppendorf and was used as an inoculum for testing.

Screening of antibacterial activity

Sensitivity of different bacterial strains to various

extracts was measured in terms of zone of inhibition using agar diffusion assay. The plates containing Mueller-Hinton agar media were spread with 0.2 ml of the inoculum equivalent to McFarland 0.5 (15×10^7 CFU/mL) turbidity standard. Wells were cut out from agar plates using a sterilized stainless steel borer and filled with 0.1 ml (500 µg) of the extract. The plates were inoculated with different bacterial cultures and incubated at 37°C up to 24 h, and the diameter of the resultant zone of inhibition was measured. The bacteria with a clear zone of inhibition were considered to be sensitive. 20 µl of Ciprofloxacin (20 µg/ml in DMSO) was used as a positive control and 20 µl of DMSO was used as a negative control, and plates were incubated for 24 h at 37°C.

Determination of MIC

The MIC of ethanol, petroleum ether, and hexane tuber extracts of *A. paeonifolius* was determined by agar diffusion assay. The plates containing Mueller-Hinton agar media were spread with 0.2 ml of the inoculum equivalent to McFarland 0.5 (15×10^7 CFU/ml) turbidity standard. Wells were prepared in agar plates using a sterilized stainless steel borer, and extracts were loaded. The concentrations of ethanol, petroleum ether, and hexane tuber extracts of *A. paeoniifolius* were used in this test, ranging from 10, 20, 30, and 40 µg/ml. Each plate contained four wells, including a control, as 20 µl of Ciprofloxacin (20 µg/ml in DMSO) was used as a positive control. The lowest concentration of the extract that showed a zone of inhibition against the respective organisms was taken as MIC.

Collection and Isolation of Probiotic Bacteria

Bacterial cultures were isolated from five different fermented and dairy food samples: ice cream, curd, kimchi, sauerkraut, and yogurt. Pure cultures were maintained on nutrient agar slants at 4 °C until further use.

Preparation of Plant Extracts and Mueller-Hinton Agar Plates

Plant extracts of *A. paeoniifolius* (AP) were prepared using three different solvents: ethanol (AP-E), petroleum ether (AP-PE), and hexane (AP-H). The extracts were filtered, concentrated, and stored at 4 °C until further use in antibacterial activity testing. Mueller-Hinton agar (MHA) plates were prepared and inoculated with freshly cultured bacterial isolates. Three replicates were maintained for each organism.

Well Preparation and Antibacterial Activity Assay

Wells of 7 mm diameter were aseptically punched into the agar using a sterile cork borer. For ethanolic extracts (AP-E), three wells were prepared per plate. For petroleum ether (AP-PE) and hexane (AP-H) extracts, two wells were prepared per plate, including one for the negative control. Each well was loaded with 70 µl of the respective extract. The corresponding solvents served as negative controls: ethanol for AP-E, petroleum ether for AP-PE, and hexane for AP-H. A chloramphenicol disc was placed on each plate as a positive control. All plates were incubated at 37 °C for 24 h. Following incubation, antibacterial activity was assessed by measuring the diameter of the inhibition zones surrounding the wells and the control discs.

Statistical analysis

All experiments were carried out in triplicate,with each experiment repeated a minimum of three times. Data were analyzed using SPSS version 17.0, and a p-value of ≤0.01 was regarded as statistically significant.

Results and Discussion

Qualitative Phytochemical Analysis

The qualitative phytochemical screening of *A. paeoniifolius* tuber extracts demonstrated clear differences in the distribution of secondary metabolites across solvents (Table 1). Alkaloids were strongly detected in the ethanol extract (++) and moderately in the petroleum ether extract (+), but were absent in the hexane extract. Flavonoids were abundant in the hexane extract (++), weakly present in ethanol (+), and undetected in petroleum ether. Terpenoids showed strong positivity in both petroleum ether and hexane

Table 1: Qualitative Phytochemical analysis of *Amorphophallus paeoniifolius*

Phytochemical	Test	Ethanol Extract	Petroleum Ether Extract	Hexane Extract
Alkaloids	Dragendorff's test	**++**	**+**	**-**
Flavonoids	Ferric chloride test	**+**	**-**	**++**
Terpenoids	Salkowski test	**-**	**++**	**++**
Phenols	Lead acetate test	**+**	**+**	**+**
Tannins	Ferric chloride test	**++**	**-**	**-**
Steroids	Liebermann-Burchard's test	**_**	**-**	**-**
Saponins	Frothing test	**-**	**-**	**-**
Carbohydrates	Molisch test	+	+	+

Presence (+), Absence (-)

extracts (++), whereas they were not detected in ethanol. Phenolic compounds were consistently observed across all three extracts (+). Tannins were found in high levels in the ethanol extract (++), but absent in petroleum ether and hexane. Steroids and saponins were not detected in any of the extracts. Carbohydrates were uniformly present (+) in ethanol, petroleum ether, and hexane extracts. Overall, ethanol extract was particularly rich in alkaloids, tannins, and phenols, while petroleum ether and hexane extracts favored the extraction of terpenoids and flavonoids. Previous studies have reported a diverse range of phytochemical constituents in *A. paeoniifolius*, including alkaloids, flavonoids, steroids, tannins, carbohydrates, reducing sugars, amino acids, glycosides, saponins, phenols, and glucomannans (Dey *et al*., 2010; Salunke *et al*., 2018). Sen *et al*. (2024) further confirmed that aqueous extracts contain alkaloids, flavonoids, reducing sugars, carbohydrates, and tannins, whereas ethanolic extracts additionally reveal the presence of steroids and triterpenoids. These findings are consistent with the present study, where ethanol demonstrated a broader spectrum of phytochemical recovery.

