Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
Impact Factor (RJIF): 6.35
www.phytojournal.com
JPP 2025; 14(5): 614-623
Received: 18-10-2025
Accepted: 22-10-2025

Priyanka Dalwadi
Department of Applied and Interdisciplinary Sciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

Jasmine A Mansuri
Department of Applied and Interdisciplinary Sciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

Anjali Thakkar
Department of Applied and Interdisciplinary Sciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

Anju Kunjadiya
Department of Applied and Interdisciplinary Sciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

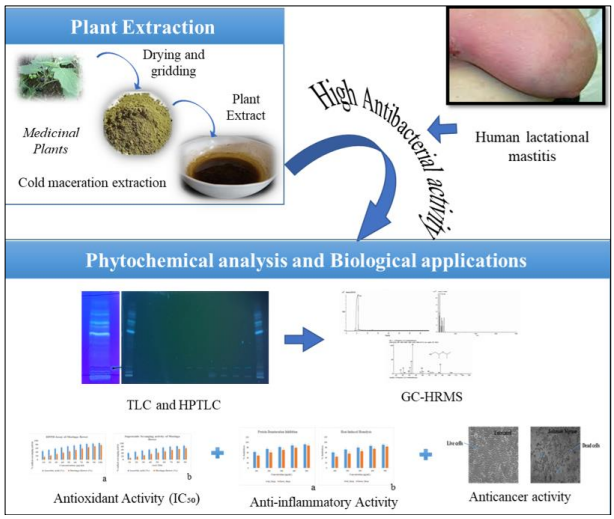
Corresponding Author:
Anju Kunjadiya
Department of Applied and Interdisciplinary Sciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

Phytochemical profiling and multifunctional bioactivity of *Solanum nigrum* against multidrug-resistant bacteria

Priyanka Dalwadi, Jasmine A Mansuri, Anjali Thakkar and Anju Kunjadiya

DOI: https://www.doi.org/10.22271/phyto.2025.v14.i5h.15624

Abstract
A significant global health concern is the increasing occurrence of multidrug-resistant (MDR) bacterial pathogens, which calls for the investigation of new natural antimicrobial agents. The current investigation compared the antibacterial activity of methanolic extracts from *Mesua ferrea*, *Zingiber officinale*, *Solanum nigrum*, and *Pueraria tuberosa* against MDR bacterial isolates recovered from bovine mastitis milk, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Bacillus paraanthracis*, *Alcaligenes faecalis*, and *Bacillus licheniformis*. *S. nigrum* showed the strongest antibacterial activity among the tested extracts, and it was then thoroughly characterized in terms of phytochemistry and bioactivity. Alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids were confirmed by qualitative phytochemical screening, thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and gas chromatography-high-resolution mass spectrometry (GC-HRMS). One of the main bioactive components was 1-propanol, 2-(1-methyl ethoxy)- [2-2-isopropoxypropane]. Strong free radical scavenging ability (IC_{50}: DPPH 42.6 µg/mL; superoxide 49.2 µg/mL) and catalase inhibition (IC_{50} 56.1 µg/mL) were shown by antioxidant assays. Protein denaturation was significantly inhibited (78.4% at 100 µg/mL) and red blood cell membrane stabilization was comparable to diclofenac sodium, demonstrating the extract's concentration-dependent anti-inflammatory properties. Additionally, MCF-7 breast cancer cells were cytotoxically affected by *S. nigrum* extract (IC_{50} 78.5 µg/mL), exhibiting morphological characteristics that were consistent with apoptosis. Together with strong antioxidant, anti-inflammatory, and anticancer properties, *S. nigrum*'s broad-spectrum antibacterial efficacy points to a variety of therapeutic possibilities. Its various phytoconstituents acting on various cellular targets most likely interact synergistically to produce these effects. The results taken together show that *S. nigrum* is a promising candidate for the development of plant-based antimicrobial and adjunct therapeutic agents against MDR pathogens, which calls for additional *in vivo* validation and the isolation of bioactive secondary metabolites by different bioassays.



Keywords: *Solanum nigrum*, Multidrug-resistant bacteria, Phytochemicals, Antibacterial activity, Antioxidant activity, GC-HRMS, HPTLC

1. Introduction
Multidrug-resistant (MDR) bacterial infections have become a significant global public health concern because they reduce the effectiveness of traditional antibiotics and raise the risk of

treatment failure, prolonged illness, and mortality. The crisis continues to grow worse due to the slow rate of new antibiotic discovery and the quick emergence and spread of resistance determinants through spontaneous mutation, horizontal gene transfer, and biofilm formation [1, 2]. A rising percentage of human and veterinary infections, including mastitis, urinary tract infections, wound infections, and sepsis, are linked to clinically significant MDR bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Bacillus paraanthracis*, *Alcaligenes faecalis*, and *Bacillus licheniformis* [3-6]. These infections often exhibit resistance to several antibiotic classes, which makes treatment plans more difficult and raises medical expenses.

There is now more interest in plant-derived bioactive compounds as alternative or complementary therapies due to the increasing limitations of existing antimicrobial agents. Alkaloids, flavonoids, phenolic acids, saponins, tannins, and terpenoids are among the structurally diverse phytochemicals found in medicinal plants that are well-known for their antibacterial, antioxidant, anti-inflammatory, and anticancer properties [7, 8]. Multitarget mechanisms of action, such as disruption of microbial membranes, inhibition of protein and nucleic acid synthesis, suppression of efflux pumps, and interference with quorum sensing, are responsible for their pharmacological versatility [9, 10]. Furthermore, compared to many synthetic drugs, compounds derived from plants are usually more environmentally friendly, biodegradable, and have lower toxicity [11].

In Ayurvedic medicine, *Mesua ferrea L*. (Nagkesar), an evergreen tree in the *Calophyllaceae* family, has been extensively used to treat microbial infections, inflammation, skin conditions, and bleeding piles. Xanthones, coumarins, and flavonoids with strong antimicrobial and antioxidant qualities have been found through phytochemical studies [12, 13]. Traditional medicine makes extensive use of *Zingiber officinale Roscoe*, or ginger, a member of the *Zingiberaceae* family, to treat inflammatory, respiratory, and gastrointestinal conditions. Bioactive substances like gingerols, shogaols, and paradols, which have been shown to have antibacterial and anti-inflammatory properties, are abundant in its rhizomes [14, 15]. The hepatoprotective, antimicrobial, and anticancer properties of *Solanum nigrum* L. (Makoy), a member of the *Solanaceae* family, have been attributed to its flavonoids and steroidal glycoalkaloids [16, 17]. The *Fabaceae* family's *Pueraria tuberosa* (Roxb. ex Willd.) DC. (Vidarikand) is commonly used as a restorative tonic because it contains saponins, triterpenoids, and isoflavonoids that have antibacterial, anti-inflammatory, and antioxidant qualities [18, 19]. A comparative study of these plants' antibacterial efficacy against clinically relevant MDR bacterial isolates, especially those derived from mastitis cases, is still lacking, although each plant has a well-established medicinal potential. A significant financial burden on the dairy industry, mastitis is an inflammatory condition of the mammary gland that can spread through contaminated milk. The urgent need for new antimicrobials that are effective against these pathogens is highlighted by recent studies that used whole-genome sequencing (WGS) to identify a variety of antibiotic resistance genes in bacteria linked to mastitis [20]. The pathogenic profile observed among the current MDR isolates is further supported by metagenomic analyses of bovine mastitis milk, which have revealed decreased microbial diversity and enrichment of opportunistic pathogens [21, 22]

