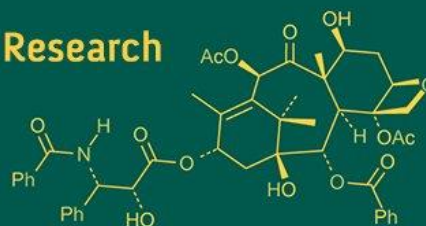


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(5): 12-21
www.biochemjournal.com
Received: 16-01-2026
Accepted: 21-02-2026

Mit Sathvara
Department of Biochemistry,
College of Basic science and
Humanities, S. D. Agricultural
University, Sardarkrushinagar,
Gujarat, India

Smita Singh
Department of Biochemistry,
College of Basic science and
Humanities, S. D. Agricultural
University, Sardarkrushinagar,
Gujarat, India

Ramesh V
Department of Dairy
Chemistry, G.N. Patel College
of Dairy Science, Kamdhenu
University, Sardarkrushinagar
Campus, Gujarat, India

Abidali G Bhagat
Department of Microbiology,
College of Basic science and
Humanities, S. D. Agricultural
University, Sardarkrushinagar,
Gujarat, India

Corresponding Author:
Smita Singh
Department of Biochemistry,
College of Basic science and
Humanities, S. D. Agricultural
University, Sardarkrushinagar,
Gujarat, India

Phytochemical profiling and evaluation of antimicrobial, anti-hemolytic and cytotoxic activities of different parts of *Phyllanthus emblica* varieties (Anand-1, Banarasi, Chakaiya and Kanchan)

Mit Sathvara, Smita Singh, Ramesh V and Abidali G Bhagat

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5Sa.8303>

Abstract

The present study evaluated the phytochemical composition and biological activities of different parts of four *Phyllanthus emblica* varieties; Chakaiya, Kanchan, Anand-1 and Banarasi using solvents of varying polarity; methanol, ethyl acetate, water and chloroform. The average fruit weight ranged from 23.90 g (Kanchan) to 30.07 g (Chakaiya), with the pulp comprising the major portion (92-94%) of the total fruit mass. The phytochemical screening revealed that methanolic extracts exhibited the richest composition, showing strong presence of phenols, flavonoids, tannins, alkaloids and quinones, whereas chloroform extracts were largely inactive. The pulp consistently exhibited the highest diversity of phytochemicals, followed by the seedcoat and seed. Antibacterial assays demonstrated that methanolic and ethyl acetate extracts were highly effective against both Gram-positive and Gram-negative bacteria, with the strongest activity observed in the seed methanolic extract from Anand-1 variety (28 ± 1.2 mm against *Bacillus cereus*). Among all treatments, the methanolic pulp extracts of *Phyllanthus emblica* exhibited distinct antifungal efficacy, showing inhibition against *Fusarium oxysporum* and *F. udum*, with the Chakaiya and Kanchan varieties producing the largest inhibition zones (14-15 mm). Further, anti-hemolytic assay validated the strong bioactivity of these extracts, as they recorded the lowest IC₅₀ values (42.30-54.74 µg/mL), surpassing ethyl acetate, aqueous, and chloroform extracts. Furthermore, specific cytotoxicity against MDA-MB cells was observed, an effect unique to the Kanchan variety's methanolic pulp extract. Overall, solvent polarity and fruit part markedly influenced both extractive efficiency and bioactivity. Overall, the methanolic pulp extracts of *Phyllanthus emblica*, especially from the Anand-1 and Chakaiya varieties, demonstrated pronounced antimicrobial and anti-hemolytic efficacy, highlighting their promise for future development into therapeutic agents and functional food formulations.

Keywords: *Phyllanthus emblica*, phytochemicals, antimicrobial activity, antifungal activity, anti-hemolytic assay, Cytotoxic activity

1. Introduction

One of the most valuable medicinal plants in the *Phyllanthaceae* family, *Phyllanthus emblica* L. is widely distributed throughout tropical and subtropical regions including India, Sri Lanka, Malaysia, Thailand and China (Guan *et al.*, 2022) [12]. In India, its cultivation spans several states such as Uttar Pradesh, Gujarat, Madhya Pradesh, Maharashtra and Tamil Nadu with well-known varieties including Chakaiya, Banarasi, Francis, Kanchan, Krishna, Anand-1, Anand-2, NA-7 and NA-10 (Pawar *et al.*, 2024) [24]. Traditionally, *P. emblica* has been an integral component of Ayurvedic formulations such as *triphala* and *chyananprash* attributed to its rejuvenating and restorative properties (Lim, 2012) [16].

Pharmacological investigations have shown that *P. emblica* exhibits a broad spectrum of biological activities including antioxidant, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, nephroprotective and antidiabetic effects (Guan *et al.*, 2022; Tiwana *et al.*, 2025) [12, 37]. These effects are attributed to its rich phytochemicals profile; phenolics, flavonoids, tannins, alkaloids, terpenoids as well as its high ascorbic acid content (Pareek *et al.*, 2022; Suci and Syahputra, 2024) [23, 34]. Earlier studies have mainly emphasized the fruit pulp, yet other parts such as the seed and seedcoat have also been reported to contain significant bioactive compounds like fatty acids, phytosterols and polyphenols suggesting their unexplored medicinal potential (Avinash *et al.*, 2024) [5].

The qualitative phytochemical screening of solvent extracts is an essential step in identifying the classes of bioactive molecules responsible for these biological activities (Halim *et al.*, 2022) ^[14]. Also fruits rich in phenolic and flavonoid compounds are known to play a vital role in combating microbial infections and protecting erythrocytes from oxidative damage (Nath and Dhivya, 2019) ^[21]. Many of these compounds have also garnered significant interest for their potential cytotoxic effects against various cancer cell lines. Therefore, assessing both the antimicrobial and anti-hemolytic activities of *P. emblica* extracts can offer insights into its therapeutic relevance.

In view of the growing interest in natural remedies as safer alternatives to synthetic drugs, the present study aims to evaluate the phytochemical screening, antimicrobial, anti-hemolytic and cytotoxic activities of solvent extracts from different parts (pulp, seedcoat and seed) of four *Phyllanthus emblica* varieties (Chakaiya, Kanchan, Anand-1 and Banarasi). The comparative analysis across multiple solvents provides a better understanding of the extractive efficiency and bioactivity profile of these medicinally significant fruits.

2. Materials and Methods

2.1 Collection of fruits

The fruits of *Phyllanthus emblica* (Amla) varieties namely Anand-1, Banarasi, Chakaiya and Kanchan were collected from the Agro-forestry Research Station, SDAU, S.K. Nagar, Gujrat.

