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PHYTOCHEMICAL PROFILING AND CHROMATOGRAPHIC EVALUATION OF *ALLIUM SATIVUM* AND *TERMINALIA CHEBULA*: IMPLICATIONS FOR PEPTIC ULCER MANAGEMENT AND ANTIMICROBIAL RESISTANCE MITIGATION

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ABSTRACT

Peptic ulcer disease (PUD) remains a significant gastrointestinal disorder worldwide, largely associated with *Helicobacter pylori* infection and exacerbated by antimicrobial resistance (AMR). The current study investigates the phytochemical composition of *Allium sativum* (garlic) and evaluates its potential therapeutic role in managing PUD, *H. pylori* eradication, and AMR mitigation. Preliminary phytochemical screening of garlic extracts using various solvents confirmed the presence of bioactive constituents including alkaloids, phenolics, flavonoids, tannins, terpenoids, and saponins, with ethanolic extracts showing the highest concentrations. Quantitative analysis further revealed notable levels of alkaloids (2.53 mg/g), phenols (2.70 mg/g), flavonoids (2.13 mg/g), and terpenoids (2.18 mg/g), all of which are known for their gastroprotective and antimicrobial properties. High-Performance Thin Layer Chromatography (HPTLC) profiling confirmed the presence of key phytoconstituents such as alliin, gallic acid, rutin, and lupeol. These compounds are documented to inhibit *H. pylori*, reduce oxidative stress, and promote mucosal healing. High-Performance Liquid Chromatography (HPLC) further quantified allicin, a potent sulfur-containing compound, with a retention time of 16.789 minutes. Allicin exhibits broad-spectrum antibacterial activity and disrupts bacterial biofilms, aiding in *H. pylori* eradication and reducing the development of resistance. The study highlights the therapeutic relevance of *A. sativum* in gastrointestinal health and as a natural alternative or adjunct to conventional ulcer therapies. Its phytochemical complexity and multi-targeted mechanisms make it a promising candidate in addressing both peptic ulcer disease and the global challenge of antimicrobial resistance.

KEYWORDS: *Allium sativum, Terminalia chebula*, Peptic ulcer disease, Antimicrobial resistance, HPTLC, HPLC.

1. INTRODUCTION

1.1 Peptic ulcer disease: A global and national burden

Peptic ulcer disease (PUD) is a big health problem worldwide. This occurs when the lining of the stomach or the initial section of the small intestine becomes eroded. In 2019, it accounted for approximately 267,000 deaths worldwide, with India contributing nearly 15% of these deaths, primarily due to complications such as bleeding and perforation (GBD 2019 Collaborators, 2020; WHO, 2021; ICMR, 2022). Duodenal ulcers represent 70% of cases, whereas gastric ulcers account for 30%, often linked to *Helicobacter pylori* infection and NSAID use (Malfertheiner et al., 2024; Zhang et al., 2024). The contributing factors include stress, smoking, alcohol consumption, diet, and genetics (Kumar et al., 2022; Kim et al., 2023).

1.2 Diagnosis and treatment

Helicobacter pylori infection is diagnosed using both non-invasive and invasive methods. Noninvasive techniques include the urea breath test, which identifies labeled carbon dioxide following urease activity, stool antigen testing, and serological detection of anti-*H. pylori* antibodies. pylori antibodies (Katelaris et al., 2023; Gatta et al., 2022). Invasive procedures, typically performed during upper gastrointestinal endoscopy, include histological analysis, rapid urease testing, and microbial culture from biopsy samples (Sugano et al., 2023). The treatment approaches, corresponding medications, durations, and associated adverse effects are presented in Table 1.

Table 1: Modern ways to treat peptic ulcers caused by *Helicobacter pylori* include different drugs, how long to take them, and their side effects.

S. No.	Method	Drugs	Duration	Adverse Effects	Reference(s)
1	Triple Therapy	Proton pump inhibitor (such as omeprazole 20 mg twice daily) combined with Amoxicillin 1 g twice daily and Clarithromycin 500 mg twice daily	10–14 days	Diarrhea, taste disturbances, nausea, allergic reactions, antibiotic resistance	Khosravi & Nassaji 2023, Chey et al. 2024
2	Bismuth Quadruple Therapy	PPI twice daily, Bismuth subsalicylate 300 mg four times daily, Tetracycline 500 mg four times daily, and Metronidazole 500 mg three times daily	10–14 days	Dark stools, metallic taste, nausea, constipation, dizziness	Graham et al. 2023, Malfertheiner et al. 2023
3	Non-Bismuth Quadruple Therapy	PPI twice daily, Amoxicillin 1 g twice daily, Clarithromycin 500 mg twice daily, and Metronidazole 500 mg twice daily	10–14 days	GI upset, diarrhea, antibiotic-associated colitis	Aghaizu et al. 2022
4	High-Dose Dual Therapy	Vonoprazan 20 mg BID + Amoxicillin 1 g TID	14 days	Mild GI symptoms (lower than quadruple therapy)	Chey et al. 2024
5	Rifabutin-Based Therapy	PPI twice daily, Amoxicillin 1 g twice daily, and Rifabutin 150 mg twice daily	14 days	Myelotoxicity (rare), elevated liver enzymes	USFDA Talicia Approval 2023, Tshibangu-Kabamba et al. 2024
6	Hybrid Therapy	Begin by taking PPI and Amoxicillin for 7 days. Following this, continue with PPI, Amoxicillin, Clarithromycin, and Metronidazole for an additional 7 days.	14 days	Similar to concomitant therapy but lower adverse events	Georgopoulos et al. 2024

1.3 An overview of *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs (NSAIDs)

Helicobacter pylori, a gram-negative, spiral bacterium that survives gastric acidity by producing urease, which neutralizes stomach acid (Ashraf et al., 2024; Malfertheiner et al., 2024). It spreads primarily via the fecal–oral route and adheres to gastric epithelial cells through adhesins such as BabA and SabA (Okuda et al., 2022; Yamaoka, 2023). Virulence factors, such as CagA and VacA, disrupt mucosal integrity, promote inflammation, and contribute to ulcerogenesis and carcinogenesis (Cover & Blanke, 2023; Hatakeyama, 2022). NSAIDs, widely used analgesics, damage the gastric mucosa by inhibiting COX-1 and reducing protective prostaglandin levels (FitzGerald & Patrono, 2021). Co-administration of PPIs or COX-2 inhibitors can reduce gastrointestinal harm (Bally et al., 2022).

