RESEARCH PAPER

Phytochemical profiling and In Vitro Evaluation of the Biological activities of *Mirabilis jalapa Linn*. Stem Extracts

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ABSTRACT

Medicinal plants serve as a valuable source of bioactive compounds with significant therapeutic potential. Mirabilis jalapa Linn. (Nyctaginaceae) has traditionally been used to treat various ailments, owing to its anti-inflammatory, antioxidant, and antimicrobial properties. The objective of the present study was to investigate the phytochemical constituents and assess the antimicrobial, anti-inflammatory, antioxidant, and cytotoxic activities of stem extracts of *Mirabilis jalapa Linn.* The stem powder of *Mirabilis jalapa Linn.* was extracted using a soxhlet apparatus with different solvents. Preliminary qualitative and quantitative phytochemical analyses were carried out following standard protocols. The antibacterial activity of the extracts was evaluated by the disc diffusion method against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. Antifungal activity was assessed against Candida albicans and Aspergillus niger. The anti-inflammatory potential was determined using the protein denaturation assay. Antioxidant activity was determined by applying the DPPH and Nitric Oxide scavenging assay. The cytotoxic activity of the ethanol extract was evaluated against SH-SY5Y human neuroblastoma cells using the MTT assay. Phytochemical analysis revealed the presence of alkaloids, triterpenoids, flavonoids, tannins, carbohydrates, saponins, and unsaturated hydrocarbons in the various solvent extracts of *Mirabilis jalapa Linn.* Quantitative analysis revealed the presence of alkaloid, tannin, saponin and flavonid. Among the extracts, the ethanol extract demonstrated the strongest antibacterial activity, exhibiting inhibition zones of 33 mm against *Escherichia coli* and 31 mm against *Pseudomonas aeruginosa*. It also showed appreciable antifungal activity against Candida *albicans* (15.33 ± 0.33 mm) and *Aspergillus niger* (12.33 ± 0.33 mm). The anti-inflammatory activity increased with concentration, producing inhibition of protein denaturation from 43.86 to 88.07 % over the same concentration range. The antioxidant potential of the ethanol extract was found to be concentration dependent, exhibiting significant free radical scavenging activity in the DPPH and Nitric oxide assay, with percentage inhibition increasing from 12.12 to 61.30 % for DPPH and 13.10 - 69.10 % for nitric oxide at concentrations of 100–500 µg/mL. Furthermore, the ethanol extract exhibited marked cytotoxic activity against SH-SY5Y human neuroblastoma cells, causing a dose-dependent reduction in cell viability from 87.78 to 27.91%, thereby indicating its promising antiproliferative potential. The study demonstrates that *Mirabilis jalapa Linn* stem possesses potent antimicrobial, anti-inflammatory, antioxidant, and cytotoxic properties, thereby supporting its ethnomedicinal significance and underscoring its potential as a natural source of bioactive molecules for future therapeutic applications.

Keywords *Mirabilis jalapa Linn*, Phytochemical screening, Antimicrobial activity, Anti-inflammatory activity, Anti oxidant activity, Cytotoxicity, SH-SY5Y cells, MTT assay.

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INTRODUCTION

Medicinal plants have served as an indispensable source of therapeutic agents for centuries and remain an integral component of both traditional and modern healthcare systems. Owing to their rich diversity of phytochemicals, medicinal plants have contributed

significantly to the discovery and development of numerous pharmaceutical drugs. In recent years, the rapid emergence of antimicrobial resistance, the increasing incidence of chronic inflammatory diseases, oxidative stress-related disorders, and various forms of cancer have accelerated the search for safer, more effective, and naturally derived therapeutic agents. Consequently, medicinal plants have gained considerable scientific attention as promising reservoirs of biologically active compounds with multiple pharmacological activities (1-3).

Plants synthesize a wide range of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, glycosides, saponins, steroids, and coumarins, which exhibit diverse biological properties. These phytoconstituents possess antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antiviral, immunomodulatory, and anticancer activities through various mechanisms such as scavenging reactive oxygen species, modulating inflammatory mediators, regulating cell-signaling pathways, and inducing apoptosis in malignant cells. Furthermore, many plant-derived compounds demonstrate synergistic effects with conventional therapeutic agents, thereby enhancing efficacy while minimizing adverse effects and delaying the development of drug resistance (4-5).