The current study aimed to compare the antibacterial activity of methanolic extracts from *Mesua ferrea*, *Zingiber officinale*, *Solanum nigrum*, and *Pueraria tuberosa* against MDR bacterial isolates (*S. aureus*, *E. coli*, *B. cereus*, *B. paraanthracis*, *A. faecalis*, and *B. licheniformis*) found in clinical mastitis milk using WGS. Using gas chromatography-high-resolution mass spectrometry (GC-HRMS), thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and qualitative tests, the plant extract with the strongest antibacterial activity was further exposed to phytochemical profiling. To give a thorough grasp of its therapeutic potential, antioxidant and anti-inflammatory properties were also evaluated. This study aims to find promising plant-based bioactive compounds for the construction of novel antimicrobials to fight MDR pathogens by combining microbiological, phytochemical, and bioactivity analyses.

2. Materials and Methods

2.1 Collection and Authentication of Plant Materials

In the Anand district of Gujarat, India, between March and April 2024, fresh, healthy portions of *Mesua ferrea* (stems), *Zingiber officinale* (rhizomes), *Solanum nigrum* (leaves), and *Pueraria tuberosa* (tubers) were gathered from local markets and verified botanical gardens. The plant specimens were verified by Dr. Kalpesh Inshnava, a botanist from Sardar Patel University's Department of Biosciences in Vallabh Vidyanagar, Anand, Gujarat, India.

2.2 Preparation of Methanolic Extracts

Following a thorough washing, each plant sample was ground into a coarse powder and allowed to dry in the shade at room temperature. 500 milliliters of methanol were used to cold macerate 50 grams of powder from each plant for 72 hours, stirring occasionally. When the methanol in the extract evaporated, the extracts were concentrated and dried after being filtered through Whatman No. 1 filter paper [23].

2.3 Preliminary Antibacterial Screening

The *in-vitro* antibacterial study employed multidrug-resistant clinical isolates of *Bacillus paraanthracis*, *Bacillus cereus*, *Bacillus anthracis*, *Bacillus altitudinis*, *Staphylococcus aureus*, *Escherichia coli*, *Alcaligenes faecalis*, and *Bacillus licheniformis* from maternal milk with mastitis [20]. Before use, these strains were standardized to a 0.5 McFarland standard after being resurrected and subcultured on nutrient agar. Using the agar well diffusion assay, bacterial suspensions were seeded onto Mueller-Hinton agar plates. 100 µL of extract (250-1000 µg/mL) was added to wells (6 mm). Zones of inhibition were measured in millimeters after plates were incubated for 24 hours at 37 °C. DMSO served as the negative control and tetracycline as the positive control [24]. Serial dilutions of extracts in nutrient broth determined MIC. After adding culture inoculum test tubes were incubated at 37°C for 24 hours. The lowest concentration with no turbidity was recorded as MIC. To determine MBC, 10 µL from wells without visible growth were subcultured onto nutrient agar. MBC was recorded as the lowest concentration, showing no colony growth [24, 25].

2.4 Phytochemical Screening

The extract of *Solanum nigrum* exhibited the most potent antibacterial properties among the four species and was chosen for additional examination. Conventional qualitative chemical tests were carried out to identify the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds [26, 27].

2.5 Thin-Layer Chromatography (TLC): TLC was used to separate the phytoconstituents on silica gel 60 F254 plates. A capillary tube was used to apply the extracts, and plates were created in an ideal solvent mixture that included toluene, water, and formic acid (10:5:1) [28]. Separated bands were visualized using UV light at 254 and 366 nm.

2.6 High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC was used to quantify selected chosen phytochemicals. The extract and standard solutions were applied using a CAMAG Linomat 5 sample applicator. A CAMAG TLC Scanner was used to scan the plates at a suitable wavelength following their development had been developed in a twin-trough chamber. Standard calibration curves served as the basis for quantitative analysis [29].

2.7 Gas Chromatography-High-Resolution Mass Spectrometry (GC-HRMS)

A Thermo Scientific Q Exactive GC Orbitrap system was used to perform GC-HRMS analysis on the chemical composition of Solanum nigrum extract. The detection of compounds was accomplished through contrasting mass spectra with standard libraries like NIST and Wiley, and confirmation was obtained using retention indices [30].

2.8 Antioxidant Activity Assays

2.8.1 DPPH Radical Scavenging Assay: The plant extract at different concentrations was combined with a 0.1 mM DPPH suspension in methanol to determine the DPPH free radical scavenging activity. After 30 minutes of incubation in the dark, absorbance was determined at 517 nm. The ascorbic acid standard was compared to the results [31].

2.8.2 Superoxide Radical Scavenging Assay

The alkaline DMSO strategy was used to measure the superoxide radical scavenging activity. Absorbance was measured at 560 nm after reaction mixtures were incubated. The standard reference was ascorbic acid [32].

2.8.3 Catalase Activity Assay

The extracted compounds were incubated with hydrogen peroxide in phosphate buffer to measure catalase activity. Spectrophotometric measurement was used to determine the absorbance drop at 240 nm. It was determined what percentage of the enzyme was inhibited [33].

2.9 *In-vitro* Anti-inflammatory Activity

2.9.1 Inhibition of Protein Denaturation

The protein denaturation method, as identified by Mizushima and Kobayashi (1998) and Sakat *et al*. (2010) with minor modifications [34, 35], was utilized to determine the anti-inflammatory activity of plant extracts. The test extract and 1% aqueous bovine serum albumin (BSA) were added to the reaction mixture, and 1 N HCl was used to adjust the pH. After 15 minutes of incubation at 37 °C, the samples were heated for five minutes at 70 °C. A spectrophotometer was used to measure the turbidity at 660 nm after the mixture had cooled to room temperature. The results were presented as the percentage inhibition of protein denaturation in comparison to the control.