Furthermore, the plant's richness in secondary metabolites underpins its broad therapeutic potential. As noted by Sharma and Kumar (2025), *A. paeoniifolius* exhibits a wide range of pharma-cological activities, including antibacterial, anti-oxidant, anticancer, gastroprotective, anal-gesic, antidiabetic, anthelmintic, antidiarrheal, antiviral, anticonvulsant, and central nervous system depressant effects. The presence of these bioactive compounds validates the ethnomedicinal uses of the plant and supports further investi-gation into its pharmacological applications.

Quantitative Phytochemical Analysis

The quantitative estimation of phytochemicals in *A. paeoniifolius* tuber extracts revealed significant variation depending on the solvent used (Table 2). The ethanol extract contained the highest levels of alkaloids (3.58 ± 0.59 mg/g), followed by petroleum ether (2.46 ± 0.21 mg/g), while alkaloids were absent in the hexane extract. Flavonoid content was most abundant in the hexane extract (2.21 ± 0.74 mg/g), present at lower levels in ethanol (1.35 ± 0.36 mg/g), and undetected in petroleum ether. Terpenoid concentration was highest in the petroleum ether extract (3.74 ± 0.45 mg/g), moderate in hexane

Table 2: Quantitative phytochemical analysis of *Amorphophallus paeoniifolius*

S. No.	Tests	*Amorphophallus paeoniifolius*		
		Ethanol Extract	**Petroleum Ether Extract**	**Hexane Extract**
1	TAC (mg/g)	3.58 ± 0.59	2.46 ± 0.21	-
2	TFC (mg /g)	1.35 ± 0.36	-	2.21 ± 0.74
3	TTC (mg /g)	-	3.74 ± 0.45	1.76 ± 0.19
4	TPC (mg/g)	2.61 ± 0.42	1.28 ± 0.34	-
5	TTC (mg/g)	2.84 ± 0.38	-	-

Values are mean ± SD (n = 3)

(1.76 ± 0.19 mg/g), and absent in ethanol. Phenolic content was greatest in ethanol (2.61 ± 0.42 mg/g), comparatively lower in petroleum ether (1.28 ± 0.34 mg/g), and absent in hexane. Tannins were detected exclusively in ethanol (2.84 ± 0.38 mg/g), while undetectable in petroleum ether and hexane extracts. Overall, ethanol extract was rich in alkaloids, phenols, and tannins; petroleum ether extract showed high terpenoid and moderate alkaloid levels; and hexane extract was particularly enriched in flavonoids and terpenoids.

Alkaloids are a diverse group of plant secondary metabolites characterized by complex structural variability, distinct biosynthetic pathways, and a wide range of pharmacological activities (Velu *et al*., 2018). In the present study, quantitative analysis revealed that the ethanolic extract of *A. paeoniifolius* was particularly rich in phenolic compounds, flavonoids, and tannins, supporting its selection as the primary extract for further investigation (Bhuvaneswari and Sivasubramanian, 2023). The high alkaloid content observed in the ethanol extract suggests potential anticancer and antioxidant activities, as previously reported (Diniso *et al*., 2022).

Flavonoids, a major class of polyphenolic compounds, are known for their potent antioxidant and anti-inflammatory properties. They act as free radical scavengers, neutralizing reactive species such as superoxide anions, hydroxyl radicals, and lipid peroxyl radicals. This activity contributes to the protection of cellular components such as membranes, proteins, and DNA from oxidative damage, thereby playing a critical role in preventing diseases associated with oxidative stress (Michalak, 2022). Phenolic compounds, another important class of secondary metabolites, have been widely recognized for their role in reducing the risk of chronic conditions, including cancer, cardiovascular diseases, and age-related degenerative disorders (Mukherjee *et al*., 2024). Their antioxidant capacity and metal-chelating properties make them crucial for cellular defense mechanisms. Terpenoids, predominantly extracted using nonpolar solvents, exhibit a wide spectrum of biological activities, including anticancer, anti-inflammatory, antibacterial, antiviral, antimalarial, and hypoglycemic effects. Additionally, they enhance transdermal absorption and are involved in immunoregulation, antioxidation, neuroprotection, and the modulation of insecticide resistance (Yang *et al*., 2020; Bhuvaneswari and Sivasubramanian, 2023).

Antibacterial Activity

The antibacterial potential of different solvent extracts of *A. paeoniifolius* was evaluated against *E. coli, E. faecalis, S. dysenteriae,* and *E. aerogenes* bacterial pathogens using the agar well diffusion assay. The inhibition zones were measured in millimeters and compared with positive (Ciprofloxacin) and negative (DMSO) controls (Table 3).