Mirabilis jalapa Linn., commonly known as the Four O'Clock plant, belongs to the family Nyctaginaceae. It is a perennial herbaceous plant widely distributed throughout tropical and subtropical regions of the world and is extensively cultivated as an ornamental plant because of its attractive, fragrant, and brightly coloured flowers that typically bloom in the late afternoon. In addition to its ornamental value, *M. jalapa* has attracted considerable scientific interest due to its rich phytochemical composition and diverse pharmacological activities (6, 7).

The plant has been used extensively in traditional systems of medicine, including Ayurveda, folk medicine, and indigenous healthcare practices, for the management of numerous ailments. Different parts of the plant, including the roots, leaves, flowers, and seeds, possess significant medicinal properties. The roots are traditionally employed as a purgative, diuretic, laxative, anti-inflammatory, analgesic, antimicrobial, and vermifuge agent and are also used in the treatment of constipation, urinary tract disorders, arthritis, dysentery, abdominal pain, and edema. Leaf preparations are commonly applied topically to promote wound healing and to treat boils, abscesses, skin infections, insect bites, burns, and inflammatory conditions. The leaves are also reported to exhibit antimicrobial, antioxidant, anti-inflammatory, and hepatoprotective activities (8,9).

The flowers have been traditionally used for the treatment of herpes lesions, skin pigmentation disorders, and fungal infections, while the seeds have been reported to possess antiviral, antimicrobial, and immunomodulatory properties. Furthermore, extracts of *M. jalapa* have demonstrated antioxidant, cytotoxic, anticancer, antidiabetic, antinociceptive, and immunomodulatory activities in several experimental studies, which are largely attributed to the presence of bioactive phytoconstituents such as alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, glycosides, steroids, and saponins (10-11). These pharmacological properties support the traditional therapeutic uses of the plant and highlight its potential as a valuable source of bioactive molecules for the development of novel therapeutic agents.

Despite the extensive traditional use and reported pharmacological activities of *Mirabilis jalapa Linn.*, most scientific investigations have focused on the roots, leaves, flowers, and whole plant extracts. To the best of our knowledge, there are no published reports on the phytochemical evaluation and the anti-inflammatory, antioxidant, antimicrobial and cytotoxic activities of the stem of *Mirabilis jalapa Linn.* Therefore, the present study was undertaken to investigate the phytochemical constituents of different solvent extracts of *M. jalapa Linn* stem and to evaluate their antimicrobial, antioxidant, anti-inflammatory and cytotoxic activities using appropriate in vitro methods. The findings of this study are expected to provide scientific evidence for the medicinal potential of the stem and establish it as a promising source of bioactive compounds for the development of novel therapeutic agents.

MATERIALS AND METHODS

Phytochemical Screening of *Mirabilis jalapa Linn.* stem Extract

The phytochemical constituents present in different solvent extracts of the fresh stem of *Mirabilis jalapa Linn.* were identified and characterized through qualitative and quantitative phytochemical analyses. The extracts were evaluated for the presence of major secondary metabolites, including flavonoids, alkaloids, glycosides, saponins, tannins, phytosterols, and terpenoids. Furthermore, the biological activities of the extracts, including antioxidant, anti-inflammatory, antimicrobial, and cytotoxic effects, were systematically investigated to establish their therapeutic potential (12-14).

Collection and Authentication of plant material

Fresh stems of *Mirabilis jalapa Linn.* were collected from Erode District, Tamil Nadu, India. The plant material was taxonomically authenticated by the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore. A voucher specimen was deposited at the institute for future reference and

assigned the accession number BSI/SRC/5/23/2023/Tech-280.

Extracts preparation

The collected stems were thoroughly washed to remove adhering impurities, shade-dried at room temperature, and pulverized into a coarse powder. The powdered material was successively extracted using different solvents like benzene,chloroform, dichloromethane, ethanol and aqueous in a soxhlet apparatus. The resulting extracts were concentrated under reduced pressure using a rotary evaporator and stored in airtight containers at an appropriate temperature until further analysis.