2.2 Phytochemical screening of various part of *P. emblica* varieties

Phytochemical screening of all *P. emblica* extracts was carried out to identify the presence of major classes of bioactive compounds.

2.2.1 Test for tannins

One ml of the extract was mixed with 1ml of 5% ferric chloride solution. The formation of a brown-black colour indicated the presence of tannins (Sultana *et al.*, 2009) ^[35].

2.2.2 Test for saponins

One ml of the extract was diluted with 2ml of Water and shaken vigorously in a graduated cylinder for 15 minutes. The formation of a persistent layer of foam indicated the presence of saponins (Selvaraj *et al.* 2014) ^[30].

2.2.3 Test for quinones

One ml of the extract was mixed with 1ml of concentrated sulfuric acid. The appearance of a dark red colour indicated the presence of quinones (Singh *et al.*, 2015) ^[33].

2.2.4 Test for flavonoids

One ml of the extract was treated with 1ml of 2N sodium hydroxide solution. The development of a yellow colour indicated the presence of flavonoids (Savithramma *et al.* 2011) ^[29].

2.2.5 Test for alkaloids

One ml of the extract was mixed with 2ml of concentrated hydrochloric acid. A few drops of Mayer's reagent were then added. The formation of a green colour indicated the presence of alkaloids (Singh *et al.*, 2015) ^[33].

2.2.6 Test for glycosides

One ml of the extract was added to 3ml of chloroform followed by a few drops of 10% ammonium solution. A pink colour formation indicated the presence of glycosides (Zhao *et al.*, 2015) ^[41].

2.2.7 Test for terpenoids

One ml of the extract was mixed with 2ml of chloroform and a few drops of concentrated sulfuric acid were added. A reddish-brown colouration at the interface indicated the presence of terpenoids (Savithramma *et al.* 2011) ^[29].

2.2.8 Test for phenols

One ml of the extract was combined with 1ml of Water followed by the addition of a few drops of 10% ferric chloride solution. The development of a dark blue colouration confirmed the presence of phenols (Selvaraj *et al.* 2014) ^[30].

2.3 Antimicrobial activity

The antimicrobial activity of various extracts from different parts (pulp, seedcoat and seed) of *P. emblica* varieties was assessed using the agar well diffusion method with slight modifications as per the procedure described by Manandhar *et al.* (2019) ^[17].

2.3.1 Antibacterial activity

The antibacterial activity of the extracts was evaluated against two Gram-positive (*S. aureus*, *B. cereus*) and two Gram-negative (*E. coli*, *S. Typhi*) strains using the agar well diffusion method. Fresh bacterial suspensions were adjusted to an OD of 0.1 at 600 nm (Ahmed *et al.*, 2023) ^[1] and uniformly swabbed (100 μ L) onto Mueller-Hinton agar plates. Wells (8 mm) were made with a sterile cork borer and filled with 100 μ L of each extract. DMSO and ampicillin served as negative and positive controls, respectively. Plates were kept at 4°C for 1 h to allow diffusion, then incubated at 37°C for 24 h, after which inhibition zones (mm) were measured.

2.3.2 Antifungal activity

Antifungal activity was evaluated against *Fusarium oxysporum* f. sp. *ricini* and *F. udum* using the agar well diffusion method. Spore suspensions were adjusted to an OD of 0.1 at 600 nm (Ahmed *et al.*, 2023) ^[1] and 100 μ L was swabbed onto sterile SDA plates. Wells (8 mm) were filled with 100 μ L of each extract; DMSO and nystatin served as negative and positive controls, respectively. Plates were kept at 4°C for 1 h to permit diffusion, then incubated at 28°C for 4-5 days. Antifungal activity was determined by measuring inhibition zone diameters (mm).

2.4 Anti-hemolytic assay

The anti-hemolytic activity of *Phyllanthus emblica* extracts was assessed following Yang *et al.* (2005) ^[39]. Fresh human blood was obtained from healthy volunteer via Health Centre, SDAU, Sardarkrushinagar and collected in EDTA-treated tubes.

Extracts from four varieties and three fruit parts were tested at concentrations of 125-1000 μ g/mL. Fresh human blood (EDTA-treated) was centrifuged at 2000 rpm for 10 min, and erythrocytes were washed with PBS to obtain a 10% hematocrit suspension. For the assay, 250 μ L of erythrocyte suspension was mixed with 500 μ L of extract (test), PBS (negative control) or water (positive control) and incubated

at 37°C for 4 hr. Samples were then diluted to 4.5 mL with PBS, centrifuged and the absorbance of the supernatant was measured at 540 nm (Yang *et al.*, 2017) ^[40]. Percent hemolysis was calculated as:

$$\text{Hemolysis rate (\%)} = [(A_{\text{extract}} - A_{\text{negative control}} / A_{\text{positive control}}) \times 100]$$

2.5 Cytotoxic activity (MTT assay)

The cytotoxic activity of methanolic extracts of *Phyllanthus emblica* was evaluated using the MTT assay against breast cancer (MDA-MB) cell line. Cells were maintained in growth medium with 10% FBS and antibiotics at 37°C in 5% CO₂. After seeding into 96-well plates and allowing 36 hr for adherence, cells were treated with serial dilutions of the extracts (stock = 2 mg/mL). Following incubation, 10 µL of MTT solution was added and plates were further incubated for formazan formation. The medium was removed; crystals were dissolved in 100 µL DMSO and absorbance was read at 570 nm. Cell viability (%) was calculated relative to untreated controls.

3. Result and Discussion

3.1 Phytochemical screening of *P. emblica* varieties

A comparative phytochemical screening was performed on the pulp, seedcoat and seed of four *Phyllanthus emblica*

varieties using solvents (methanol, ethyl acetate, water and chloroform). Eight major phytochemical groups were assessed (Tables 1 and 2). Methanolic pulp extracts showed the richest profile, with abundant phenols and quinones across all varieties and strong flavonoid presence in 'Chakaiya' and 'Kanchan'. Moderate levels of tannins, saponins, alkaloids, glycosides and terpenoids were also detected. Ethyl acetate extracts moderately showed tannins, flavonoids and phenols, whereas aqueous and chloroform extracts showed limited or absence of phytochemicals.

In seedcoat and seed, methanol again proved most efficient, yielding notable tannins and quinones along with moderate flavonoids, alkaloids and terpenoids. Other solvents showed poor presence, with chloroform largely inactive. Overall, extraction efficiency was strongly influenced by solvent polarity and plant part, with pulp extracts consistently richest in bioactive constituents.

These observations agree with previous studies (Sultana *et al.*, 2009; Singh *et al.*, 2015) ^[35, 33], which highlight methanol's superior polarity and solubility range and with Rahman *et al.* (2013) ^[26], who reported chloroform's weak performance due to low polarity. The present results confirm methanol as the most effective solvent for broad-spectrum phytochemical recovery in *P. emblica*.