1.4 Limitations and challenges of current PUD therapies

Current therapies for PUD are effective but face significant limitations. Prolonged indulgence in proton pump inhibitors (PPIs) casts a shadow over bone health, weaving a tale of heightened vulnerability to hip fractures. (20–24%) and hypomagnesemia, particularly in patients with renal issues (Chubineh & Kumar, 2023; Zhou et al., 2023). PPIs can also disrupt the gut microbiota, increasing the risk of infections, notably *Clostridioides difficile* (Freedberg et al., 2023). Rebound acid hypersecretion occurs in 44% of patients after abrupt discontinuation of PPIs (Tack et al., 2024). Moreover, *H. pylori* resistance to clarithromycin, metronidazole, and levofloxacin has reduced triple therapy efficacy to below 70% (Savoldi et al., 2023; Fallone et al., 2023), necessitating costly and side-effect-prone alternatives.

1.5 Antimicrobial resistance and the role of herbal medicines

Antimicrobial resistance (AMR) poses a growing global threat, with estimates projecting 10 million annual deaths by 2050 if left unchecked (O'Neill 2016). Resistant pathogens, such as *Klebsiella pneumoniae*, show carbapenem resistance rates of 40–60% in ICUs, while MRSA and VRSA contribute to nearly 100,000 deaths annually (Nordmann et al., 2022; Turner et al., 2023). These mechanisms include enzymatic degradation, target modification, and drug efflux (Bush & Bradford, 2020; Li et al., 2022). The WHO (2023) supports the use of herbal medicines as complementary treatments because of their multi-targeted mechanisms. Compounds such as curcumin and berberine exhibit anti-*H. pylori* activity and promote mucosal healing (Tariq et al., 2021; Yuan et al., 2023).

2. MATERIALS AND METHODS

Sample description: *Allium sativum* bulbs, commonly known as garlic, belong to the Amaryllidaceae family and are prized for antimicrobial and digestive properties. *Terminalia chebula* fruits, known as chebulic myrobalan or black myrobalan, belong to the Combretaceae family and are traditionally used for treating gastrointestinal disorders and enhancing gut health.

2.1 Qualitative preliminary phytochemical screening

Qualitative screening of *Allium sativum* bulbs and *Terminalia chebula* fruits was performed using standard phytochemical tests to identify major secondary metabolites. Water, ethanol, methanol, ethyl acetate, and chloroform extracts were prepared and analyzed following the protocols described by Khan et al. (2024) and Harborne (1998). Tests included Dragendorff's and Mayer's for alkaloids, ferric chloride for tannins, Shinoda and alkaline reagent tests for flavonoids, foam test for saponins, and Liebermann–Burchard and Salkowski tests for terpenoids and steroids. Sulfur compounds were confirmed using lead acetate and sodium nitroprusside, while anthraquinones were identified using Borntrager's test (Indian Pharmacopoeia, 2022).

2.2 Quantitative phytochemical screening

Quantitative analyses were performed on ethanolic extracts using established spectrophotometric methods. Alkaloids were estimated using the Harborne method (1973), as modified by Adegbaju et al. (2021). Phenolic content was measured using the Folin–Ciocalteu method (Singleton & Rossi, 1965; Akinmoladun et al., 2020). Flavonoids were assessed using the aluminum chloride method (Ordonez et al., 2006; Sharma et al., 2021). Tannins were determined using the Folin–Denis reagent (Schenderl, 1970; Olayemi et al., 2022), while saponins were quantified according to Obadoni and Ochuko (2001). Terpenoids were analyzed using the method outlined by Ghorai et al. (2012), with minor alterations by Mehta et al. (2021), and the results were expressed in standard equivalents.

2.3 HPTLC of *Allium sativum*

The garlic extract in ethanol was spun at 3000 rpm for 5 minutes, and 6 µL of the supernatant liquid was applied onto silica gel 60 F_{254} HPTLC plates using a CAMAG LINOMAT 5 applicator (Reich & Schibli, 2007). Plates were developed in suitable mobile phases and scanned at 254 nm, 366 nm, and visible light using a CAMAG system. Alkaloids were identified using ethyl acetate–methanol–water (10:1.35:1) with Dragendorff's reagent, phenolics with toluene–ethyl acetate–formic acid (5:3:1) and aluminum chloride, and terpenoids using n-hexane–ethyl acetate (7.2:2.9) and anisaldehyde–sulfuric acid reagent (Reich & Schibli, 2007).

2.4 HPTLC of *Terminalia chebula*

The extract of *Terminalia chebula* was prepared in DMSO and applied (8 mm bands) on silica gel 60 F_{254} plates using a CAMAG LINOMAT 5. The mobile phase (toluene:ethyl acetate:formic acid:methanol, 3:3:0.8:0.7) was selected based on Indian Herbal Pharmacopoeia (2002) standards. After development to 80 mm, the plates were air-dried, derivatized with alcoholic ferric chloride, and visualized under UV light (254 nm) and visible light. Densitometric scanning and data analysis were performed using a CAMAG TLC SCANNER and winCATS software (Gupta et al., 2012). This HPTLC protocol helps standardize herbal products and authenticate their phytochemical profiles (Mukherjee, 2002).