Qualitative test for Extract

Qualitative phytochemical screening of the extracts was carried out using standard phytochemical methods to identify the presence of various secondary metabolites, including alkaloids, phenols, carbohydrates, tannins, flavonoids, saponins, triterpenoids, glycosides, proteins, and unsaturated hydrocarbons.

Alkaloids

Five millilitres of each of the five different solvent extracts of *Mirabilis jalapa Linn.* stem were transferred separately into individual test tubes and concentrated to obtain a residue. The resulting residue was dissolved in 1.5 mL of 2% (v/v) hydrochloric acid, followed by the addition of three drops of Mayer's reagent, prepared by dissolving 0.679 g of mercuric chloride ($HgCl_2$) and 2.5 g of potassium iodide (KI) in 50 mL of distilled water. The appearance of a cream or white precipitate indicated the presence of alkaloids.

Phenols

The five different solvent extracts of *Mirabilis jalapa Linn.* stem were taken separately in individual test tubes. To each extract, 2 mL of 2% ferric chloride ($FeCl_3$) solution was added and mixed thoroughly. The development of a blue, green, or black coloration indicated the presence of phenolic compounds.

Flavonoids

The five different solvent extracts of *Mirabilis jalapa Linn.* stem were taken separately and mixed with a few pieces of magnesium followed by a dropwise addition of concentrated. HCl. The formation of a pink scarlet color after a few minutes indicated the presence of flavonoids.

Tannins

About two millilitres of 5% $FeCl_3$ was added to 2 mL of the five different solvent extracts of *Mirabilis jalapa Linn.* Stem separately and observed for the formation of yellow or brown precipitates. The formation of a yellow or brown precipitate indicated the presence of tannins.

Reducing sugars

A total of 2.5 mL of Benedict's solution (17 g of trisodium citrate dihydrate, 10 g of Na_2CO_3 , and 1.74 g of copper sulfate pentahydrate in 100 mL of distilled water) was taken in a test tube followed by adding 0.5 g of five different solvent extracts of *Mirabilis jalapa* Linn. Stem separately and then warmed in a hot water bath for about 5 min. The formation of green/red or yellow coloration indicated the presence of reducing sugar.

Saponins

The five different solvent extracts of *Mirabilis jalapa* Linn. stem were mixed with 5 mL of distilled water and then shaken vigorously. The formation of stable foam indicated the presence of saponins.

Coumarin

A single pellet of KOH was dissolved in 1.0 mL of ethanol. Then, 1.0 mL of five different solvent extract of *Mirabilis jalapa* Linn. Stem solution were added separately. The formation of precipitate indicated the presence of coumarins.

Terpenoids

A small amount of five different solvent extract were separately dissolved in chloroform and an equal volume of conc. H_2SO_4 was added. Reddish-brown coloration at the junction of two liquids indicated the presence of terpenoids.

Quinones

About one millilitre of conc. H_2SO_4 was added to 1.0 mL of five different solvent plant extract solution separately. The formation of red coloration indicated the presence of quinones.

Sterols

About two milliliters of five different solvent plant extract was separately mixed with chloroform. Overall, 1–2 mL of acetic anhydride was added followed by adding one or two drops of conc.H_2SO_4 from the side of the test tube. An array of red, blue, and green colors indicated the presence of sterols.

Glycosides

About two milliliters of glacial acetic acid, one drop each of 5% $FeCl_3$ and conc. H_2SO_4 was added to 5 mL of five different solvent plant extract. The appearance of a brown ring indicated the presence of glycosides.

Proteins

About 1.0 ml five different solvent plant extract were mixed separately with 2.0 mL of Millon's reagent and observed for a white precipitate that turns red on gentle heating. The red color formation indicated the presence of proteins.