Table 1: Phytochemical screening of *P. emblica* parts with different solvents

(A) Variety: Chakaiya		P				Sc				S			
Sr.	Phytochemical constituents	M	E	C	W	M	E	C	W	M	E	C	W
1.	Tannins	++	++	-	++	+++	+	-	-	+++	+	-	-
2.	Saponins	++	+	-	+	+	+	-	+	+	+	-	+
3.	Quinones	+++	++	-	+	+++	++	-	+	++	+	-	+
4.	Flavonoids	+++	++	-	++	+++	++	-	+	++	+	-	+
5.	Alkaloids	++	++	+	-	+	+	-	-	+	+	-	-
6.	Glycosides	++	+	-	-	+++	+	-	-	++	++	-	+
7.	Terpenoids	++	+	+	-	++	+	+	-	++	+	+	-
8.	Phenols	+++	+++	-	++	+++	+++	-	+	++	++	-	+
(B) Variety: Kanchan		P				Sc				S			
Sr.	Phytochemical constituents	M	E	C	W	M	E	C	W	M	E	C	W
1.	Tannins	++	++	-	++	+++	+	-	-	+++	+	-	-
2.	Saponins	++	+	-	+	+	+	-	+	+	+	-	+
3.	Quinones	+++	++	-	+	+++	++	-	+	++	+	-	+
4.	Flavonoids	+++	++	-	++	++	++	-	+	++	+	-	+
5.	Alkaloids	++	++	+	-	+	+	-	+	+	+	-	+
6.	Glycosides	++	+	-	-	+++	+	-	-	++	++	-	+
7.	Terpenoids	++	+	+	-	++	+	+	-	++	+	+	-
8.	Phenols	+++	+++	-	++	+++	++	-	+	++	+	-	+

+++ = Significantly present, ++ = Moderately present, + = Poorly present, - = Absent; (P= Pulp, Sc= Seed coat, S= Seed, M= Methanol, E= Ethyl acetate, C= Chloroform, W= Water)

Table 2: Phytochemical screening of *P. emblica* parts with different solvents

(C) Variety: Anand-1		P				Sc				S			
Sr.	Phytochemical constituents	M	E	C	W	M	E	C	W	M	E	C	W
1.	Tannins	++	++	-	++	+++	+	-	-	+++	+	-	-
2.	Saponins	++	+	-	+	+	+	-	+	+	+	-	+
3.	Quinones	+++	++	-	+	+++	++	-	+	++	+	-	+
4.	Flavonoids	+++	++	-	++	++	++	-	+	++	+	-	+
5.	Alkaloids	++	++	+	-	+	+	-	+	+	+	-	+
6.	Glycosides	++	+	-	-	+++	+	-	-	++	++	-	+
7.	Terpenoids	++	+	+	-	++	+	+	-	++	+	+	-
8.	Phenols	+++	+++	-	++	+++	++	-	+	++	+	-	+
(D) Variety: Banarasi		P				Sc				S			
Sr.	Phytochemical constituents	M	E	C	W	M	E	C	W	M	E	C	W
1.	Tannins	++	++	-	++	+++	+	-	-	+++	+	-	-
2.	Saponins	++	+	-	+	+	+	-	+	+	+	-	+

3.	Quinones	+++	++	-	+	+++	++	-	+	++	+	+	+
4.	Flavonoids	++	++	-	+	+	+	-	+	+	+	-	+
5.	Alkaloids	++	++	-	-	++	+	-	-	+	+	-	-
6.	Glycosides	++	+	-	-	++	+	-	-	++	++	-	+
7.	Terpenoids	++	+	+	-	++	+	+	-	++	+	+	-
8.	Phenols	+++	++	-	++	++	+	-	+	++	+	-	+

+++ = Significantly present, ++ = Moderately present, + = Poorly present, - = Absent; (P= Pulp, Sc= Seed coat, S= Seed, M= Methanol, E= Ethyl acetate, C= Chloroform, W= Water)

3.2 Antimicrobial activity of *P. emblica* varieties

3.2.1 Antibacterial activity

The present study demonstrated that the antibacterial activity of *Phyllanthus emblica* extracts strongly depended on the solvent system. Extracts from the pulp, seedcoat, and seed of four varieties were tested against two Gram-positive (*S. aureus*, *B. cereus*) and two Gram-negative (*E. coli*, *S. Typhi*) strains (Table 3; Fig 1-4). The negative control (DMSO) showed no inhibition, while the positive control (Ampicillin) exhibited strong activity (17-29 mm).

A clear dependency on solvent polarity was observed for extraction efficacy. aqueous and chloroform extracts were inactive, whereas methanolic and ethyl acetate extracts

displayed broad-spectrum antibacterial effects. Methanol consistently yielded the largest inhibition zones, confirming its higher efficiency in extracting active antibacterial constituents. Among plant parts, the pulp showed the strongest activity, followed by the seedcoat and seed.

Varietal differences were evident, with Anand-1 showing the highest antibacterial potential. Its seed methanolic extract recorded the maximum inhibition zone (28 ± 1.2 mm) against *B. cereus*, and the pulp methanolic extract showed high activity against *E. coli* (26 ± 1.4 mm) and *B. cereus* (26 ± 1.1 mm). Kanchan and Chakaiya also demonstrated notable inhibition, whereas Banarasi displayed comparatively weak activity.

Table 3: Antibacterial activity of different solvent extracts derived from fruit parts of *P. emblica* varieties