Among the tested extracts, the ethanol extract exhibited the strongest inhibitory activity against all bacterial strains tested (Fig. 1). The maximum activity was recorded against *E. faecalis* and *S. dysenteriae* (20 mm each), which was nearly equivalent to Ciprofloxacin (20–21 mm). The

Table 3: Antibacterial activity of different solvent extracts of *A. paeoniifolius* against test bacteria

S. No.	Test Bacteria	Inhibition Zone (mm)				
		NC (DMSO)	Ethanol Extract	Petroleum Ether Extract	Hexane Extract	PC (Ciprofloxacin)
1	*Escherichia coli*	0	19	17	18	20
2	*Enterococcus faecalis*	0	20	16	16	20
3	*Shigella dysenteriae*	0	20	17	17	21
4	*Enterobacter aerogenes*	0	18	18	18	19

NC: Negative Control; PC: Positive Control

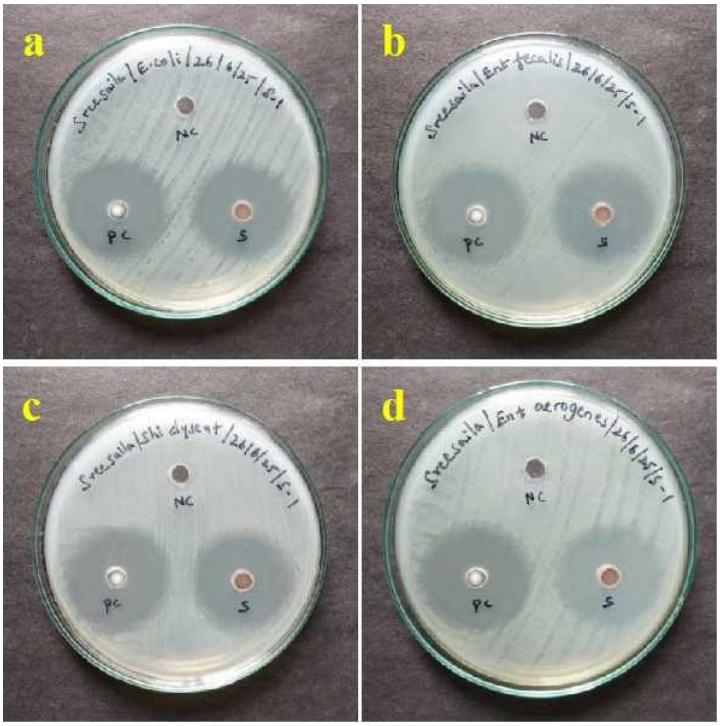

Fig. 1: Antibacterial activity of ethanolic extracts of *Amorphophallus paeoniifolius* against selected pathogenic bacteria: (a) *E. coli* (b) *E. faecalis* (c) *S. dysenteriae* and (d) *E. aerogenes.*

lowest inhibition (18 mm) was observed against *E. aerogenes*.

The petroleum ether extract showed moderate inhibition (16–18 mm), with the highest zone against *E. aerogenes* (18 mm) and the lowest against *E. faecalis* (16 mm) (Fig. 2).

Similarly, the hexane extract displayed activity in the range of 16–18 mm (Fig. 3). The highest inhibition was observed against *E. coli* and *E. aerogenes* (18 mm each), while *E. faecalis* showed the least sensitivity (16 mm).

Overall, the ethanol extract demonstrated superior antibacterial efficacy compared to petroleum ether and hexane extracts, and its effect was nearly comparable to the standard antibiotic Ciprofloxacin. DMSO (negative control) did not

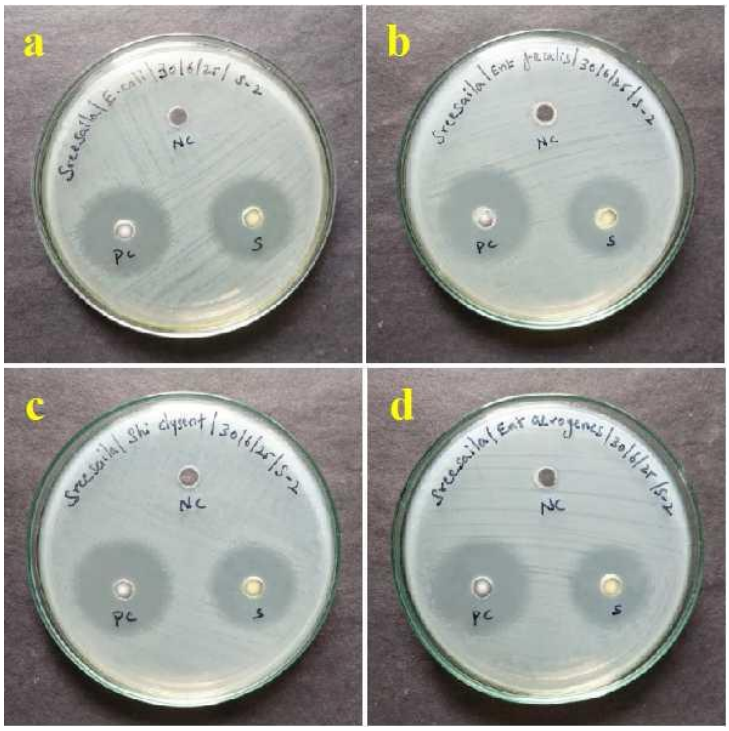

Fig. 2: Antibacterial activity of petroleum ether extracts of *Amorphophallus paeoniifolius* against selected pathogenic bacteria: (a) *E. coli* (b) *E. faecalis* (c) *S. dysenteriae* and (d) *E. aerogenes.*