Quantitative test of Extract

Alkaloid

The total alkaloid content of the stem different extract of *Mirabilis jalapa Linn.* was determined using a standard gravimetric method and expressed as a percentage. Briefly, 5 g of the sample was transferred into a clean beaker and 20 % acetic acid solution was

added. The mixture was stirred thoroughly and allowed to stand for 4 h to facilitate complete extraction of alkaloids. The extract was then filtered, and the filtrate was concentrated on a water bath until its volume was reduced to one-fourth of the original volume. Concentrated ammonium hydroxide was added dropwise to the concentrated filtrate until complete precipitation of the alkaloids occurred. The resulting precipitate was collected by filtration, dried to a constant weight, and weighed. The total alkaloid content was calculated using the following equation:

Percentage of alkaloids (%) = (Weight of dried alkaloid residue / Weight of sample) × 100

Saponins

The total saponin content (TSC) of the different stem extracts of *Mirabilis jalapa Linn.* was determined by the vanillin–sulfuric acid colorimetric method using diosgenin as the reference standard. Diosgenin standard solutions (20–120 µg/mL) were prepared in methanol to construct a calibration curve. An aliquot of 3 mL of each sample extract or standard solution was transferred into a clean test tube. To this, 0.5 mL of 8 % (w/v) vanillin solution and 5 mL of 72 % sulfuric acid were added, and the mixture was vortexed thoroughly. The reaction mixture was incubated in a water bath at 60°C for 10 min, followed by rapid cooling in an ice bath for 5 min to terminate the reaction. The absorbance was measured at 544 nm against a reagent blank using a UV–Visible spectrophotometer (15). The concentration of saponins was determined from the diosgenin standard calibration curve and expressed as milligrams of diosgenin equivalents per gram of dry extract (mg DE/g extract) using the following equation:

Percentage of saponins (%) = (mg of diosgenin equivalent / g of sample) × 100

Flavonoid

The total flavonoid content of the stem different extract of *Mirabilis jalapa Linn.* was determined using the aluminium chloride colorimetric method. About 1 mg quercetin stock solution (mg/ml or 1000µg/ml) was made by dissolving 1mg quercetin in 1 mL methanol. The standard solution was serially diluted to produce solutions with concentrations of 100 µg/mL, 150 µg/mL, 200 µg/mL, 250 µg/mL, 300 µg/mL and 350 µg/mL. Briefly, 2 mL of the sample different extract was dissolved in distilled water, followed by the addition of 5 % sodium nitrite solution. The reaction mixture was allowed to stand for 5 min. Subsequently, 5 % aluminium chloride solution and 4 % sodium hydroxide solution were added, and the final volume was adjusted to 5 mL with distilled water. The mixture was shaken thoroughly and incubated at room temperature for 10 min to allow colour development. The intensity of the pink colour formed was measured spectrophotometrically, and the total flavonoid content was calculated using quercetin as the reference standard and expressed as a percentage (16).

Percentage of flavonoids (%) = (mg of quercetin equivalent / g of sample) × 100

Tannins

The tannins were determined by Folin-Ciocalteu method. About 1 mg tannic acid stock solution (mg/ml or 1000µg/ml) was made by dissolving 1mg tannic acid in 1 ml methanol. The standard solution was serially diluted to produce solutions with concentrations of 100 µg/mL, 150 µg/mL, 200 µg/mL, 250 µg/mL, 300 µg/mL and 350 µg/mL. Test tube containing 7.5 mL of distilled water and 0.5 mL of FolinCiocalteu phenol reagent, 1 mL of 35 % sodium carbonate solution and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Test solution were prepared by 10 mg of plant different extracts were separately dissolved in 10 mL of methanol (1000 µg/mL) and further it was diluted to get 100 µg/mL of methanol. The above procedure was followed for test solution. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample (17).