Sr. No	Variety	Parts	Extract	Diameter of inhibition zone (mm)			
				<i>S. aureus</i>	<i>B. Cereus</i>	<i>E. coli</i>	<i>S. Typhi</i>
1	Chakaiya	P	E	12 ± 1.4	19 ± 1.0	20 ± 1.2	18 ± 1.0
			M	17 ± 1.1	20 ± 1.2	24 ± 1.5	18 ± 1.3
		Sc	E	10 ± 1.4	14 ± 1.3	16 ± 1.2	14 ± 0.7
			M	15 ± 1.3	16 ± 1.3	19 ± 1.1	16 ± 1.6
		S	M	12 ± 1.1	14 ± 0.9	20 ± 1.3	12 ± 1.1
2	Kanchan	P	E	13 ± 1.3	20 ± 1.1	21 ± 1.0	13 ± 1.1
			M	17 ± 1.2	25 ± 1.2	24 ± 1.3	14 ± 1.2
		Sc	E	4 ± 1.4	15 ± 1.6	14 ± 1.8	11 ± 0.9
			M	11 ± 1.2	18 ± 0.8	21 ± 1.5	12 ± 1.2
		S	M	16 ± 1.2	15 ± 1.0	16 ± 1.3	15 ± 1.6
3	Anand-1	P	E	12 ± 1.4	21 ± 1.4	20 ± 1.5	18 ± 0.9
			M	15 ± 1.2	26 ± 1.1	26 ± 1.4	19 ± 1.1
		Sc	E	4 ± 1.3	14 ± 1.4	16 ± 1.8	10 ± 1.0
			M	9 ± 1.5	15 ± 1.3	22 ± 1.6	13 ± 1.3
		S	M	12 ± 1.1	28 ± 1.2	20 ± 1.3	21 ± 1.4
4	Banarasi	P	E	6 ± 1.6	16 ± 0.9	14 ± 1.4	8 ± 1.1
			M	13 ± 1.2	18 ± 1.2	20 ± 1.1	10 ± 1.3
		Sc	E	4 ± 1.3	16 ± 1.6	9 ± 1.7	9 ± 1.5
			M	11 ± 1.4	17 ± 1.1	15 ± 1.2	12 ± 0.9
		S	M	9 ± 1.3	12 ± 0.8	11 ± 1.6	12 ± 1.2
Ampicillin				17	29	28	23
DMSO				0	0	0	0

Values are presented as mean $\pm$ S.D, n=3. - = indicates the no zone of inhibition; (P= Pulp, Sc= Seed coat, S= Seed, C= Chloroform, E= Ethyl acetate, M= Methanol, W= Water)

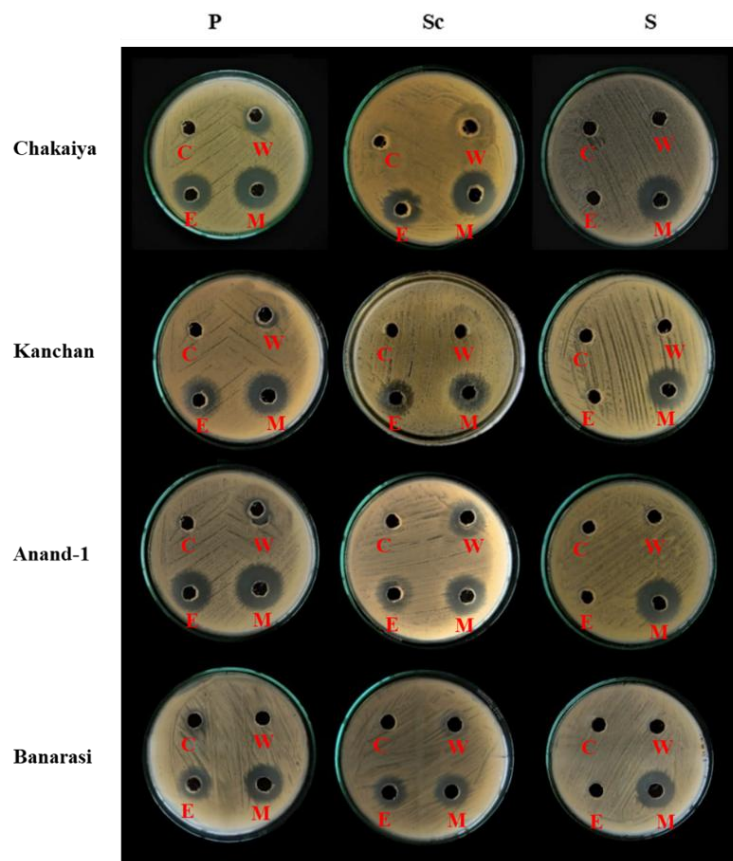


Fig 1: Antibacterial activity of different extracts of *P. emblica* varieties against *Staphylococcus aureus* using agar well diffusion method; (P= Pulp, Sc= Seed coat, S= Seed, C= Chloroform, E= Ethyl acetate, M= Methanol, W= Water)

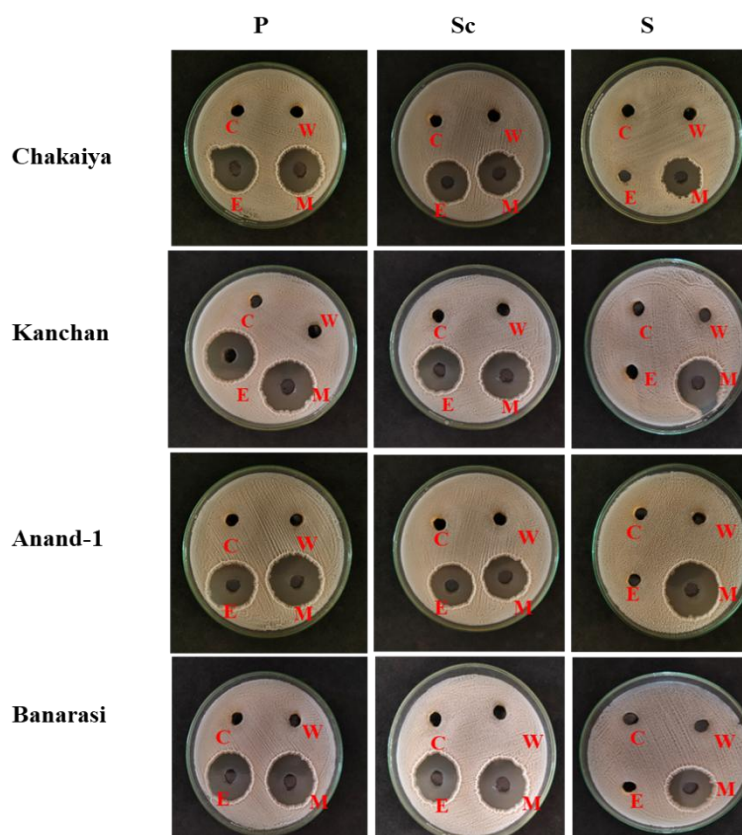


Fig 2: Antibacterial activity of different extracts of *P. emblica* varieties against *Bacillus cereus* using agar well diffusion method; (P= Pulp, Sc= Seed coat, S= Seed, C= Chloroform, E= Ethyl acetate, M= Methanol, W= Water)