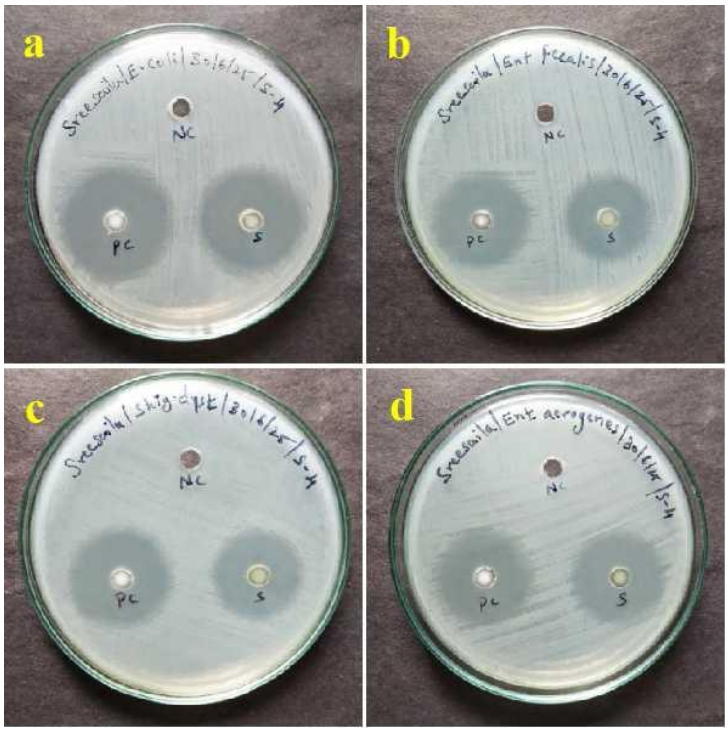

Fig. 3: Antibacterial activity of Hexane extracts of *Amorphophallus paeoniifolius* against selected pathogenic bacteria: (a) *E. coli* (b) *E. faecalis* (c) *S. dysenteriae* and (d) *E. aerogenes.*

Table 4: MIC of different solvent extracts of *Amorphophallus paeoniifolius* against test bacteria

S. No.	Test Bacteria	MIC (µg/mL)	Ethanol	Petroleum Ether	Hexane	PC (Ciprofloxacin)
1	*Escherichia coli*	10	NZ	NZ	NZ	21
		20	NZ	NZ	NZ	
		30	12	13	10	
		40	19	15	12	
2	*Enterococcus faecalis*	10	NZ	NZ	NZ	17
		20	NZ	NZ	NZ	
		30	15	11	10	
		40	16	13	13	
3	*Shigella dysenteriae*	10	NZ	NZ	NZ	18
		20	NZ	NZ	NZ	
		30	13	12	12	
		40	16	13	14	
4	*Enterobacter aerogenes*	10	NZ	NZ	NZ	19
		20	NZ	NZ	NZ	
		30	14	14	14	
		40	16	17	16	

NZ: No Zone

show any inhibition, confirming that the activity was due to the plant extracts.

Minimal Inhibitory Concentration (*MIC*)

The antibacterial potency of *A. paeoniifolius* extracts was further evaluated by determining the minimal inhibitory concentration (MIC) against selected bacterial pathogens. The MIC values indicate the lowest concentration of the extract that effectively inhibits bacterial growth (Table 4).

The ethanol extract exhibited the strongest antibacterial activity, with MIC values ranging from 12–15 µg/ml at 30 µg/ml, and increased inhibition at 40 µg/ml. *E. coli* was the most sensitive strain, showing inhibition at 12 µg/ml (30 µg/ml well) and 19 µg/ml (40 µg/ml well). *S. dysenteriae* showed similar sensitivity, whereas *E. faecalis* and *E. aerogenes* required slightly higher concentrations to achieve comparable inhibition (Fig. 4).

The petroleum ether extract showed moderate activity, with MIC values ranging from 11–14 µg/mL at 30 µg/ml and 13–17 µg/ml at 40 µg/ml (Fig. 5). *E. aerogenes* exhibited the highest sensitivity, while *E. faecalis* was less sensitive.

The hexane extract displayed the lowest but significant antibacterial effect, with MIC values between 10–14 µg/ml at 30 µg/ml and 12–16 µg/ml at 40 µg/ml (Fig. 6). *E. coli* responded at the lowest MIC, followed by other tested bacteria.

Among the various solvent extracts of *A. paeoniifolius*, the ethanolic extract consistently exhibited the strongest antibacterial activity, followed by petroleum ether and hexane extracts. This trend suggests that ethanol is the most effective solvent for extracting antimicrobial compounds from the tuber. In comparison, the standard antibiotic ciprofloxacin (used as a positive control) demonstrated superior inhibition across all tested bacterial strains, with inhibition zones ranging from 16–21 mm, confirming its efficacy. Notably, no antibacterial activity was observed at lower extract concentrations (10–20 µg/ml), indicating that the minimum inhibitory concentration (MIC) for all tested extracts likely exceeds 20–30 µg/ml.

The enhanced antibacterial efficacy of the ethanol extract can be attributed to its ability to solubilize a wide range of polar bioactive compounds, including alkaloids, phenolic