2.5 HPLC of *Allium sativum*

HPLC was used to check the amounts of alliin and allicin in the ethanolic extract of *Allium sativum*. Fresh garlic bulbs (10 g) were crushed and extracted using 12 mL of methanol–water (1:1, v/v). After various extraction durations (5–30 min), the samples were centrifuged at 5000 rpm and filtered through 0.22 µm filters (Al-Taai et al., 2019). Chromatographic analysis was conducted using a Shimadzu HPLC system equipped with a C18 column. (4.6 mm × 250 mm) and methanol–water (50:50, v/v) as the mobile phase. The detection process utilized a UV detector set to 240 nm. Quantification of allicin content was achieved by analyzing the retention times and peak areas. with standard calibration curves (Al-Taai et al., 2019).

2.6 HPLC of *Terminalia chebula*

HPLC analysis of *Terminalia chebula* fruit extract was performed using a reverse-phase C18 column (4.6 mm × 150 mm, 5 µm) and a mobile phase of methanol, acetic acid, and deionized water (15:5:80, v/v/v). The flow rate was 1.0 mL/min, with UV detection at 280 nm and an injection volume of 20 µL (Chauhan & Chaurasiya 2017). Standard gallic acid solutions (10–100 µg/mL) were prepared to generate a calibration curve, against which the phenolic content in the sample (20 µg/mL) was measured. To ensure accuracy, each sample underwent filtration through a 0.22 µm syringe filter. This method reliably quantifies gallic acid and related phenolics (Rajani & Ashok, 2009).

3. RESULTS

3.1 Preliminary Phytochemical Screening of *Allium sativum* and *Terminalia chebula*

Phytochemical screening of *Allium sativum* (AS) and *Terminalia chebula* (TC) revealed a broad range of bioactive compounds. Ethanolic extract of AS showed high presence (+++) of alkaloids, terpenoids, glycosides, and carbohydrates, while phenols, flavonoids, tannins, and saponins were found in moderate to high amounts. Alkaloids (2.53 mg/g), phenols (2.70 mg/g), saponins (2.39 mg/g), and terpenoids (2.18 mg/g) were In TC, the ethanolic extract showed very high levels (+++) of phenols and tannins and moderate amounts of alkaloids, saponins, and glycosides. The quantitative values were notably high for tannins (45.3 mg/g), phenols (28.5 mg/g), and flavonoids (12.75 mg/g), indicating strong antioxidant and antimicrobial potential. Table 2 & 4 presents the results of the phytochemical screening of garlic powder and *Terminalia chebula* fruits powder in various solvents.

Table 2: Phytochemical analysis of various solvent extracts of garlic powder.

Phytochemicals	Aqueous	Ethanol	Methanol	Chloroform	Ethyl acetate
Alkaloids	1+	3+	3+	2+	2+
Phenols	1+	2+	-	-	1+
Flavonoids	1+	2+	-	-	1+
Tannins	1+	2+	2+	-	-
Saponins	1+	2+	2+	2+	2+
Terpenoids	2+	3+	3+	1+	2+
Steroids	-	1+	1+	-	-
Carbohydrate	1+	3+	2+	1+	1+
Glycosides	2+	3+	-	2+	1+
Amino acids	1+	2+	-	-	-
Proteins	1+	2+	-	-	-

Key: 1+ means present in low quantity; 2+ means present in medium-high quantity; 3+ means present in very high quantity; - means not present.

The ethanolic extracts of garlic powder were exposed for estimation of Alkaloids, Total phenols, Flavonoids, Tannins and Saponins, which were found at higher concentrations in the qualitative tests. The outcomes of quantitative tests of phytochemical constituents of ethanolic extracts of garlic power and *Terminalia chebula* fruits powder are shown in Table 3 & 5.

Table 3: Quantitative tests of phytochemical constituents of ethanolic extracts of garlic powder.

Biologically active components (mg/g sample)	Garlic powder (mean± SD)
Alkaloids	2.53±0.20
Total phenols	2.70±0.18
Flavonoids	2.13±0.15
Tannins	2.15±0.13
Saponins	2.39±0.18
Terpenoids	2.18±0.27

Table 4: Qualitative phytochemical analysis of various solvent extracts of *Terminalia chebula* fruits.

Phytochemicals	Aqueous	Ethanol	Methanol	Chloroform	Ethyl acetate
Alkaloids	1+	3+	2+	1+	2+
Phenols	2+	3+	3+	1+	2+
Flavonoids	1+	2+	2+	-	1+
Tannins	3+	3+	3+	2+	2+
Saponins	1+	2+	1+	-	1+

Terpenoids	1+	2+	2+	1+	2+
Steroids	-	1+	1+	-	-
Carbohydrates	2+	3+	2+	1+	2+
Glycosides	2+	3+	1+	2+	2+
Anthraquinones	1+	2+	-	-	-

Key: 1+ means present in low quantity; 2+ means present in medium-high quantity; 3+ means present in very high quantity; - means not present.

Table 5: Quantitative tests of phytochemical constituents of ethanolic extract of *Terminalia chebula* fruits.

Biologically active components (mg/g sample)	***T. chebula* fruits (mean ± SD)**
Total Phenols	28.50 ± 1.20
Flavonoids	12.75 ± 0.85
Tannins	45.30 ± 2.10
Saponins	8.40 ± 0.60
Alkaloids	5.20 ± 0.45
Terpenoids	6.80 ± 0.55
Anthraquinones	3.10 ± 0.25
Glycosides	7.90 ± 0.70

3.2 HPTLC of *Allium sativum* and *Terminalia chebula*

HPTLC analysis confirmed the presence of alkaloids, phenols, and terpenoids in AS extracts. Peaks corresponding to compounds such as alliin, allyl disulfide, gallic acid, caffeic acid, rutin, chlorogenic acid, lupeol, β-caryophyllene and citral were identified. Thirteen alkaloid peaks and 15 phenolic peaks were observed with significant areas and Rf values, indicating a diverse phytochemical profile. In TC, HPTLC performed at 254 nm and post derivatization with alcoholic ferric chloride revealed 7 and 5 peaks, respectively, confirming the presence of ellagic acid and related phenolics. Prominent peaks with high area percentages further confirmed the high phenolic content of TC. Reference compounds used here were Colchicine to compare the alkaloid compound, quercetin for the phenolic compound, and lupeol for terpenoids. Samples were analyzed under natural light and UV light at two different wavelengths (366 and 254 nm).