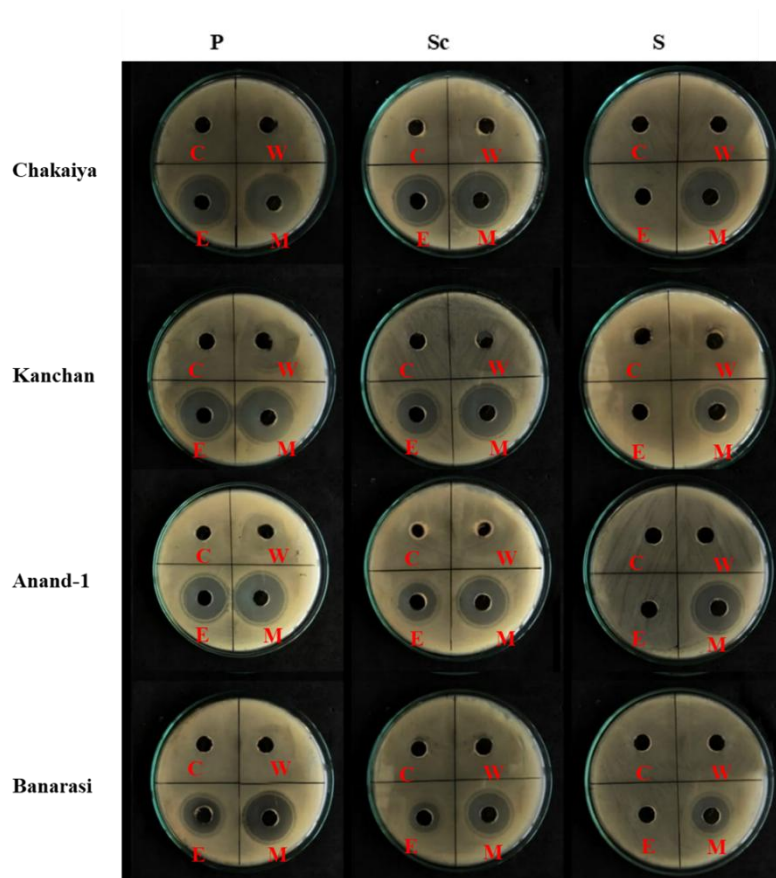


Fig 3: Antibacterial activity of different extracts of *P. emblica* varieties against *Escherichia coli* using agar well diffusion method; (P= Pulp, Sc= Seed coat, S= Seed) (C= Chloroform, E= Ethyl acetate, M= Methanol, W= Water)

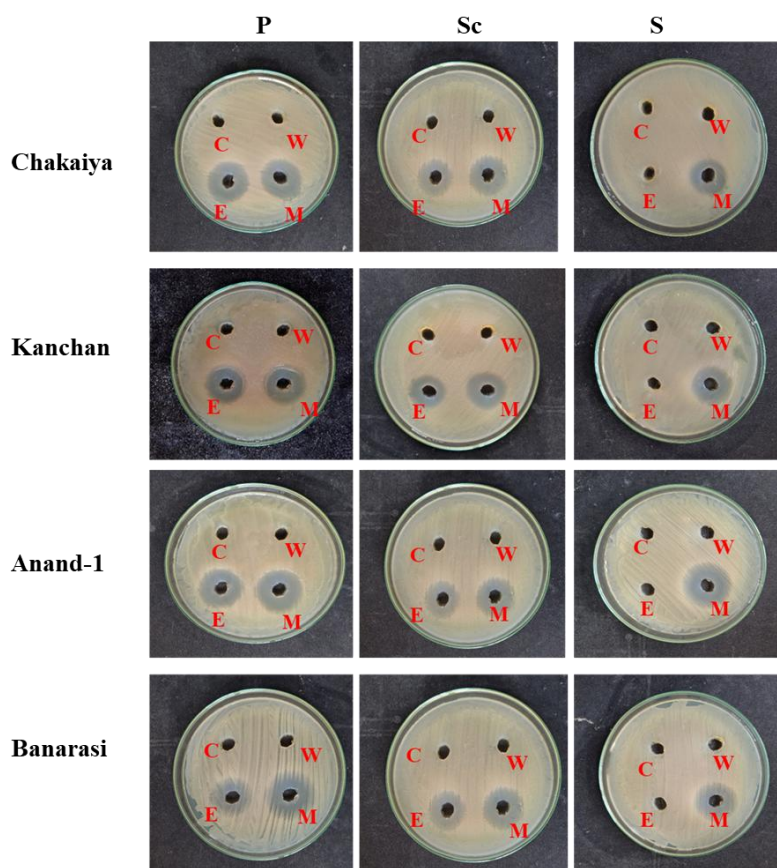


Fig 4: Antibacterial activity of different extracts of *P. emblica* varieties against *Salmonella Typhi* using agar well diffusion method; (P= Pulp, Sc= Seed coat, S= Seed) (C= Chloroform, E= Ethyl acetate, M= Methanol, W= Water)

These findings are consistent with earlier reports. The inhibition zones for *S. aureus* (9-16 mm) align with Anbuselvi and Jha (2015) [2], while moderate ethyl acetate activity agrees with Goyal *et al.* (2022) [11]. The methanolic pulp extracts in this study showed stronger *E. coli* inhibition (up to 26 mm) than the 14.2 mm reported by Anusha (2019) [3], and comparable to values observed by Ghosh and Dey (2022) [10]. The pronounced inhibition of *B. cereus* (up to 28 ± 1.2 mm) parallels the results of Phyto *et al.* (2020) [25], confirming the potent antibacterial efficacy of *P. emblica* particularly the methanolic pulp and seed extracts of Anand-1.

The superior performance of Anand-1 is chemically driven by its high concentration of gallic acid, ellagic acid, rutin and quercetin (Tiwana *et al.*, 2025) [38]. Molecular docking studies reveal that ellagic acid and quercetin bind specifically to penicillin-binding protein 3 (PBP-3) and tyrosyl-tRNA synthetase in bacteria (Saini *et al.*, 2024) [28]. This interaction inhibits cell wall synthesis and protein translation, respectively. Furthermore, these phenolics and tannins act by binding to bacterial surface proteins, causing the leakage of intracellular contents and ultimately leading to cell death (Cahyaningrum *et al.*, 2025) [8].

3.2.2 Antifungal activity of *P. emblica* varieties

The antifungal efficacy of *P. emblica* extracts was assessed against *Fusarium oxysporum* f. sp. *ricini* and *Fusarium udum* using the agar well diffusion method. Nystatin served as the positive control and DMSO was the negative control. Results are presented in Table 4.

A significant finding was that only methanolic pulp extracts exhibited observable antifungal activity. Ethyl acetate, chloroform and water extracts were ineffective, indicating their failure to extract the relevant antifungal constituents. Against *F. oxysporum* f. sp. *ricini*, the methanolic pulp

extract of the Chakaiya variety showed the highest inhibition (15 mm), followed by Kanchan (14 mm), Banarasi (12 mm) and Anand-1 (5 mm). A similar trend was observed against *F. udum*, with Chakaiya again showing strong activity (14 mm), followed by Kanchan (12 mm), Banarasi (10 mm) and Anand-1 (9 mm).