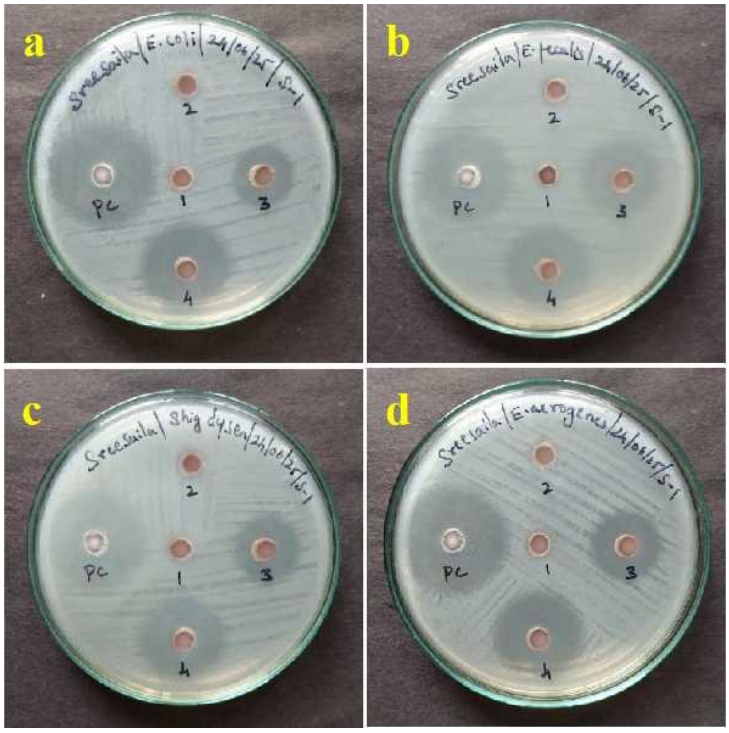

Fig. 4: Minimal Inhibitory Concentration (MIC) of Ethanol Extract of *Amorphophallus paeoniifolius* against selected bacterial strains (a) *E. coli* (b) *E. faecali*, (c) *S. dysenteria*, and (d) *E. aerogenes.*

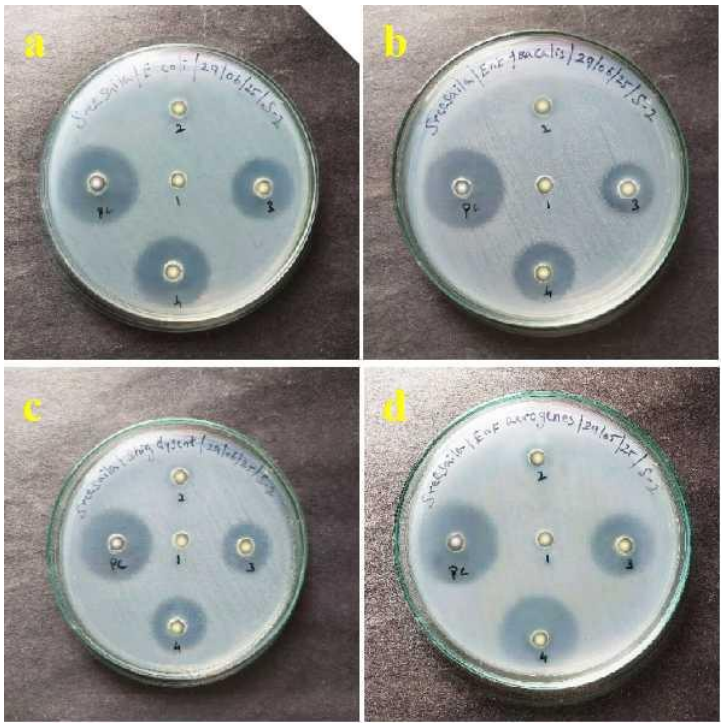

Fig. 5: Minimal Inhibitory Concentration (MIC) of Petroleum Ether Extract of *Amorphophallus paeoniifolius* against selected bacterial strains (a) *E. coli* (b) *E. faecalis* (c) *S. dysenteriae* and (d) *E. aerogenes.*

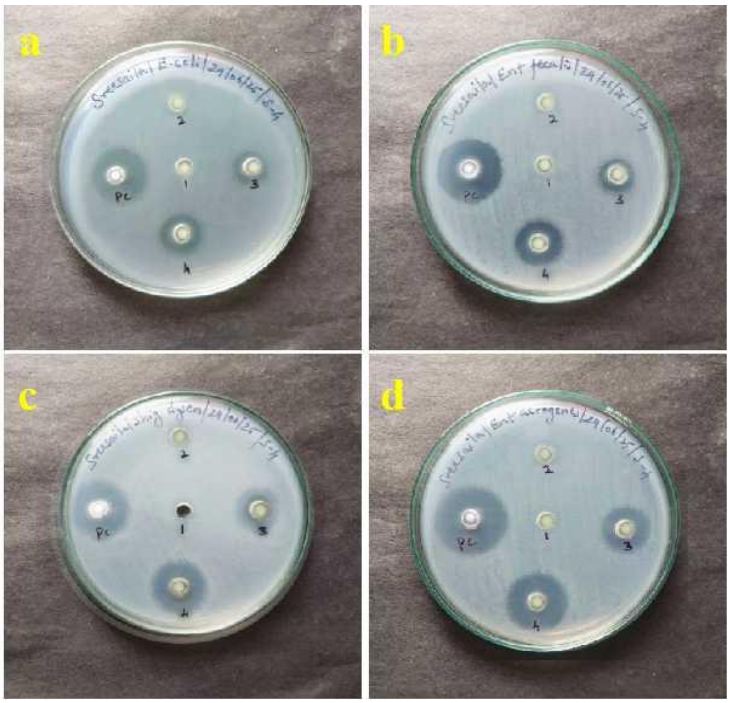

Fig. 6: Minimal Inhibitory Concentration (MIC) of Hexane Extract of *Amorphophallus paeoniifolius* against selected bacterial strains (a) *E. coli* (b) *E. faecalis* (c) *S. dysenteriae* and (d) *E. aerogenes.*

compounds, flavonoids, and tannins, many of which are well-documented for their antimicrobial properties (Wink, 2015). This is consistent with previous reports by Pandey and Gupta (2013), who attributed the antibacterial effects of *A. paeoniifolius* to the presence of flavonoids, saponins, tannins, alkaloids, phenolics, and triterpenoids.