Fig 1 shows densitogram of garlic power (1a colchicine and 1b sample extract), Fig 2 densitogram of garlic powder (2c Quercetin and 2d sample extract), Fig 3 shows densitogram of garlic powder (3e Lupeol and 3f sample extract), 4 shows pre-derivatization HPTLC analysis of *T. chebula* extract and 5 shows post derivatization HPTLC analysis of *T. chebula* extract.

Alkaloid profile: The HPTLC alkaloid profiles of colchicine and ethanolic extract of garlic powder are shown in Fig 1a and 1b and Table 3.

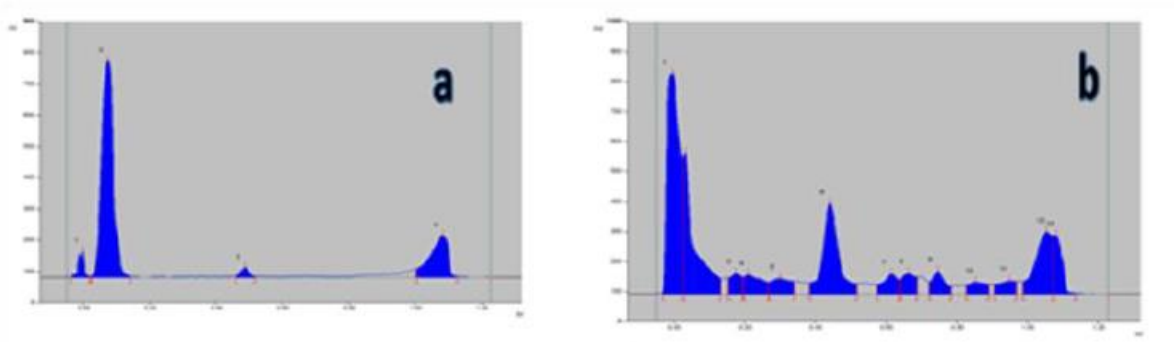

Fig 1: Densitogram illustrating the ethanolic extract from garlic powder. 1a shows Reference alkaloid (Colchicin) and 1b shows Sample (Alkaloid profile).

Table 6: HPTLC alkaloid profile of *Allium sativum* ethanolic extract.

Track	Peak	Rf	Height	Area	Entity	Alkaloid Name	Ref.
Standard	2	0.13	694.5	17,438.6	Colchicine	Colchicine	Reich & Schibli, 2007
Sample 1	1	0.03	732.7	18,448.1	Alkaloid 1	Alliin	Kanaki & Rajani, 2005
Sample 2	2	0.13	473.5	10,793.7	Alkaloid 2	Allyl disulfide	Mathew et al., 2009
Sample 3	3	0.20	70.5	1,671.1	Unknown	–	--
Sample 4	4	0.27	63.3	2,424.2	Unknown	–	--
Sample 5	5	0.34	52.4	2,087.6	Unknown	–	--
Sample 6	6	0.52	302.2	9,748.1	Alkaloid 3	Gloriosine	Mehta et al., 2021
Sample 7	7	0.64	66.9	2,018.6	Unknown	–	--
Sample 8	8	0.69	69.6	1,951.7	Unknown	–	--
Sample 9	9	0.79	75.6	2,002.1	Unknown	–	--
Sample 10	10	0.89	39.0	1,444.8	Unknown	–	--
Sample 11	11	0.97	44.5	1,570.9	Unknown	–	--
Sample 12	12	1.07	206.9	7,025.7	Unknown	–	--
Sample 13	13	1.14	197.2	3,398.9	Alkaloid 4	Colchicine derivative	Reich & Schibli, 2007

Phenolic profile: A yellowish-blue glowing area at UV 366 nm was seen, showing that phenolic compounds are in the sample. The sample and standard chromatograms are shown in Figure 2c and 2d.

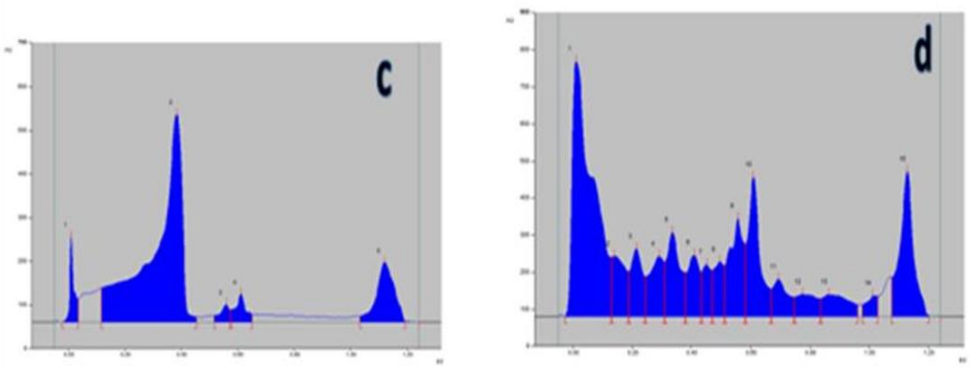

Fig. 2: Densitogram illustrating the ethanolic extract of garlic powder. 2c shows Standard phenol (Quercetin) and 2d shows Sample (Phenol profile)

Table 7: HPTLC phenolic profile of *Allium sativum* ethanolic extract.