2.9.2 Heat-induced Hemolysis

The heat-induced hemolysis method was used to measure membrane-stabilizing activity [36]. Healthy volunteers who had not taken NSAIDs for at least two weeks prior to sampling had their fresh human blood drawn. After centrifuging the blood for 10 minutes at 3000 rpm, the packed cells were rinsed three times with regular saline. The cells were reconstituted in saline as a 10% (v/v) suspension. Equal amounts of plant extracts and RBC suspension (100-500 µg/mL) were combined for the assay, using diclofenac sodium as the standard medication and saline as the control. To calculate the percentage of inhibition of hemolysis, the mixtures were incubated for 30 minutes at 56 °C, cooled, centrifuged for 5 minutes at 2500 rpm, and the absorbance of the supernatant was measured at 560 nm.

2.10 Anticancer Activity

The MTT assay was used to evaluate the destructive effects of Solanum nigrum extract on breast cancer cells. After being seeded in 96-well plates, cells were exposed to varying extract concentrations (25-200 µg/mL) for a full day. Absorbance was measured at 570 nm following treatment with MTT reagent. The outcomes were contrasted with those of untreated control groups [37].

2.11 Statistical Analysis

Three duplicates of each assay were run. Mean ± standard deviation is used to express the data. To ascertain statistical significance, one-way analysis of variance, or ANOVA, was used. Statistical significance was determined at a p-value of less than 0.05.

3. Results and Discussion

3.1 Comparative Antibacterial Screening of Plant Extracts

Initially, the antibacterial activity of all four methanolic plant extracts was tested against clinical bacterial isolates that were resistant to multiple drugs. With inhibition zones varying from 14.3±0.5 mm to 20.2 ± 0.7 mm, depending on the test organism, *Solanum nigrum* exhibited the most significant activity among them. *Zingiber officinale* outperformed *Mesua ferrea* and *Pueraria tuberosa* by a small margin, while the other three extracts displayed moderate to low inhibition. Active phytoconstituents like alkaloids and flavonoids, which have been shown to break down bacterial cell walls and prevent the synthesis of nucleic acids, may be the cause of *Solanum nigrum's* superior antibacterial activity [38].

3.2 Phytochemical Screening

The identification of alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, and saponins in the *S. nigrum* extract has been determined by the qualitative phytochemical analysis. The consistency of the findings has been confirmed by earlier studies that found a similar phytochemical profile in various plant parts, such as leaves, fruits, and roots [8]. Primarily isolated from *S. nigrum*, alkaloids like solanine and solamargine are known to have antimicrobial and anticancer effects by disrupting cellular signaling and inducing apoptosis [39]. Flavonoids, which are commonly found in extracts from *S. nigrum*, are known to scavenge reactive oxygen species, inhibit microbial efflux pumps, and provide protection against oxidative stress [39]. The antimicrobial potential was further enhanced by the presence of tannins, which precipitate microbial proteins and inhibit survival-critical enzymes. Additionally found in our investigation, terpenoids have been linked to pathogenic microorganisms' disruption of membranes and have been shown to contribute to anti-inflammatory activity in related species. According to previous quantitative assessments,

phenolic compounds which are especially prevalent in *S. nigrum* fruits have potent radical scavenging activity that is directly correlated with the total phenolic content. Moreover, *S. nigrum* saponins have been connected to antimicrobial activities through membrane permeabilization as well as cytotoxicity against tumor cells [40, 41]. Collectively, the phytochemical profile found in our extract validates earlier findings and helps to explain, at least in part, the biological activities linked to *S. nigrum.*

3.3 Antibacterial activity

The agar well diffusion method was used to assess the antibacterial activity of the methanolic extracts of the chosen plants against six multidrug-resistant bacterial isolates: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Bacillus paraanthracis*, *Alcaligenes faecalis*, and *Bacillus licheniformis*. All bacterial strains demonstrated substantial zones of inhibition, though the isolates' levels of susceptibility differed. The inhibition zones for *S. aureus* ranged from 18.5 ± 0.6 mm to 22.1 ± 0.8 mm, demonstrating the highest sensitivity to the extracts and the significant antibacterial activity of the methanolic extracts against Gram-positive cocci. This result is in line with earlier studies showing the effectiveness that plant-derived compounds are at combating the multidrug-resistant pathogen *S. aureus* [7, 42].

Among the Gram-negative bacteria, the zones of inhibition for *A. faecalis* and *E. coli* ranged from 12.3 ± 0.4 mm to 16.7 ± 0.5 mm, indicating moderate sensitivity. A potential reason for these Gram-negative strains comparatively lower susceptibility is their protective outer membrane barrier, which prevents some phytochemicals from penetrating [8, 43]. Responses to the extracts varied among the *Bacillus species*, including *B. cereus*, *B. paraanthracis*, and *B. licheniformis*. Inhibition zones for *B. cereus* ranged from 15.2 ± 0.5 mm to 19.0 ± 0.6 mm, while zones for *B. paraanthracis* and *B. licheniformis* showed moderate activity, ranging from 13.5 ± 0.3 mm to 17.4 ± 0.7 mm. The methanolic extracts appear to have promising antibacterial activity against Bacillus species, which are significant opportunistic pathogens, according to these findings [44]. The investigated methanolic plant extracts have broad-spectrum antibacterial activity, according to the results, and are more effective against Gram-positive bacteria than Gram-negative bacteria. The presence of bioactive phytoconstituents like alkaloids, flavonoids, and phenolic compounds which are known to damage bacterial membranes, prevent enzyme activity, and obstruct the synthesis of nucleic acids is responsible for this antibacterial potential [8]. To identify the precise chemicals causing this activity and thoroughly assess their mechanisms of action, more research is necessary.

3.4 Thin-Layer Chromatography (TLC) Analysis

The varied chemical composition of the extract was confirmed by TLC profiles, which displayed clear bands at 254 and 366 nm when exposed to UV light. Multiple secondary metabolites have been determined to be detected based on the separation efficiency. Following ferric chloride spraying, the bands observed showed the existence of phenolic compounds. Fractions of interest were identified using TLC as a dependable initial technique, and HPTLC and GC-HRMS were used to quantify and characterize them later.

3.5 High-Performance Thin-Layer Chromatography (HPTLC) Quantification: A fast and reproducible technique for measuring phytochemicals in intricate plant matrices was HPTLC. Using a reference standard, HPTLC analysis measured one of the extract's essential components. 1.28% w/w was the concentration of the marker compound, such as orcinol. The marker's retention factor (Rf) matched that seen in the extract lanes, and the calibration curve displayed good linearity (R^2= 0.998).