These results confirm that methanol is an effective solvent for extracting antifungal compounds from *P. emblica* pulp. The Chakaiya and Kanchan varieties consistently demonstrated superior activity, implying a higher concentration of bioactive antifungal phytochemicals.

The inhibition zones observed in this study (ranging from 12-15 mm in effective varieties) align directly with previous research. Arora and Bharti (2014) [4] reported significant activity (12-16 mm) for methanolic amla pulp extracts against *Fusarium oxysporum*. This efficacy is also supported by findings from Bhalodia *et al.* (2011) [7] (13.2 mm zone), Goyal *et al.* (2022) [11] (13-16 mm), and Mazumder *et al.* (2008) [18] (11-13 mm) against *Fusarium* spp., reaffirming the antifungal potency of methanol-extracted pulp components.

The significant inhibition zones observed for Chakaiya and Kanchan are attributed to their high levels of hydrolyzable tannins, such as emblicanin A and B (Cárdenas and Laverde 2021) [9]. These tannins function as biological surfactants that compromise fungal cell membrane integrity. This leads to a dose-dependent leakage of water-soluble carbohydrates and intracellular proteins from the fungal hyphae. Transcriptomic profiling suggests that these phytochemicals also down-regulate key metabolic pathways, including the MAPK signalling pathway and porphyrin metabolism, effectively shutting down fungal growth and pathogenicity (Rohilla *et al.*, 2023) [27].

Table 4: Antifungal activity of methanolic extracts derived from fruit parts of *P. emblica* varieties

Sr. No.	Variety	Parts	Extract	Diameter of inhibition zone (mm)	
				<i>F. oxysporum</i> f. sp. <i>ricini</i> (mm)	<i>F. udum</i> (mm)
1	Chakaiya	P	M	15 ± 1.1	14 ± 1.2
2	Kanchan			14 ± 1.3	12 1.5
3	Anand-1			5 ± 1.2	9 ± 1.4
4	Banarasi			12 ± 1.5	10 ± 1.3
Nystatin				17	20
DMSO				0	0

Values are presented as mean ± S.D, n=3; (P= Pulp, M= Methanol)

Values are presented as mean ± S.D, n=3; (P= Pulp, M= Methanol)

3.3 Anti-hemolytic activity of *P. emblica* varieties

The anti-hemolytic activity of pulp, seedcoat and seed extracts from four *Phyllanthus emblica* varieties was assessed using different solvents (Table 5). IC₅₀ values varied notably with both plant part and solvent system. Methanolic pulp extracts showed the strongest activity with the lowest IC₅₀ values (42.30-54.74 µg/mL), followed by ethyl acetate (74.94-151.08 µg/mL), water (131.58-305.13 µg/mL) and chloroform extracts, which were least effective. Across all varieties, pulp extracts consistently outperformed seedcoat and seed extracts, confirming the pulp as the richest source of anti-hemolytic compounds. Overall solvent efficiency followed the order: Methanol > Ethyl acetate > Water > Chloroform, while plant parts followed: Pulp > Seed coat > Seed.

Compared to previous findings by Sharif *et al.* (2023), who reported an IC₅₀ of 101.08 µg/mL for methanolic fruit extract, the present study recorded much lower IC₅₀ values (42-55 µg/mL), indicating stronger hemolysis inhibition and higher concentrations of active phenolic and flavonoid constituents in the tested materials.

The protective effect is chemically linked to Amla's rich phenolic and flavonoid content. Phytochemicals like catechin and epicatechin stabilize the erythrocyte membrane by neutralizing free radicals (Tao *et al.*, 2022) [36]. Furthermore, these compounds specifically regulate the 15-lipoxygenase (15-LOX) mediated pathway, preventing the transformation of arachidonic acid into inflammatory mediators that otherwise cause membrane rupture (Saini *et al.*, 2024) [28].

Table 5: Anti-hemolytic activity (IC50) of different solvent extracts derived from fruit parts of *P. emblica* varieties

Sr. No.	Variety	Parts	C	E	M	W
1	Chakaiya	P	341.58	77.44	44.85	135.18
		Sc	543.25	101.79	71.62	183.27
		S	684.81	148.82	82.85	212.52
2	Kanchan	P	438.83	83.32	54.74	131.58
		Sc	747.92	117.21	80.21	219.55
		S	655.97	151.08	100.2	305.13
3	Anand-1	P	305.89	74.94	42.31	167.86
		Sc	612.50	123.78	71.21	184.78
		S	847.19	128.99	82.72	180.82
4	Banarasi	P	411.54	84.22	43.26	137.30
		Sc	-	111.63	67.04	159.31
		S	-	133.99	86.00	236.18
Factor		SE(m)		C.D. at 1%		CV%
Part		0.147		0.443		5.72
Solvent		0.170		0.662		
Part x solvent		0.295		1.108		
(P= Pulp, Sc= Seed coat, S= Seed, C= Chloroform, E= Ethyl acetate, M= Methanol, W= Water)						

(P= Pulp, Sc= Seed coat, S= Seed, C= Chloroform, E= Ethyl acetate, M= Methanol, W= Water)

3.4 Cytotoxic activity of *P. emblica* varieties

The cytotoxic effects of methanolic pulp extract of four Amla varieties were evaluated against a breast cancer cell line (MDA-MB) using an MTT assay. Among the tested varieties, only the Kanchan variety exhibited significant cytotoxic activity. This toxicity was specific, occurring at the 0.5 mg/ml concentration, where it reduced cell viability to approximately 47-56% (Fig 5). In contrast, the other varieties did not show a similar cytotoxic effect.

Shashank *et al.* (2021) [32] reported that methanolic extracts of Amla exhibited cytotoxic effects. Similarly, Baliga *et al.* (2011) [6] emphasized Amla's broad anticancer potential, noting that its efficacy is strongly influenced by the variety, extraction method and dosage. Amla varieties possess higher levels of bioactive compounds such as polyphenols

and tannins, which may account for their enhanced cytotoxic properties.

Kanchan's specific efficacy is chemically attributed to its unique accumulation of bioactive polyphenols and tannins. Studies show that Kanchan can contain exceptionally high levels of ellagic acid compared to other cultivars (Zhao *et al.*, 2015) [41]. These components, along with emblicanin A and B, induce apoptosis in MDA-MB-231 cells by modulating the cell cycle at the G2 phase and interfering with cyclin B regulation (Natsakulmongkon and Poeaim 2026) [22]. This results in internucleosomal DNA fragmentation and membrane blebbing specifically in malignant cells, while showing four-fold lower sensitivity toward normal fibroblasts (Huang *et al.*, 2022) [15].