Track	Peak	Rf	Height	Area	Entity	Phenolic Name	Reference
Standard	2	0.12	194.8	31681.7	Quercetin	Quercetin (standard)	Rutin & Gupta, 2016
Sample 1	1	0.13	673.2	35205.3	Unknown	–	–
Sample 2	2	0.19	192.0	6594.5	Unknown	–	–
Sample 3	3	0.25	206.1	6440.2	Unknown	–	–
Sample 4	4	0.32	180.3	6415.1	Unknown	–	–
Sample 5	5	0.39	250.8	8292.9	Unknown	–	–
Sample 6	6	0.45	183.3	5484.8	Unknown	–	–
Sample 7	7	0.48	159.8	3460.3	Phenolic 1	Gallic acid	Aguirre et al., 2018
Sample 8	8	0.53	168.9	4303.6	Unknown	–	–
Sample 9	9	0.60	299.9	10674.6	Phenolic 2	Caffeic acid	Markham et al., 2010
Sample 10	10	0.69	424.9	12732.4	Phenolic 3	Rutin	Rutin & Gupta, 2016
Sample 11	11	0.76	119.2	4206.3	Unknown	–	–
Sample 12	12	0.86	76.6	3450.5	Unknown	–	–
Sample 13	13	0.91	73.2	2281.5	Unknown	–	–
Sample 14	14	0.99	70.0	2637.2	Unknown	–	–
Sample 15	15	1.22	354.1	17653.4	Phenolic 4	Chlorogenic acid	Spigno & De Faveri, 2007

Terpenoid profile: Figure 3e, 3f, and Table 5 show the HPTLC terpenoid profiles of the standard and ethanolic extract of garlic powder.

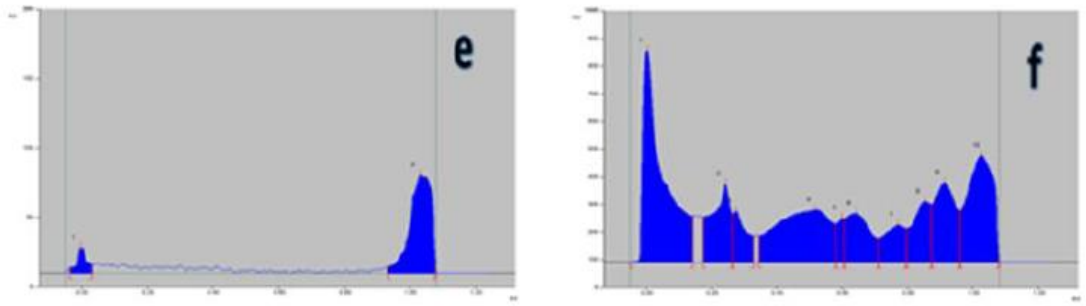

Fig 3: Densitogram of ethanolic extract of garlic powder. 3e shows Standard Terpenoids (Lupeol) and 3f shows Sample (Terpenoids profile).

Table 8: List of peaks of Terpenoids with Rf values, height, and area.

Track	Peak	Rf	Height	Area	Entity	Terpenoid name	Reference
Standard	2	1.08	70.8	3368.1	Lupeol	Lupeol	Qiao et al., 2015
Sample	1	0.14	765.3	34061.3	Not known	Possibly α-Pinene	Das et al., 2020
Sample	2	0.26	282.8	11298.6	Terpenoid 2	β-Caryophyllene	Zhang et al., 2017
Sample	3	0.33	183.8	5362.7	Terpenoid 3	γ-Terpinene	Al-Bayaty et al., 2014
Sample	4	0.58	187.6	22691.7	Terpenoid 4	Geraniol	Kim et al., 2021
Sample	5	0.62	159.5	2549.6	Not known	Unknown	—
Sample	6	0.72	176.1	9314.7	Terpenoid 5	Linalool	Rao et al., 2019
Sample	7	0.81	134.6	6160.4	Not known	Unknown	—
Sample	8	0.86	219.8	8468.2	Terpenoid 6	β-Myrcene	Egharevba & Kunle, 2010
Sample	9	0.96	287.0	13087.6	Terpenoid 7	Citral	Srinivasan et al., 2022
Sample	10	1.08	384.2	21768.6	Terpenoid 1	Lupeol (confirmed)	Qiao et al., 2015

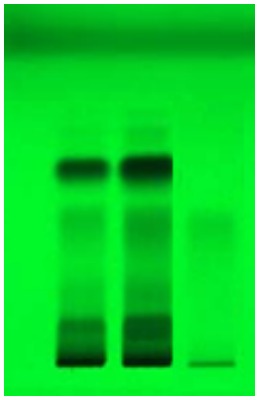

Fig 4: HPTLC analysis of T. chebula extract at 254 nm (From Left to Right; Track 1 - 2µl of ethanolic extract, Track 2 - 5µl of ethanolic extract, Track 3 - 2µl of Ellagic acid).

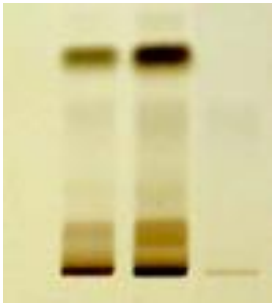

Fig 5: HPTLC analysis of T. chebula extract post-derivatization (From left to right:Track 1: 2µl of the ethanolic extract, Track 2: 5µl of the ethanolic extract, Track 3: 2µl of Ellagic acid).

Table 9: HPTLC profile of ethanolic extract of *T. chebula* pre-derivatization.