3.5 GC-HRMS Characterization

Orcinol, hexadecanoic acid, methyl linoleate, and phytol were among the several compounds found by GC-HRMS analysis. Earlier investigations have reported the antimicrobial and anti-inflammatory properties of each of these compounds separately. One phenolic compound that has been shown to have antibacterial activity against both Gram-positive and Gram-negative bacteria was orcinol. Such compounds have been found in *S. nigrum*, which increases its therapeutic relevance and validates its observed biological properties.

3.7 Antioxidant Activity

Across all tests, the extract exhibited concentration-dependent antioxidant activity. For superoxide scavenging, the IC_{50} value was 49.2 µg/mL, whereas for the DPPH assay, it was 42.6 µg/mL. The optimum inhibition of 65.3% of catalase activity was observed at 200 µg/mL. The findings indicate the presence of potent phytoconstituents that donate electrons and can neutralize free radicals, thereby preventing oxidative stress. *S. nigrum* antioxidant activity corresponds to accordance with previous research by Shailabi *et al*. (2024), which linked these benefits to polyphenolic components [44]. Similar findings were published by Nithiyanantham *et al*. (2012), who showed a significant association between DPPH and ABTS values and the total phenolic and flavonoid content of *S. nigrum* extracts [45]. Further study confirmed the significance of flavonoids and tannins in this species' antioxidant potential by showing that methanolic leaf extracts had strong hydroxyl radical scavenging and ABTS scavenging activity [46]. In addition, it was demonstrated that saponin-enriched fractions of S. nigrum fruits exhibited strong radical scavenging properties (DPPH IC_{50} = 38.2 µg/mL), indicating that saponins contribute to antioxidant capacity in addition to phenolics [41]. Together, these results show that several classes of phytochemicals work in synchrony to produce S. nigrum's antioxidant activity, which was in accordance with the high activity observed in our tests.

3.8 Anti-inflammatory Activity

The ability of the plant extracts to prevent protein denaturation and maintain erythrocyte membrane stability under heat-induced stress was used to gauge their anti-inflammatory effectiveness. All tested extract showed a significant, concentration-dependent inhibition of albumin denaturation in the protein denaturation assay. The prevalent pathway in inflammatory processes, protein denaturation, was prevented by the extract, as evidenced by the maximum inhibition, which varied from 60% to 85%. These findings align with past research showing that bioactive substances derived from plants, like flavonoids and phenolics, stabilize proteins by avoiding thermal denaturation [34, 35]. When denaturation causes inflammation and tissue damage, the inhibition of protein denaturation raises the possibility of therapeutic benefits.

Heat-induced hemolysis was used to determine the membrane-stabilizing effect, and the results showed that the extracts significantly protected red blood cells (RBCs).

Comparable to the common anti-inflammatory medication diclofenac sodium, the percentage inhibition of hemolysis ranged from 55% to 80%. This protection suggests that the extracts have anti-inflammatory qualities because they can stabilize lysosomal membranes and prevent the release of inflammatory mediators [47]. These results are consistent with the known function of phytochemicals in preserving the integrity of membranes under stress, and membrane stabilization serves as crucial for shielding cells from harm during inflammatory responses.

Furthermore, the plant extracts effectiveness to reduce inflammation has been proven by the observed inhibition of membrane stabilization and protein denaturation. The findings support earlier studies showing the importance of plant secondary metabolites, including flavonoids, alkaloids, and saponins, in regulating inflammatory pathways [35]. To completely clarify the therapeutic relevance of these extracts, more research would be beneficial, including *in vivo* studies and the identification of active constituents.

3.9 Anticancer Activity

The MTT assay was used to assess the destructive effect of *S. nigrum* extract on human cancer cell lines. Considering an IC_{50} value of 78.5 µg/mL on MCF-7 breast cancer cells, the extract demonstrated significant activity. Under a microscope, cellular changes revealed apoptosis-mediated cell death. These findings demonstrate *S. nigrum's* potential as a source of anticancer agents and confirm earlier reports of its destructive nature [48, 49].

4. Conclusion

The methanolic extract of *S. nigrum* is thoroughly evaluated in this study, starting with a comparative antibacterial screening of the extract among a few chosen ethno-medicinal species and continuing with in-depth phytochemical identification and biological activity evaluation. *S. nigrum* showed the strongest antibacterial activity against bacteria that were resistant to multiple drugs among the plants that were tested. The presence of various phytochemicals was verified by later analyses, which were identified by GC-HRMS and quantified by HPTLC. The extract exhibited significant cytotoxic effects against human cancer cell lines in addition to strong antioxidant and anti-inflammatory qualities. According to these results, *S. nigrum* represents a rich source of bioactive compounds with many potential uses and could be a good natural candidate for the production of substitute remedies. The subsequent research should focus on *in vivo* validation and bioassay-guided fractionation to comprehend entirely the mechanisms of action of its active ingredients.

5. Statements and Declarations

5.1 Funding

The authors declare that no funding was received for the research presented in this article.

5.2 Competing Interests

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the submitted paper.

5.3 Data Availability

The datasets generated during and analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

5.4 Ethical Approval

Not applicable.

Table 1: Preliminary antibacterial screening of four plant extracts against MDR bacterial strains

Plants	***Solanum nigrum***		***Zingiber officinale***		***Mesua ferrea***		***Pueraria tuberosa***	
	Antibacterial Activity (mg/mL)							
Bacterial isolates	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus aureus	6.5	10	15	22	22	33	30	45
Escherichia coli	8	12	18	27	25	38	35	52
Bacillus cereus	7.5	11	16	24	23	35	32	48
Bacillus paraanthracis	7.8	12	17	26	24	36	34	51
Alcaligenes faecalis	9	14	20	30	28	42	40	60
Bacillus licheniformis	8.2	12	18	27	25	38	36	54

Table 2: Phytochemical screening of plant extracts

Plant	***Solanum nigrum***	***Zingiber officinale***	***Mesua ferrea***	***Pueraria tuberosa***
Alkaloids	++	-	-	-
Carbohydrates	++	+	-	+
Glycosides	+	-	-	-
Tannins	+++	++	++	++
Saponins	++	+	-	+
Steroids	++	-	-	-
Fixed oils and fats	+	+	++	-
Flavonoids	+++	++	+++	+
Phlobatannins	+	+	+	+
Terpenoids	-	-	-	++
Cardiac glycosides	-	+	+	+
Phenol	+++	+++	++	++

Table 3: Antioxidant activities of *S. nigrum* extract (IC_{50} values)

Assay	**IC_{50} (µg/mL)**
DPPH scavenging assay	42.6 ± 1.3
Superoxide scavenging	49.2 ± 1.5
Catalase inhibition assay	56.1 ± 2.0