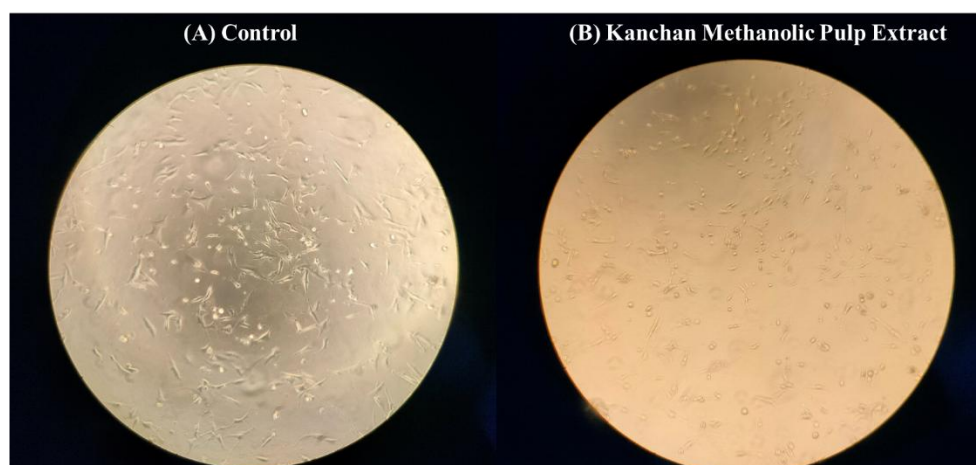


Fig 5: Cytotoxic effect on MDA-MB cells. (A) Control (B) Kanchan Methanolic Pulp Extract

Conclusion

The present investigation provides a comprehensive comparative assessment of the phytochemical composition and biological efficacy of different parts (pulp, seedcoat and seed) of four *Phyllanthus emblica* varieties Anand-1, Banarasi, Chakaiya and Kanchan extracted using solvents of varying polarity. The findings collectively highlight the profound influence of solvent type and plant part on the yield and spectrum of bioactive compounds, as well as their associated antimicrobial, antifungal, anti-hemolytic and cytotoxic potentials.

Among all tested extracts, the methanolic extracts consistently demonstrated superior extraction efficiency and biological activity, owing to their ability to solubilize a wide range of polar phytochemicals, particularly phenolics, flavonoids, tannins, alkaloids and quinones. The pulp part emerged as the richest source of these bioactive constituents, followed by the seedcoat and seed. This phytochemical abundance was directly reflected in its biological assays. The hierarchy of solvent efficacy (Methanol > Ethyl acetate > Water > Chloroform) and plant part contribution (Pulp > Seedcoat > Seed) was consistent

across all analyses, establishing a clear pattern in bioactivity correlation.

Anand-1 is scientifically identified as a leader for antibacterial applications due to its flavonoid-PBP-3 binding affinity. Chakaiya is the superior antifungal candidate, acting through tannin-mediated membrane disruption. Most notably, the Kanchan variety stands out as a high-potential source for anticancer molecules, specifically targeting triple-negative breast cancer pathways through G2 cell cycle arrest.

This varietal specificity highlights the need for a targeted approach in the development of natural therapeutic formulations and functional foods. Future research should focus on high-resolution metabolomic isolation of these individual compounds to maximize the clinical translation of *Phyllanthus emblica* in modern healthcare.

Acknowledgment

The author acknowledges the S.D. Agricultural University for providing financial support and conducive working during this study.

Conflict of interests

The authors declare that there are no financial/commercial conflicts of interest that could have appeared to influence the work reported in this paper.

Data availability statements

Data sharing is not applicable to this article.

Ethics declaration

Ethics declaration statement is not applicable.

References

- Ahmed A, Aggarwal P, Bajwa U. Effect of fruit acidulants and storage conditions on the physicochemical, microbiological and sensory characteristics of paneer. *East Afr J Sci Technol Innov.* 2023;4.
- Anbuselvi S, Jha AK. Antibacterial and phytochemical analysis of *Phyllanthus emblica* seed and pulp. *Int J Curr Pharm Res.* 2015;7(2):79-81.
- Anusha B. Evaluation of antimicrobial activity of *Phyllanthus emblica* fruit extracts against human pathogens. *Int J Sci Res Rev.* 2019;8(5):1002-1008.
- Arora DS, Bharti M. Antifungal activity of *Phyllanthus emblica* fruit extracts and its synergistic effect with commercially available antifungal drugs. *J Pharm Phytochem.* 2014;3(1):1-5.
- Avinash PG, Hamid R, Shams R, *et al.* Recent insights into the morphological, nutritional and phytochemical properties of Indian gooseberry (*Phyllanthus emblica*) for the development of functional foods. *Plants.* 2024;13(5):574. DOI:10.3390/plants13050574.
- Baliga MS, Dsouza JJ. Amla (*Emblca officinalis* Gaertn), a wonder berry in the treatment and prevention of cancer. *Eur J Cancer Prev.* 2011;20(3):225-239. DOI:10.1097/cej.0b013e32834473f4.
- Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. *In vitro* antimicrobial activity of *Psoralea corylifolia* seeds extract against some pathogenic micro-organisms. *Ayurpharm Int J Ayurveda Allied Sci.* 2011;1(2):32-38.
- Cahyaningrum PL, Sudaryati NLG, Bogoriani NW, *et al.* Phytochemical profiling, antibacterial properties and toxicity of Amla fruit tea (*Phyllanthus emblica* L.): An *in vitro* and *in silico* study. *J Multidiscip Appl Nat Sci.* 2025. DOI:10.47352/jmans.2774-3047.284.
- Cárdenas-Laverde D, Barbosa-Cornelio R, Coy-Barrera E. Antifungal activity against *Fusarium oxysporum* of botanical end-products: An integration of chemical composition and antifungal activity datasets to identify antifungal bioactives. *Plants.* 2021;10(12):2563. DOI:10.3390/plants10122563.
- Ghosh A, Dey S. Comparative analysis of antimicrobial activities of raw and processed extracts of *Phyllanthus emblica* L. against *E. coli* and *S. aureus*. *J Microbiol Experiment.* 2022;10(1):34-39.
- Goyal M, Pareek A, Sharma P. Evaluation of antimicrobial activity of various solvent extracts of *Phyllanthus emblica* seed coat. *J Pharm Phytochem.* 2022;11(4):620-625.
- Guan Q, Feng DP, Duan BZ, Fan M. Research progress on chemical constituents and pharmacological effects of *Phyllanthus emblica* and predictive analysis of quality markers. *Zhong Cao Yao.* 2022;1-14.
- Gupta A, Mishra AK, Mishra P. Nutritional and therapeutic potential of *Emblca officinalis*: A review. *Int J Pharm Biol Arch.* 2014;5(5):809-818.
- Halim B, Syahputra RA, Adenin I, *et al.* Determination of phytochemical constituent, antioxidant activity, total phenol and total flavonoid of extract ethanol *Phyllanthus emblica* fruit. *Pharm J.* 2022;14(1):63-67.
- Huang H, Tan P, Li M, *et al.* Quality analysis combined with mass spectrometry imaging reveal the difference between wild and cultivated *Phyllanthus emblica* Linn.: From chemical composition to molecular mechanism. *Arab J Chem.* 2022;15(6):103790. DOI: 10.1016/j.arabjc.2022.103790.
- Lim TK, editor. *Edible Medicinal and Non-Medicinal Plants: Volume 4, Fruits.* Springer Netherlands; 2012.
- Manandhar S, Luitel S, Dahal RK. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *J Trop Med.* 2019;20(1):1-5.
- Mazumder PM, Sasmal D, Mandal SC. Evaluation of antifungal potential of *Phyllanthus emblica* fruit extracts against some plant pathogenic fungi. *Pharm Mag.* 2008;4(14):120-123.
- Mbatchou VC, Kosoono SM. Extraction and phytochemical analysis of medicinal plants with anti-diabetic properties. *J Pharm Phytother.* 2011;3(5):107-114.
- Mishra AP, Mahanta CL. Phenolic content and antioxidant activity of *Phyllanthus emblica* L. (amla) as affected by drying methods. *Indian J Nat Prod Resour.* 2015;5(1):56-60.
- Nath A, Dhivya R. Phytochemical analysis of *Phyllanthus emblica* Linn. using different solvents. *Int J Curr Microbiol Appl Sci.* 2019;8(5):1760-1767.
- Natsakulmongkon P, Poeaim S. Bioactive potential of *Gardenia obtusifolia* extracts: Antibacterial effects of the ethyl acetate fraction against clinical pathogens. *J Pure Appl Microbiol.* 2026;20(1):470. DOI:10.22207/jpam.20.1.34.
- Pareek V, Li Q, Jing L, *et al.* Current advances on the phytochemical composition and pharmacological activities of *Phyllanthus emblica*. *Front Pharmacol.* 2022. DOI:10.3389/fphar.2022.1017268.