Peak	Starting Rf	Starting height	Max Rf	Max height	Max %	Ending Rf	Ending height	Area	Area %
1	0.06	4.2	0.10	198.7	13.56	0.12	0.7	4582.7	6.41
2	0.14	0.5	0.17	43.4	2.96	0.21	0.4	1136.6	1.59
3	0.24	4.7	0.33	158.7	10.83	0.38	0.1	8354.1	11.68
4	0.38	0.3	0.45	644.7	43.99	0.50	38.8	25569.2	35.74
5	0.51	41.7	0.53	53.6	3.66	0.55	22.7	1470.6	2.06
6	0.55	22.6	0.57	38.1	2.60	0.59	26.4	843.9	1.18
7	0.59	25.7	0.74	328.2	22.40	0.91	1.5	29580.8	41.35

Table 10: HPTLC profile of ethanolic extract of *T. chebula* post-derivatization.

Peak	Starting Rf	Starting height	Max Rf	Max height	Max %	Ending Rf	Ending height	Area	Area %
1	0.10	25.1	0.11	44.0	6.83	0.12	1.3	415.8	1.80
2	0.14	0.0	0.17	22.2	3.45	0.20	0.2	576.1	2.49
3	0.24	0.4	0.32	54.6	8.48	0.38	6.2	3236.4	14.01
4	0.38	6.5	0.45	482.1	74.85	0.49	14.6	17793.5	77.04
5	0.49	14.7	0.53	41.1	6.38	0.56	1.7	1074.6	4.65

3.3 HPLC of *Allium sativum* and *Terminalia chebula*

HPLC of AS identified allicin with a retention time of 16.789 min, with its concentration increasing over time from 30.25% to 39.24%, indicating enhanced stability and detectability under prolonged analysis. The high allicin content suggests a strong antimicrobial potential. In TC, gallic acid was identified at 6.876 min with a calculated concentration of approximately 30.52 µg/mL, based on the calibration curve. This confirms TC's antioxidant properties of TC and supports its use in traditional medicine.

Fig 6 shows HPLC chromatogram of standard allicin, 7 shows HPLC chromatograms of of allicin at different periods (7a for 5 min., 7b for 10 min., 7c for 15 min., 7d for 20 min., 7e for 25 min. and 7f 30 min.), Fig 8 shows graph of increasing concentration with increasing run time, Fig 9 shows graph of HPLC chromatogram of *Terminalia chebula* extract, Fig 10 shows HPLC chromatogram of gallic acid, Fig 11 shows calibration curve of gallic acid estimation.

Table 11, 12, 13, 14 shows retention time and concentration of allicin at different times obtained by HPLC, chromatogram readings of T. chebula extract, chromatogram readings of gallic acid and concentration and peak area for gallic acid estimation respectively.

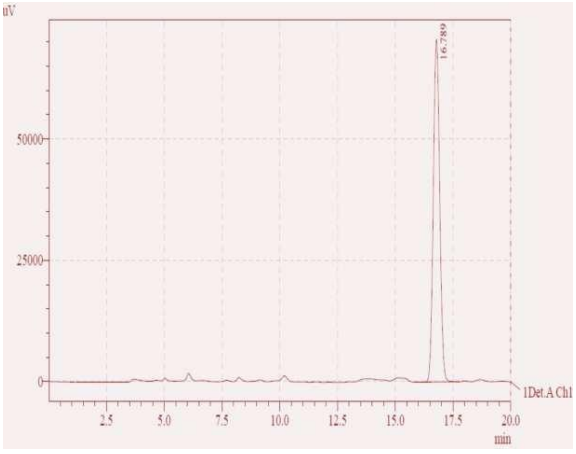

Fig 6: Chromatogram of standard allicin diagnosed by HPLC.

Table 11: Retention time and concentration of allicin at different times obtained by HPLC.

Time (min)	Ret. time (min)	Concentration%
5	16.965	30.245
10	16.975	31.838
15	16.770	32.212
20	16.770	32.259
25	16.948	33.328
30	16.951	39.244