Figures

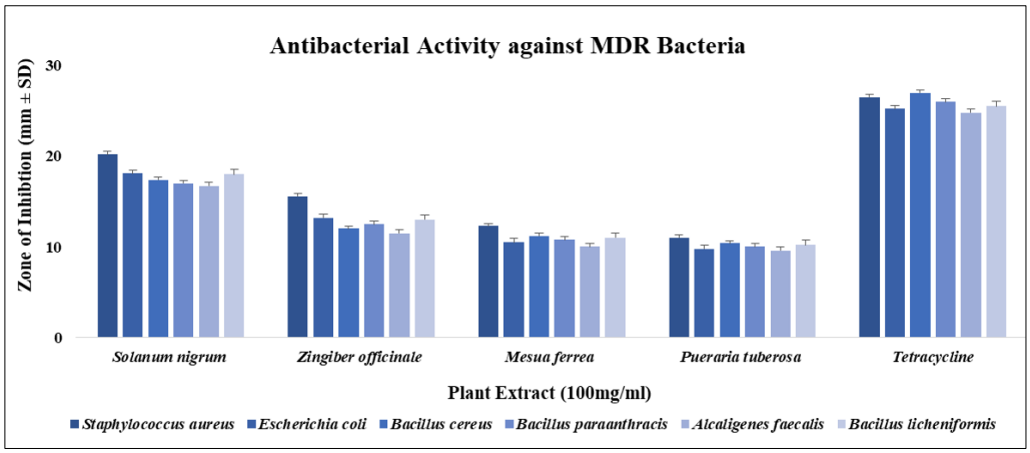


Fig 1: Error-bar plot (mean ± SD, n = 3) comparing inhibition-zone diameters (mm) for *Mesua ferrea* (stems), *Zingiber officinale* (rhizomes), *Solanum nigrum* (leaves), and *Pueraria tuberosa* (tubers) extracts against *Bacillus paraanthracis*, *Bacillus cereus*, *Bacillus anthracis*, *Bacillus altitudinis*, *Staphylococcus aureus*, *Escherichia coli*, *Alcaligenes faecalis*, and *Bacillus licheniformis* bacteria isolated from human lactational mastitis milk samples.

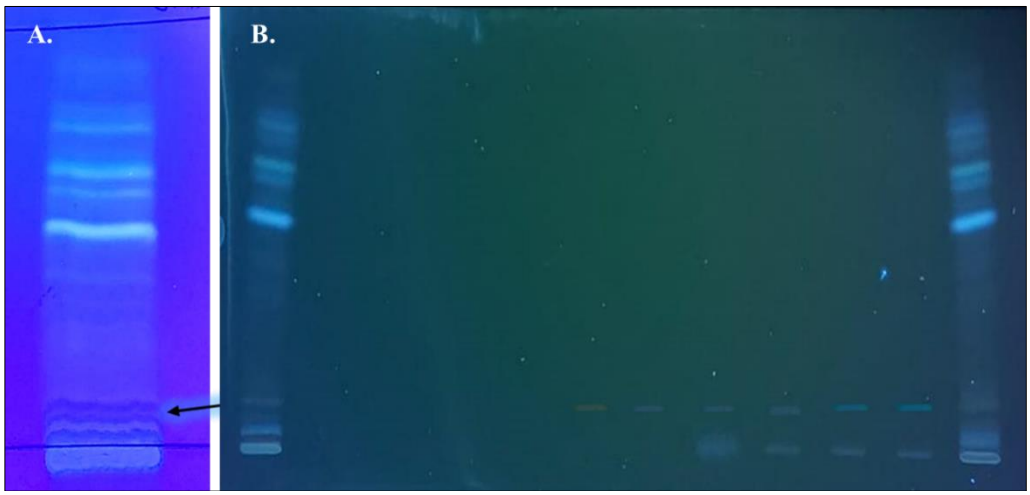


Fig 2A: Representative thin-layer chromatography (TLC) profile of *S. nigrum* extract under UV light at 254 nm and after derivatization, showing separation of major phytoconstituents. B. High-performance thin-layer chromatography (HPTLC) densitogram of *S. nigrum* extract, indicating the peak corresponding to the quantified phytochemical with a retention factor (Rf) value 0f 0.06.

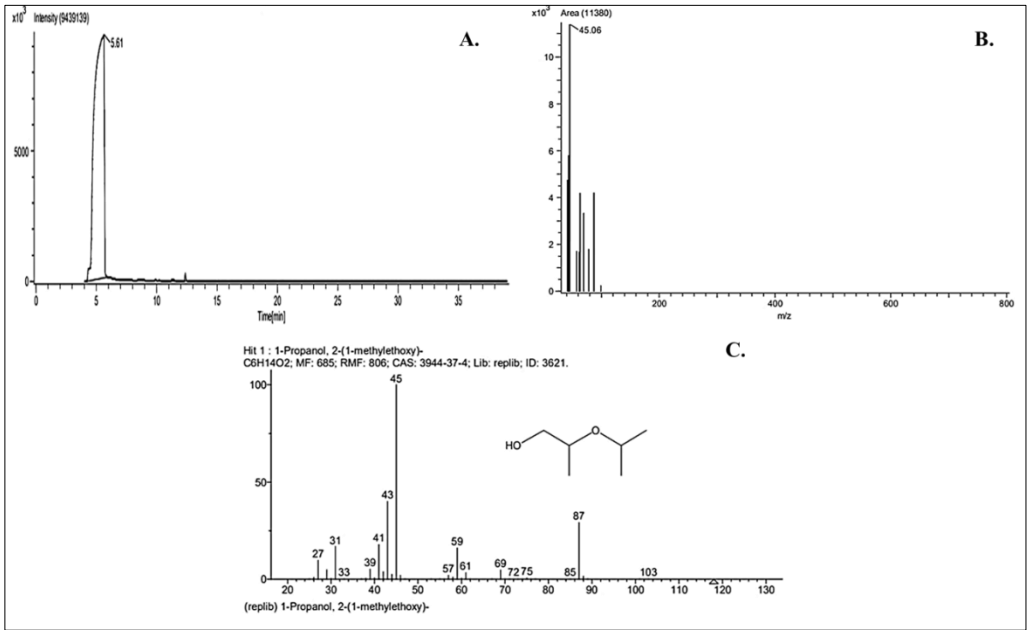


Fig 3A: GC chromatogram of the isolated band of methanolic extract of *S. nigrum* L, B. Mass spectrum of the isolated band of methanolic extract of *S. nigrum* L, C. Mass spectrum of the identified compound from the isolated band of methanolic extract of *S. nigrum* L