Earlier studies also support these findings. Shete *et al*. (2014) reported that both acetone and ethanol extracts of *Amorphophallus spp.* displayed antibacterial activity against several bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Klebsiella pneumoniae.* Similarly, Kadali *et al*. (2016) observed significant antibacterial activity of *A. paeoniifolius* tuber extracts, with ethanolic fractions producing inhibition zones ranging from 6–18 mm, compared to lower inhibition by aqueous extracts (7–9 mm). The ethanolic peel extract also showed considerable activity (7–16 mm), while the aqueous peel extract demonstrated less inhibition (6–9 mm). These differences may be attributed to ethanol's efficiency in extracting a broader spectrum of antimicrobial phytoconstituents compared to water. Moreover, Khan *et al*. (2008) reported that both aqueous and organic extracts of *Amorphophallus species* exhibited antimicrobial activity against a wide range of Gram-positive bacteria (*Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Streptococcus β-haemolyticus*) and Gram-negative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*, *Pseudomonas aeruginosa*, and *Salmonella typhi*), supporting the broad-spectrum antibacterial potential of this genus. In the present study, the superior antibacterial activity of the ethanolic extract of *A. paeoniifolius* underscores its richness in bioactive compounds with antimicrobial properties. These findings reinforce the importance of solvent selection in natural product extraction and support the potential application of *A. paeoniifolius* as a source of natural antimicrobial agents.

Selectivity and Potential for Minimal Impact on Beneficial Microbiota

The antibacterial activity of *A. paeoniifolius*

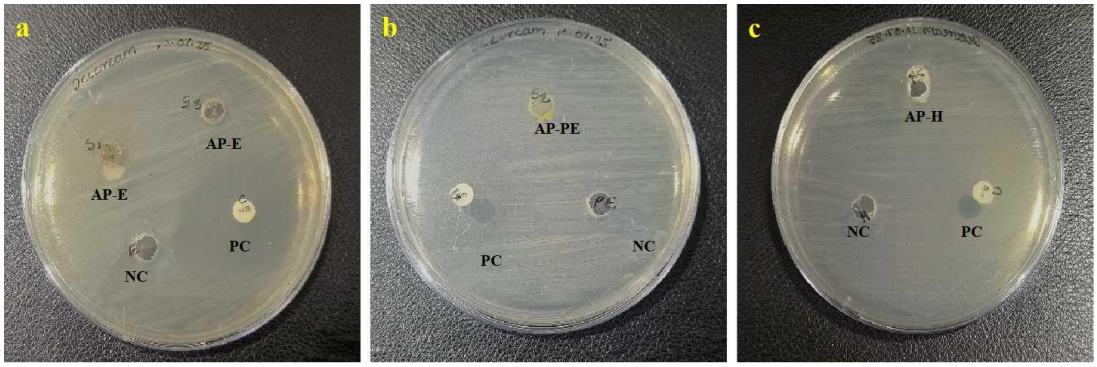

Fig. 7: Antibacterial activity of *Amorphophallus paeoniifolius* extracts against bacterial isolates from ice cream. (a) Ethanol extract (AP-E); (b) Petroleum ether extract (AP-PE); (c) Hexane extract (AP-H). PC: Positive control (ciprofloxacin); NC: Negative control.

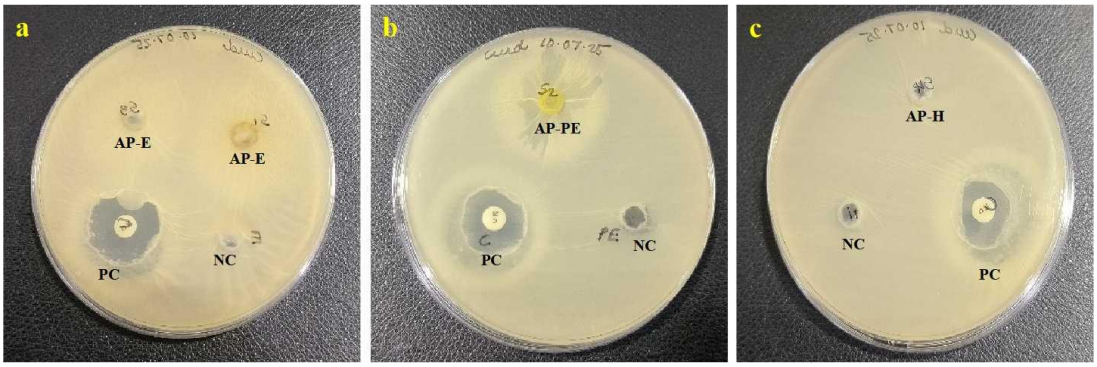

Fig. 8: Antibacterial activity of *Amorphophallus paeoniifolius* extracts against bacterial isolates from icurd. (a) Ethanol extract (AP-E); (b) Petroleum ether extract (AP-PE); (c) Hexane extract (AP-H). PC: Positive control (ciprofloxacin); NC: Negative control.

extracts prepared using ethanol (AP-E), petroleum ether (AP-PE), and hexane (AP-H) was evaluated against bacterial isolates obtained from various fermented foods, including ice cream, curd, kimchi, sauerkraut, and yogurt. The disc and well diffusion methods were employed to assess both the potency and selectivity of the extracts, with particular attention given to their impact on beneficial food-associated microbiota.