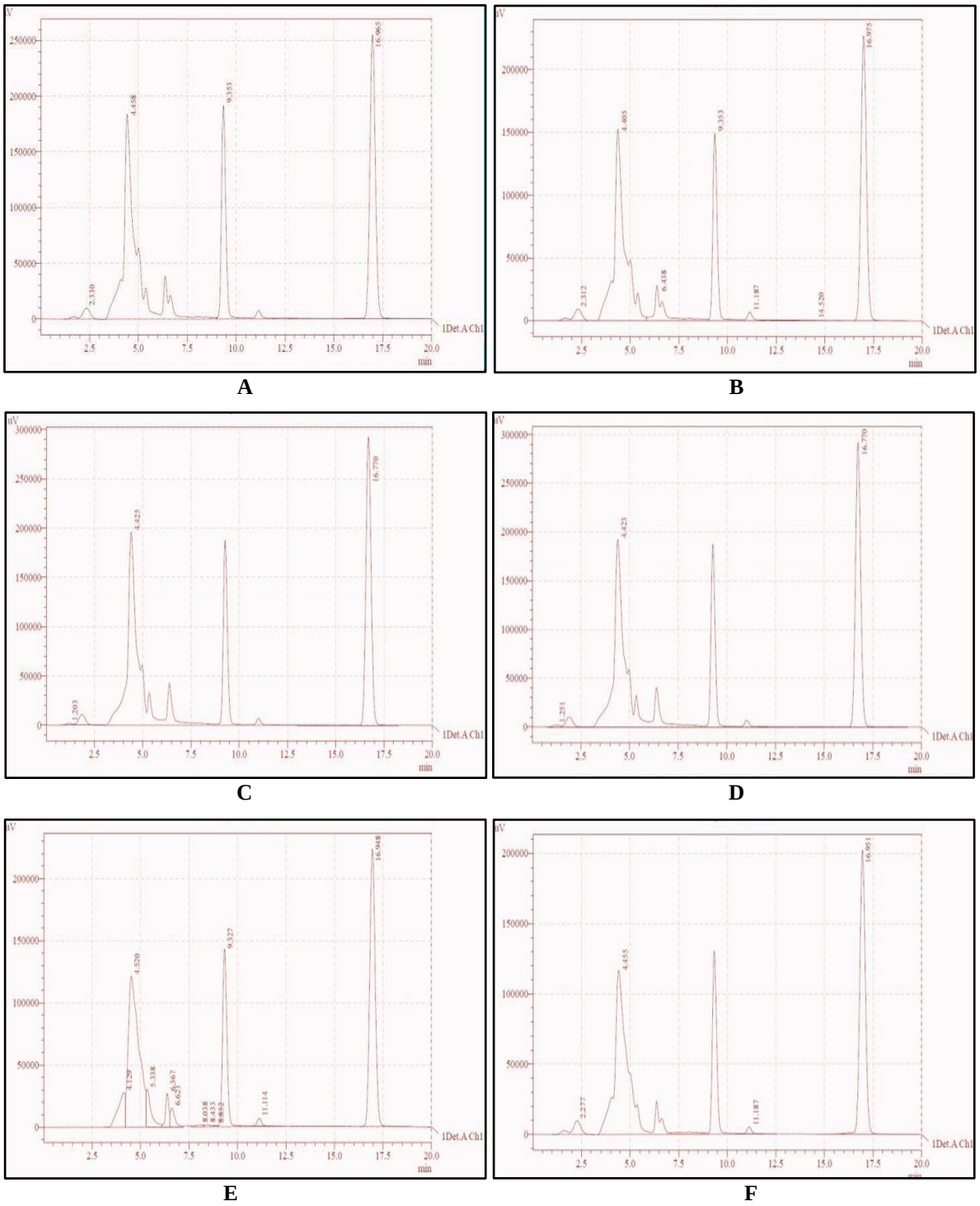

Fig 7: Six Chromatograms of allicin above in the garlic extract obtained by HPLC for the periods i.e. 7A shows 5 min., 7B shows 10 min., 7C shows 15 min., 7D shows 20 min., 7E shows 25 min., 7F shows 30 min.

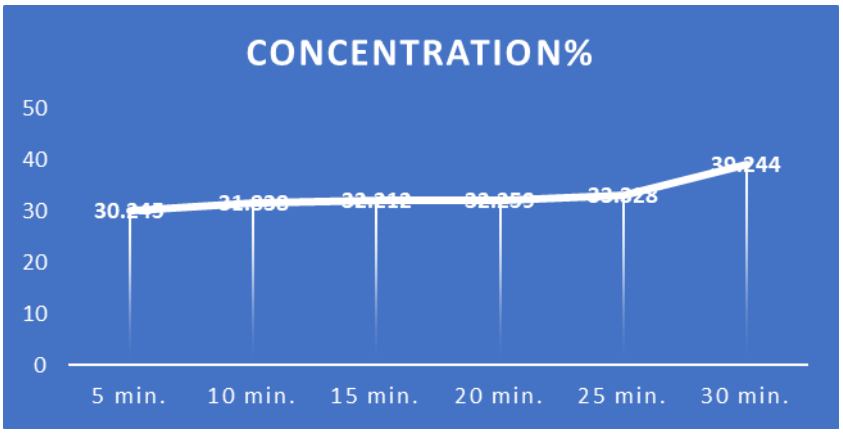

Fig. 8: Graph shows increasing concentration with increasing run time.

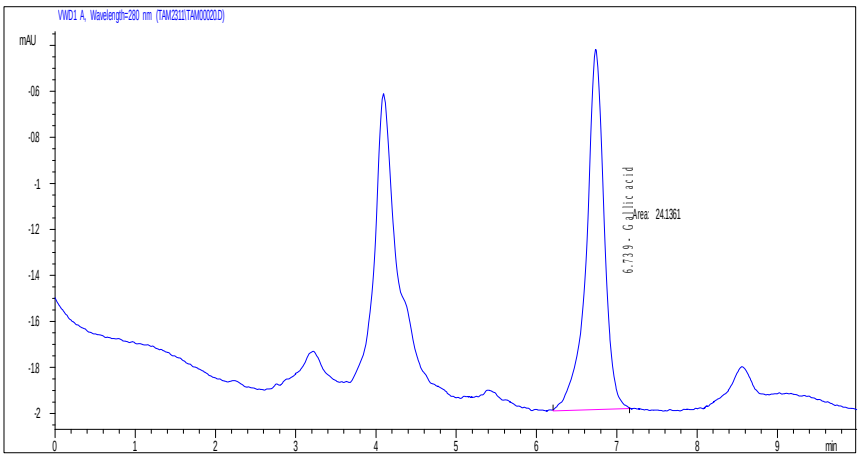

Fig 9: Graph shows HPLC chromatogram of *T.chebula* ethanolic extract.

Table 12: Numerical measurements of HPLC chromatogram of *T.chebula* ethanolic extract.

Sr. No.	Time (mins)	Area	Height	Width	Area%	Symmetry
1	6.738	124.2	1.7	0.2570	100.000	1.125

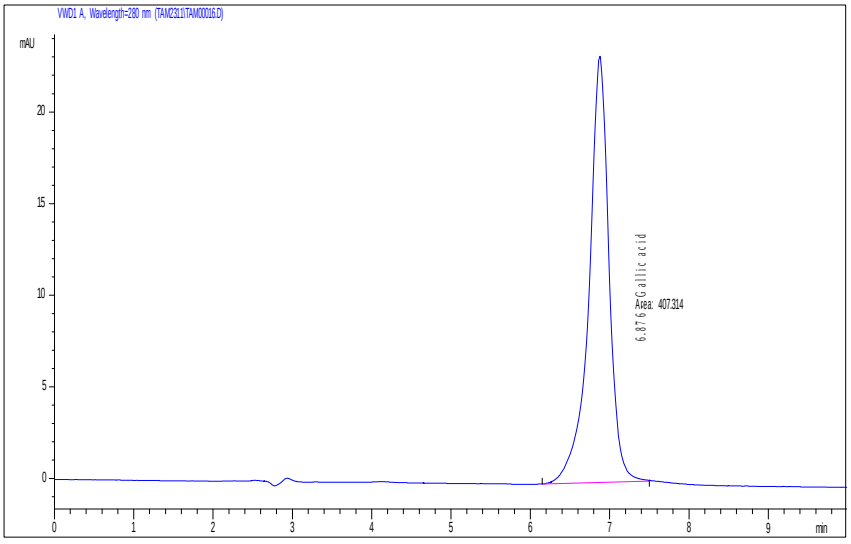

Fig 10: Graph shows HPLC chromatogram of Gallic acid.