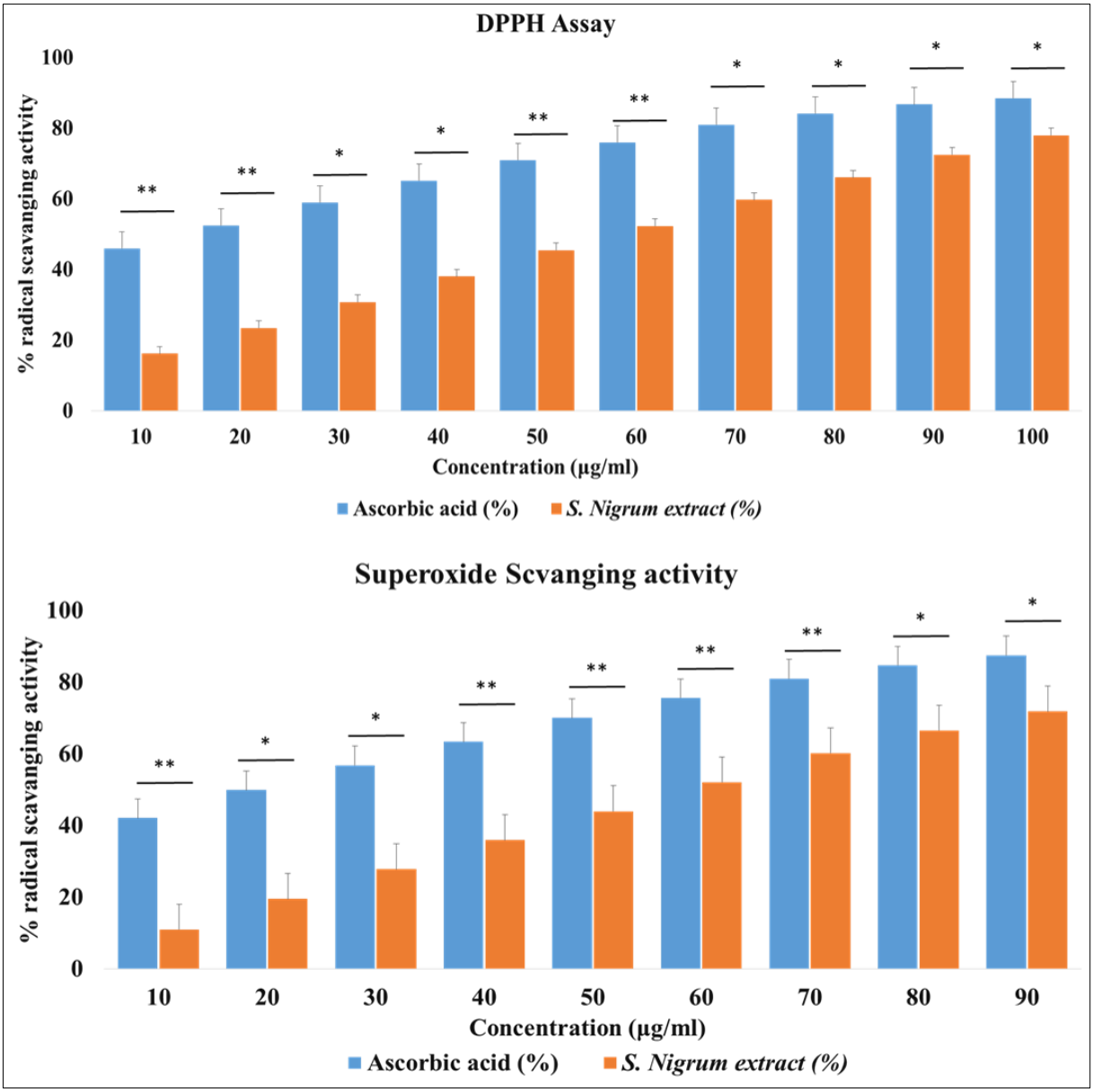


Fig 4: Dose-response curves of *S. nigrum* extract in antioxidant assays, A. DPPH, and B. Superoxide scavenging.

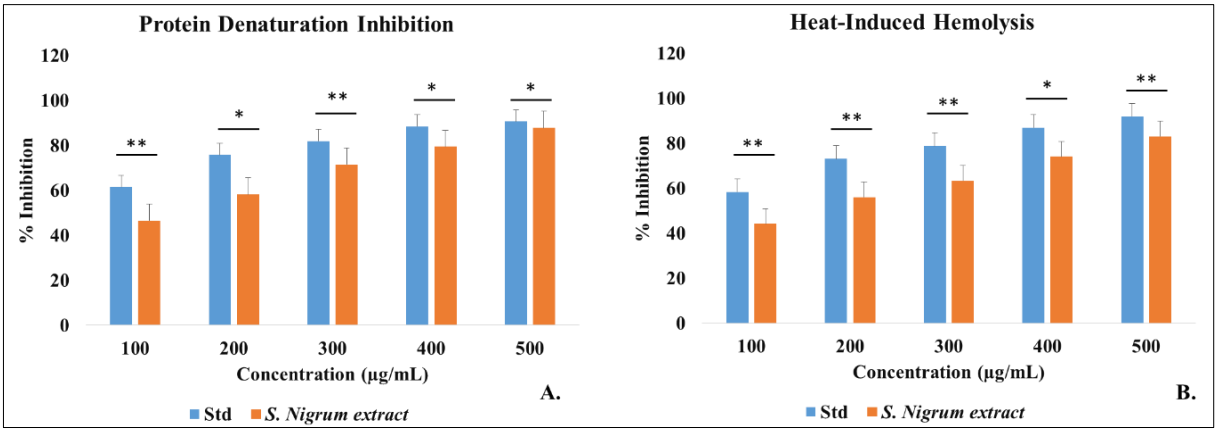


Fig 5A: Inhibition of protein denaturation by *S. nigrum* extract, illustrating its anti-inflammatory potential compared to diclofenac sodium, B. Heat-induced hemolysis by *S. nigrum* extract, illustrating its anti-inflammatory potential compared to diclofenac sodium.