The ethanol extract (AP-E) exhibited significant antibacterial activity against ice cream-associated isolates, as evident from large and well-defined inhibition zones (Fig. 7a). The activity was comparable to the positive control (PC, ciprofloxacin), and much greater than the negative control (NC), which showed no inhibition. The petroleum ether extract (AP-PE) also demonstrated antibacterial activity, though the inhibition zones were moderately smaller (Fig. 7b). The hexane extract (AP-H) showed mild antibacterial activity (Fig. 7c). These results indicate that AP-E is the most effective extract, with AP-PE providing moderate activity and AP-H showing limited efficacy.

A similar trend was observed with bacterial isolates from curd. AP-E produced prominent zones of inhibition (Fig. 8a), while AP-PE (Fig. 8b) showed moderate activity, and AP-H (Fig. 8c) exhibited minimal inhibition. The inhibition remained controlled and localized, suggesting that the extract may selectively suppress undesirable bacteria without significantly affecting beneficial strains.

Kimchi-associated bacteria, the ethanolic

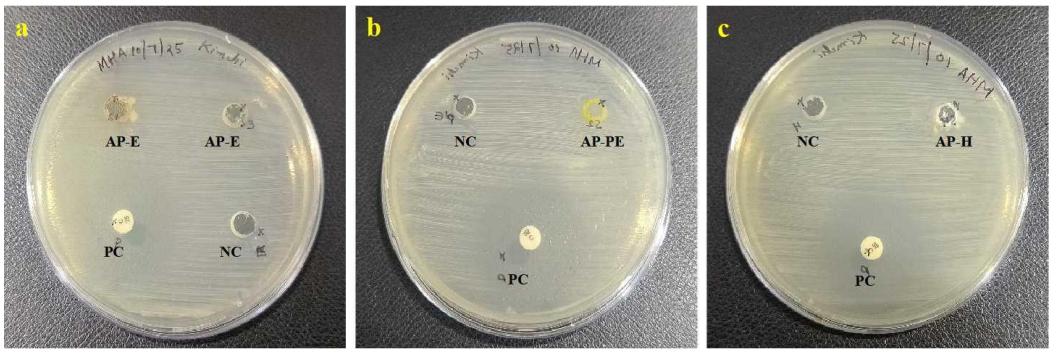

Fig. 9: Antibacterial activity of *Amorphophallus paeoniifolius* extracts against bacterial isolates from kimchi. (a) Ethanol extract (AP-E); (b) Petroleum ether extract (AP-PE); (c) Hexane extract (AP-H). PC: Positive control (ciprofloxacin); NC: Negative control.

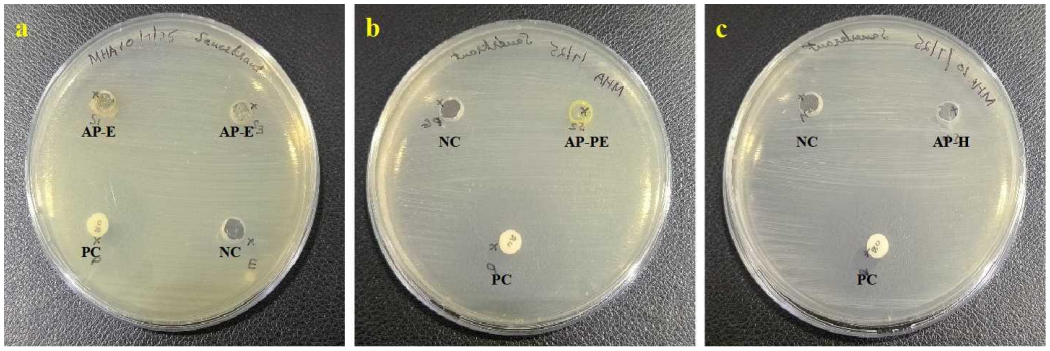

Fig. 10: Antibacterial activity of *Amorphophallus paeoniifolius* extracts against bacterial isolates from sauerkraut. (a) Ethanol extract (AP-E); (b) Petroleum ether extract (AP-PE); (c) Hexane extract (AP-H). PC: Positive control (ciprofloxacin); NC: Negative control.

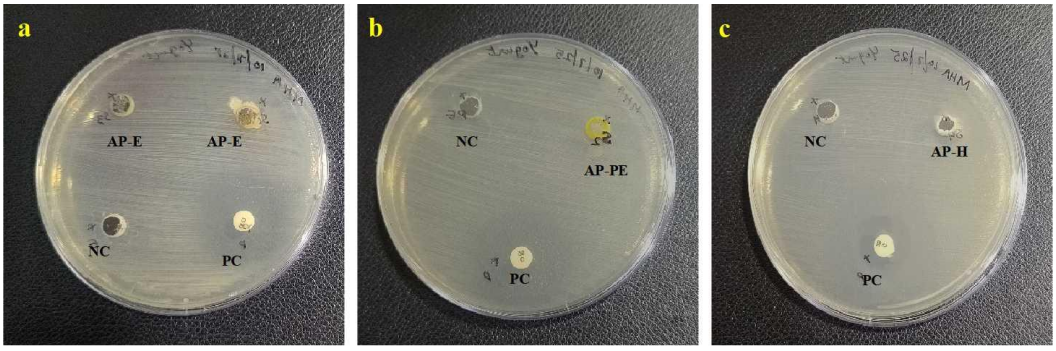

Fig. 11: Antibacterial activity of *Amorphophallus paeoniifolius* extracts against bacterial isolates from yogurt. (a) Ethanol extract (AP-E); (b) Petroleum ether extract (AP-PE); (c) Hexane extract (AP-H). PC: Positive control (ciprofloxacin); NC: Negative control.

extract again showed the highest activity (Fig. 9a), followed by moderate inhibition from AP-PE (Fig. 9b), and negligible activity from AP-H (Fig. 9c). These findings suggest that the extracts, particularly AP-E,may effectively target unwanted bacteria while preserving the beneficial microbial balance typically present in kimchi.