24. Pawar H, Hamid N, Shams R, *et al.* Recent insights into the morphological, nutritional and phytochemical properties of Indian gooseberry (*Phyllanthus emblica*) for the development of functional foods. *Plants*. 2024;13(5):574-574.
25. Phyo KMM, Win PP, Aung HT. Antibacterial activity of *Phyllanthus emblica* Linn. fruit and leaf extracts against selected pathogenic bacteria. *Univ Mandalay Res J*. 2020;11(1):45-52.
26. Rahman MM, Islam MB, Biswas M, Alam AHMK. *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res Notes*. 2013; 6:427.
27. Rohilla G, Masand P, Dhama P, *et al.* Quality by Design approach for the investigation of critical characteristics of *Phyllanthus emblica* from different vicinities. *Digit Chin Med*. 2023;6(3):272-284. DOI: 10.1016/j.dcm.2023.10.003.
28. Saini R, Kumar V, Patel CN, *et al.* Synergistic antibacterial activity of *Phyllanthus emblica* fruits and its phytocompounds with ampicillin: a computational and experimental study. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2024;397(2):857-871. DOI:10.1007/s00210-023-02624-0.
29. Savithramma R, Bhumi G. Phytochemical screening of *Thespesia populnea* (L.) Soland and *Tridax procumbens* L. *J Chem Pharm Res*. 2011;3(5).
30. Selvaraj S, Chittibabu CV, Janarthanam B. Studies on phytochemical screening, antioxidant activity and extraction of active compound (Swertiamarin) from leaf extract of *Enicostemma littorale*. *Asian J Pharm Clin Res*. 2014;7(4):240-244.
31. Sharif MA, Khan AM, Salekeen R, *et al.* *Phyllanthus emblica* (Amla) methanolic extract regulates multiple checkpoints in 15-lipoxygenase mediated inflammopathies: Computational simulation and *in vitro* evidence. *Saudi Pharm J*. 2023;31(8):101681.
32. Shashank S, Kumar KY, KS VR. Evaluation of anti-lipid peroxidation and cytotoxic activity in the methanolic extracts of Hibiscus and Amla. *J Med Plants Stud*. 2021;9(3):52-55. DOI: 10.22271/plants.2021.v9.i3a.1293.
33. Singh AK, Singh S, Makwana P. Characterization of aonla (*Embllica officinalis*) varieties under zero irrigation semi-arid conditions. *Indian J Agric Sci*. 2015;85(21):1365-1369.
34. Suci N, Syahputra H. Phytochemical constituent analysis of *Phyllanthus emblica* L. fruit nanoherbals by LC-HRMS and their antimutagenic activity and teratogenic effects. *Molecules*. 2024;29(7):1642. DOI:10.3390/molecules29071642.
35. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*. 2009;14(6):2167-2180.
36. Tao H, Zhong J, Mo Y, *et al.* Exploring the mechanism through which *Phyllanthus emblica* L. extract exerts protective effects against acute gouty arthritis: A network pharmacology study and experimental validation. *Evid Based Complement Altern Med*. 2022;2022(4):1-16. DOI:10.1155/2022/9748338.
37. Tiwana G, Cock IE, Cheesman MJ. *Phyllanthus emblica*: Phytochemistry, antimicrobial potential with antibiotic enhancement, and toxicity insights. *Microorganisms*. 2025;13(3):611. DOI:10.3390/microorganisms13030611.
38. Tiwana G, *et al.* Phytochemistry, antibacterial and toxicity properties of *Phyllanthus emblica* fruit extracts: A recent insight. *J Ethnopharmacol*. 2025.
39. Yang ZG, Sun HX, Fang WH. Haemolytic activities and adjuvant effect of *Astragalus membranaceus* saponins (AMS) on the immune responses to ovalbumin in mice. *Vaccine*. 2005;23(44):5196-5203.
40. Yang HL, Korivi M, Lin MK, *et al.* Antihemolytic and antioxidant properties of pearl powder against 2, 2'-azobis (2 amidinopropane) dihydrochloride-induced hemolysis and oxidative damage to erythrocyte membrane lipids and proteins. *J Food Drug Anal*. 2017;25(4):898-907.
41. Zhao T, Sun Q, Marques M, Witcher M. Anticancer properties of *Phyllanthus emblica* (Indian Gooseberry). *Oxid Med Cell Longev*. 2015;2015(2):1-7. DOI:10.1155/2015/950890.