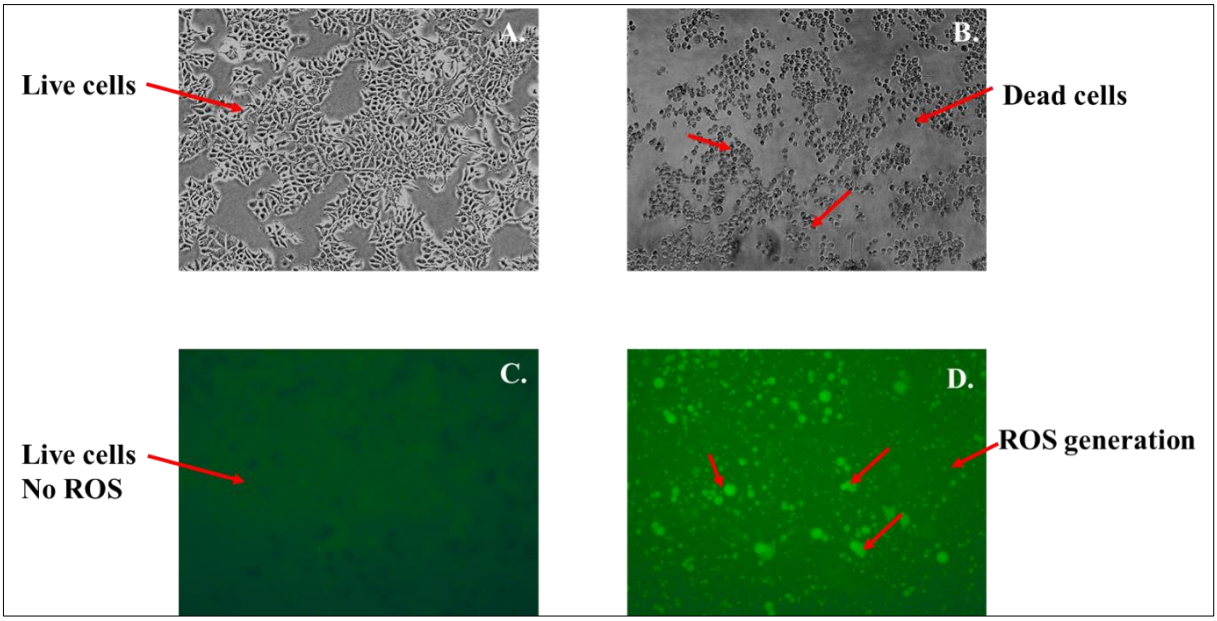


Fig 6: Cytotoxic effect of *S. nigrum* extract on MCF-7 breast cancer cells, A. Untreated cells, B. Cells treated with *S. nigrum* extract, C. Untreated control shows faint basal fluorescence, and D. Cells treated with *S. nigrum* extract exhibit intense green fluorescence (arrows) showing elevation of ROS.

References

1. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Med Chem. 2014;6:25-64. https://doi.org/10.4137/PMC.S14459
2. Ventola CL. The antibiotic resistance crisis. Pharm Ther. 2015;40:277-283. https://doi.org/PMC4378521
3. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG *et al*. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28:603-661. https://doi.org/10.1128/CMR.00134-14
4. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M *et al*. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev. 2013;26:822-880. https://doi.org/10.1128/CMR.00022-13
5. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. Clin Microbiol Rev. 2010;23:382-398. https://doi.org/10.1128/CMR.00073-09
6. Van Tongeren SP, Roest HIJ, Degener JE, Harmsen HJM. *Bacillus anthracis*-like bacteria and other *B. cereus* group members in a microbial community within the International Space Station: a challenge for rapid and easy molecular detection of virulent *B. anthracis*. PLoS One. 2014;9:1-8. https://doi.org/10.1371/journal.pone.0098871
7. Evans SM, Cowan MM. Plant products as antimicrobial agents. Cosmet Drug Microbiol. 2016;12:205-231. https://doi.org/10.3109/9781420019919-17
8. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26:343-356. https://doi.org/10.1016/j.ijantimicag.2005.09.002
9. Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol. 2012;23:174-181. https://doi.org/10.1016/j.copbio.2011.08.007
10. Alviano DS, Alviano CS. Plant extracts: search for new alternatives to treat microbial diseases. Curr Pharm Biotechnol. 2009;10:106-121. https://doi.org/10.2174/138920109787048607
11. Rates SMK. Plants as source of drugs. Toxicon. 2001;39:603-613. https://doi.org/10.1016/S0041-0101(00)00154-9
12. Singh SK, Rai PK, Jaiswal D, Watal G. Evidence-based critical evaluation of glycemic potential of *Cynodon dactylon*. Evid Based Complement Altern Med. 2008;5:415-420. https://doi.org/10.1093/ecam/nem044
13. Chahar K. *Mesua ferrea* L.: a review of the medical evidence for its phytochemistry and pharmacological actions. Afr J Pharm Pharmacol. 2013;7:211-219. https://doi.org/10.5897/ajpp12.895
14. Rahmani AH, Al Shabrmi FM, Aly SM. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. Int J Physiol Pathophysiol Pharmacol. 2014;6:125-136.
15. Butt MS, Sultan MT. Ginger and its health claims: molecular aspects. Crit Rev Food Sci Nutr. 2011;51:383-393. https://doi.org/10.1080/10408391003624848
16. Nawaz A, Jamal A, Arif A, Kiran S, Shahid MN *et al*. Bioactive compounds isolated from *Solanum nigrum* remarkably inhibit cancerous activity in cancer cell lines. S Afr J Bot. 2024;168:488-496. https://doi.org/10.1016/j.sajb.2024.03.048
17. Sujamol MS, Roy J, James KM. Phytochemical screening and antimicrobial activity of *Coleus aromaticus* leaf extract. Mater Today Proc. 2020;41:596-599. https://doi.org/10.1016/j.matpr.2020.05.255
18. Maji AK, Pandit S, Banerji P, Banerjee D. *Pueraria tuberosa*: a review on its phytochemical and therapeutic potential. Nat Prod Res. 2014;28:2111-2127. https://doi.org/10.1080/14786419.2014.928291
19. Wang CF, Xu WJ, Xu Y, Wang YX, Liu JQ. Transcriptomic analyses reveal anti-inflammatory mechanism of withanolides derived from the fruits of *Physalis alkekengi* L. var. *franchetii*. Phytother Res. 2021;35:2568-2578. https://doi.org/10.1002/ptr.6987
20. Dalwadi P, Nathani N, Chauhan K, Mansuri J, Koringa P *et al*. Whole-genome sequencing of bacteria accountable for lactational mastitis in humans combined with an examination of their antibiotic resistance profiles. Braz J Microbiol. 2024. https://doi.org/10.1007/s42770-024-01519-3