AP-E exhibited strong inhibition against sauerkraut isolates (Fig. 10a), with AP-PE showing moderate activity (Fig. 10b), and AP-H demonstrating no visible zones (Fig. 10c). Importantly, the size and clarity of the inhibition

zones suggest a selective action rather than broad-spectrum suppression, supporting the potential safety of the extract for use in fermented foods.

In yogurt-associated bacteria, AP-E again demonstrated strong antibacterial activity with large inhibition zones (Fig. 11a). AP-PE showed mild activity (Fig. 11b), while AP-H produced no significant inhibition (Fig. 11c). The negative control remained inactive in all cases, confirming the specificity of the observed effects to the extracts themselves.

The antibacterial screening of *A. paeoniifolius* extracts demonstrated that the ethanolic extract (AP-E) consistently exhibited the most potent inhibitory activity against bacterial contaminants isolated from fermented food samples. In contrast, the petroleum ether extract (AP-PE) showed moderate antibacterial effects, while the hexane extract (AP-H) exhibited minimal or no inhibitory action. The selective inhibition pattern observed, particularly with AP-E, is noteworthy suggesting that the extract can effectively target spoilage or pathogenic microorganisms without broadly suppressing beneficial microbiota.

This selectivity is particularly significant for applications in fermented foods, where microbial balance is essential for maintaining product quality, flavor profile, and the viability of probiotic organisms. The ability of *A. paeoniifolius* extracts, especially AP-E, to inhibit undesirable microbes while preserving beneficial ones highlights its potential as a natural, food-safe antimicrobial agent for use in biopreservation, functional foods, and probiotic-friendly formulations.

Probiotics, defined as live microorganisms that confer health benefits when consumed in adequate amounts, play a critical role in modulating the gut microbiota and enhancing host immunity (Hill *et al*., 2014). Prebiotics, on the other hand, are non-digestible food ingredients that selectively stimulate the growth and activity of beneficial gut bacteria (Roberfroid *et al*., 2010). The combination of both—known as synbiotics relies heavily on ensuring compatibility between prebiotic or antimicrobial additives and the probiotic strains. Hence, the integration of selective plant-based antimicrobials such as *A. paeoniifolius* extracts into synbiotic formulations demands rigorous compatibility testing, ensuring that these compounds do not compromise probiotic viability or functionality (Pandey *et al*., 2015; Markowiak and Śliżewska, 2018).

The richness of *A. paeoniifolius* in both primary and secondary metabolites, including phenolic compounds, flavonoids, and tannins further supports its potential as a functional food ingredient. Its phenolic profile, in particular, suggests strong antioxidant properties that could help improve immune function and serve as a preventive measure against infectious diseases (Lin *et al*., 2016; Ding *et al*., 2018). Moreover, the nutritional and phytochemical composition of *A. paeoniifolius* positions it as a promising value-added food or functional ingredient that could contribute to food security and health promotion, especially in regions facing nutritional challenges.

Numerous studies have confirmed the antimicrobial efficacy of plant extracts and essential oils against common foodborne pathogens and spoilage organisms (Hassanien *et al*., 2014; Moro *et al*., 2015; Youssef *et al*., 2020). These plant-derived compounds can also enhance flavor, visual appeal, and the overall functional quality of food products, particularly in fortified dairy items. Recent innovations include incorporating plant extracts either in free form or encapsulated systems to protect their bioactivity and allow controlled release during storage and digestion (Mahmoud, 2023). Taken together, these findings highlight the promising role of *A. paeoniifolius,*particularly its ethanolic extract as a natural antimicrobial and antioxidant agent in food systems. Its compatibility with beneficial microbes, bioactive phytochemical profile, and functional properties support its application in the development of safe, health-promoting, and shelf-stable functional foods.

Conclusion

This study highlights the significant phytochemical

and antibacterial potential of *Amorphophallus paeoniifolius* tuber extracts. The ethanol extract, in particular, was found to be rich in key bioactive compounds such as alkaloids, phenols, and tannins, which correlated strongly with its superior antibacterial activity. Among all tested extracts, the ethanolic fraction demonstrated the lowest minimum inhibitory concentration (MIC) and showed inhibitory effects comparable to ciprofloxacin at higher doses, underscoring its potential as a natural alternative to conventional antibiotics. Importantly, the ethanolic extract exhibited selective antibacterial effects, effectively inhibiting food-borne pathogens while preserving the viability of beneficial *Lactobacillus* species. This selective action is especially relevant for the development of synbiotic products, where maintaining a balanced gut microbiota is essential. The findings suggest that *A. paeoniifolius* may serve as a valuable ingredient in functional foods and nutraceuticals, particularly for applications aimed at modulating gut health and managing disorders associated with microbiota imbalance, such as polycystic ovary syndrome (PCOS). Overall, *A. paeoniifolius* presents a promising natural resource for the development of safe, effective, and targeted antimicrobial agents, with potential applications in both food preservation and therapeutic interventions.

Ethical Statement

No animals or human have been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Sreesaila N.P.: Conceptualization, methodology, interpretation, and manuscript preparation; *Nimmi O.S.*: Conceptualization, editing and supervision, interpretation of results, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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