Table 13: Numerical measurements of HPLC chromatogram of Gallic acid.

Sr. No.	Time (mins)	Area	Height	Width	Area%	Symmetry
1	6.877	403.2	23.1	0.2550	100.000	1.187

HPLC analysis was used to quantify the gallic acid level in the ethanolic extract. The chromatograms of the *T. chebula ethanolic* extract and standard gallic acid are illustrated in Fig 1 and 2, respectively.

3.4 Estimation of gallic acid by calibration curve method

Table 14: Concentration and peak area.

Concentration (µg/mL)	Peak Area
10	135
20	280
40	531
60	822
80	1068
100	1361
Sample	415.5

Calibration curve

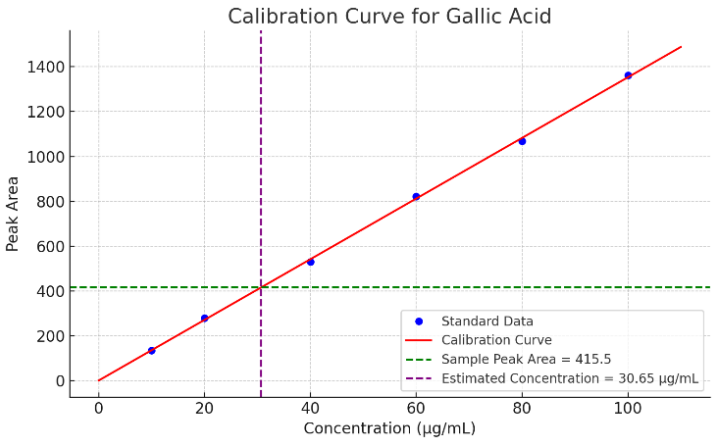

Fig. 11: Calibration curve to estimate gallic acid.

4. DISCUSSION

Preliminary phytochemical screening of *Allium sativum* and *Terminalia chebula* found that ethanol proved to be the most efficient solvent, capable of extracting a diverse array of bioactive compounds., including alkaloids, phenols, flavonoids, tannins, terpenoids, and saponins. This aligns with the findings of Sultana et al. (2009), who noted the superior polarity of ethanol in extracting diverse phytoconstituents. In *A. sativum*, alkaloids (2.53 ± 0.20 mg/g), phenols (2.70 ± 0.18 mg/g), flavonoids (2.13 ± 0.15 mg/g), tannins (2.15 ± 0.13 mg/g), and terpenoids (2.18 ± 0.27 mg/g) were found at significant concentrations. These findings support the therapeutic potential of garlic, particularly its antioxidant, anti-inflammatory, and antimicrobial activities (Ghorai et al., 2012; Akinmoladun et al., 2020). Similarly, *T. chebula* showed high levels of tannins (45.30 ± 2.10 mg/g), phenols (28.50 ± 1.20 mg/g), and flavonoids (12.75 ± 0.85 mg/g), compounds well documented for their hepatoprotective and free radical scavenging properties (Lee et al., 2005; Naik et al., 2004.).

HPTLC analysis provided detailed phytochemical fingerprinting. In *A. sativum*, alkaloids such as alliin and allyl disulfide and phenolics such as gallic acid, caffeic acid, rutin, and chlorogenic acid have been detected, supporting its complex bioactive profile (Kanaki & Rajani, 2005; Aguirre et al., 2018). Terpenoids, including lupeol and β-caryophyllene, were

also identified. In *T. chebula*, a dominant peak at Rf 0.38, attributed to ellagic acid, was observed post-derivatization with ferric chloride, confirming its phenolic richness (Govindarajan et al., 2004; Kumar et al., 2017).

HPLC confirmed the presence of these constituents with high specificity. In *A. sativum*, allicin was identified with a peak at 16.789 min, which increased with extraction time (Borlinghaus et al., 2014). In *T. chebula*, gallic acid was confirmed at 6.739 min with a concentration of 0.307 g%, which matches the literature values (Govindarajan et al., 2004). These findings validate the chemical diversity and therapeutic relevance of both plant species.

CONCLUSION

The present study highlights the phytochemical richness of *Allium sativum* and *Terminalia chebula*, supporting their traditional use in managing peptic ulcer disease (PUD), eradicating *Helicobacter pylori*, and combating antimicrobial resistance (AMR). Qualitative and quantitative analyses confirmed the presence of bioactive compounds including alkaloids, phenols, flavonoids, tannins, terpenoids, and saponins—many of which are known for their anti-ulcerogenic, gastroprotective, and antimicrobial activities. HPTLC and HPLC analyses identified and quantified key phytoconstituents such as allicin, gallic acid, and ellagic acid, all of which demonstrate significant inhibitory effects against *H. pylori*, the primary bacterial cause of PUD. Allicin, with its potent bactericidal properties, along with the phenolics in *T. chebula*, contributes to both mucosal healing and acid regulation. These phytochemicals not only support gastrointestinal health but also offer multi-targeted antimicrobial effects that reduce reliance on conventional antibiotics and help curb the emergence of resistant strains. Thus, *A. sativum* and *T. chebula* hold strong potential as natural, complementary therapies for ulcer treatment, *H. pylori* eradication, and resistance mitigation.

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