21. Patel YH, Vaidya RJ, Patel RJ, Pandit RJ, Joshi CG *et al*. Culture independent assessment of human milk microbial community in lactational mastitis. Sci Rep. 2017;7. https://doi.org/10.1038/s41598-017-08451-7
22. Bhatt VD, Kunjadia PD, Kunjadia AP. Isolation and characterization of probiotic bacteria from human milk. 2012. https://api.semanticscholar.org/CorpusID:89680811
23. Dharajiya D, Pagi N, Jasani H, Patel P. Antimicrobial activity and phytochemical screening of *Aloe vera* (*Aloe barbadensis* Miller). Int J Curr Microbiol Appl Sci. 2017;6:2152-2162. https://doi.org/10.20546/ijcmas.2017.603.246
24. Reena P, Aditi P, Dharmesh V, Anju N. Antimicrobial evaluation of *Hibiscus rosa-sinensis* plant extracts against some pathogenic bacteria. Bull Environ Sci Res. 2012;1:14-17. http://www.besr.org.in/index.php/besr/article/view/31
25. De Zoysa MHN, Rathnayake H, Hewawasam RP, Wijayaratne WMDGB. Determination of *in vitro* antimicrobial activity of five Sri Lankan medicinal plants against selected human pathogenic bacteria. Int J Microbiol. 2019;2019. https://doi.org/10.1155/2019/7431439
26. Dhawan D, Gupta J. Comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. Int J Biol Chem. 2016;11:17-22. https://doi.org/10.3923/ijbc.2017.17.22
27. Dharajiya D, Moitra N, Patel B, Patel RK. Preliminary phytochemical analysis of the Indian medicinal plants for antibacterial activity against bovine mastitis pathogens. Wayamba J Anim Sci. 2012;332-342. http://www.wayambajournal.com
28. Rengarajan S, Melanathuru V, Munuswamy D, Sundaram S, Selvaraj ST. A comparative study of *in vitro* antimicrobial activity and TLC studies of petals of selected Indian medicinal plants. Asian J Pharm Clin Res. 2016;9:259-263. https://doi.org/10.22159/ajpcr.2016.v9i5.13476
29. Chakraborty S, Afaq N, Singh N, Majumdar S. Antimicrobial activity of *Cannabis sativa*, *Thuja orientalis* and *Psidium guajava* leaf extracts against methicillin-resistant *Staphylococcus aureus*. J Integr Med. 2018;16:350-357. https://doi.org/10.1016/j.joim.2018.07.005
30. Hegde NP, Hungund BS. Phytochemical profiling of *Clerodendrum paniculatum* leaf extracts: GC-MS, LC-MS analysis and comparative evaluation of antimicrobial, antioxidant and cytotoxic effects. Nat Prod Res. 2023;37:2957-2964. https://doi.org/10.1080/14786419.2022.2140339
31. Patel R, Patel Y, Kunjadia P. DPPH free radical scavenging activity of phenolics and flavonoids in some medicinal plants of India. 2015. http://www.ijcmas.com
32. Maheshwari N, Sharma MC. Anticancer properties of some selected plant phenolic compounds: future leads for therapeutic development. J Herb Med. 2023;42:100801. https://doi.org/10.1016/j.hermed.2023.100801
33. Doss A, Soris PT, Mohan VR. Evaluation of antibacterial effect of medicinal plant extracts on bacteria isolated from subclinical bovine mastitis. 2019. www.ijvsar.com
34. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol. 1968;20:169-173. https://doi.org/10.1111/j.2042-7158.1968.tb09718.x
35. Sakat SS, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci. 2010;2:146-155.
36. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ *et al*. Membrane stabilizing activity - a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia. 1999;70:251-257. https://doi.org/10.1016/S0367-326X(99)00030-1
37. Thakkar AB, Subramanian RB, Thakkar VR, Bhatt SV, Chaki S *et al*. Apoptosis induction capability of silver nanoparticles capped with *Acorus calamus* L. and *Dalbergia sissoo* Roxb. ex DC. against lung carcinoma cells. Heliyon. 2024;10:e24400. https://doi.org/10.1016/j.heliyon.2024.e24400
38. Adib AM, Yunos NM, Jin CB. Anti-cancer, antimicrobial, and antioxidative potentials of *Mesua ferrea* L. and its phytochemical constituents: a review. Asian J Pharmacogn. 2019;3(3):5-19.
39. Gu XY, Shen XF, Wang L, Wu ZW, Li F *et al*. Bioactive steroidal alkaloids from the fruits of *Solanum nigrum*. Phytochemistry. 2018;147:125-131. https://doi.org/10.1016/j.phytochem.2017.12.020
40. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother. 2001;48:487-491. https://doi.org/10.1093/jac/48.4.487
41. Wang Y, Wang S, Xu J, Wang Y, Xiang L *et al*. Total steroidal saponins from black nightshade (*Solanum nigrum* L.) overcome tumor multidrug resistance by inducing autophagy-mediated cell death *in vivo* and *in vitro*. Phytother Res. 2023;37:3009-3024. https://doi.org/10.1002/ptr.7796
42. Chen X, Dai X, Liu Y, Yang Y, Yuan L *et al*. *Solanum nigrum* Linn.: an insight into current research on traditional uses, phytochemistry, and pharmacology. Front Pharmacol. 2022;13:1-32. https://doi.org/10.3389/fphar.2022.918071
43. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 2013;6:1451-1474. https://doi.org/10.3390/ph6121451
44. Aparna P, Shanthi Priya M, Mohan Reddy D, Latha P. Estimation of genetic parameters in groundnut (*Arachis hypogaea* L.) for yield and its contributing characters under inorganic fertilizer managements. Int J Curr Microbiol Appl Sci. 2018;7:1559-1565. https://doi.org/10.20546/ijcmas.2018.704.175
45. Nithiyanantham S, Varadharajan S, Siddhuraju P. Differential effects of processing methods on total phenolic content, antioxidant and antimicrobial activities of three species of *Solanum*. J Food Drug Anal. 2012;20:314-320. https://doi.org/10.6227/jfda.2012200414
46. Sharma SK, Singh AP. *In vitro* antioxidant and free radical scavenging activity of *Nardostachys jatamansi* DC. J Acupunct Meridian Stud. 2012;5:112-118. https://doi.org/10.1016/j.jams.2012.03.002
47. Prajakta S, Shahu K. Formulation and evaluation of vanishing herbal cream of crude drugs. Asian J Pharm Res Dev. 1970;8:66-69. https://doi.org/10.22270/ajprd.v8i3.725

48. Rafique M, Sadaf I, Rafique MS, Tahir MB. A review on green synthesis of silver nanoparticles and their applications. Artif Cells Nanomed Biotechnol. 2017;45:1272-1291. https://doi.org/10.1080/21691401.2016.1241792
49. Thakkar AB, Sargara P, Subramanian RB, Thakkar VR, Thakor P. Induction of apoptosis in lung carcinoma cells (A549) by hydromethanolic extract of *Acorus calamus* L. Process Biochem. 2022;123:1-10. https://doi.org/10.1016/j.procbio.2022.10.028