Percentage of tannins (%) = (mg of tannic acid equivalent / g of sample) × 100

Thin-Layer Chromatographic (TLC) Analysis of *Mirabilis jalapa* Extracts

Thin-layer chromatography (TLC) was carried out to identify the alkaloidal constituents present in the different extracts of *Mirabilis jalapa*. The extracts (AEMJ, EEMJ, DEMJ, and CEMJ) were applied as small spots on a pre-coated silica gel TLC plate using capillary tubes and developed in different solvent systems, including toluene:ethyl acetate (70:20), ethyl acetate:ethanol:water (100:13.5:10), chloroform:ethanol (85:15), and chloroform:diethylamine (90:10). After development, the chromatograms were visualized under UV light, and when necessary, the spots were further detected using an iodine chamber. The developed plates were then sprayed with Dragendorff's reagent, which produced characteristic orange-brown spots indicating the presence of alkaloids. The retention factor (Rf) values were calculated by dividing the distance travelled by each compound by the distance travelled by the solvent front. Among the solvent systems evaluated, ethyl acetate:ethanol:water (100:13.5:10) provided the best separation of the alkaloidal components, producing well-resolved spots with distinct Rf values, thereby confirming it as the most

suitable mobile phase for the TLC profiling of alkaloids in *Mirabilis jalapa Linn* extracts (18).

Anti microbial activity

Anti-bacterial activity

The samples were tested by the well diffusion method. Different concentration of the different extracts (100 µg/mL) was prepared by reconstituting with methanol. The antibacterial activity was evaluated against the bacterial strains *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa.* The test microorganisms were seeded into respective medium by spread plate method 10 µL (10 cells/mL) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Ampicillin (10 µg) served as the positive control and DMSO was used as the negative control. The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured in mm (19).

Antifungal Activity

The antifungal activity was tested by well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. *Candida albicans* and *Aspergillus niger* were selected as the test fungal strains for the antifungal activity assay. Different concentration of the different extracts (100 µg/mL) was prepared by reconstituting with methanol.The filter paper wells (5 mm in diameter) impregnated with 100 µg concentrations of the synthesized silver nanoparticles were placed on test organism-seeded plates. Fluconazole (10 µg well 1) used as positive control. The activity was determined after 72 hrs of incubation at 28 °C. The diameters of the inhibition zones were measured in mm (20-21).

Antioxidant activity

DPPH Radical Scavenging Activity

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517 nm.

Various concentrations of the ethanol plant extract sample (4.0 mL) were mixed with 1.0 mL of solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture were shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control (22). The percentage of inhibition in DPPH radical scavenging activity was calculated as follows; % Inhibition = A0- A1/A0 X 100.

Nitric Oxide Scavenging Assay

The nitric oxide scavenging activity of the sample was evaluated using the nitrite detection method based on the Griess–Illosvoy reaction. Nitric oxide was generated from sodium nitroprusside in an aqueous solution at physiological pH. Under these conditions, sodium nitroprusside spontaneously releases nitric oxide, which reacts with oxygen to form nitrite ions. The generated nitrite was subsequently quantified using the Griess–Illosvoy reagent. In this modified method, N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) was used instead of 1-naphthylamine as the coupling reagent for the determination of nitrite concentration. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured at 540 nm. The reaction mixture (6.0 mL) containing sodium nitroprusside (4.0 mL), phosphate buffer saline (PBS, 1.0 mL) and different concentrations (5 - 100 µg / mL) of a stem ethanol extract (1.0 mL) in DMSO was incubated at 25°C for 15 minutes after incubation, 0.5 mL of the reaction mixture containing nitrite was removed, 1.0 mL of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization and 1.0 mL of naphthyl ethylene diamine dihydrochloride was added, mixed well and allowed to stand for 30 minutes in diffused light. A pink coloured chromophore was formed (23). The absorbance of these solutions was measured at 540 nm against corresponding blank solutions. Rutin was used as a standard.

Percentage inhibittion (I) = Ao – A1/Ao X *100*

In vitro Anti-inflammatory activity

The anti-inflammatory activity of plant extract was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima and Kobayashi (1986) and Sakat *et al* (2010) followed with minor modifications. The reaction mixture (0.5 mL; pH 6.3) consisted of 0.45 ml of bovine serum albumin (5 % aqueous solution) and 0.05 mL of distilled water. pH was adjusted at 6.3 using a small amount of 1.0 N HCl. Different concentrations of ethanol plant extract was added to the reaction mixture and were incubated at 37 ºC for 20 min and then heated at 57 ºC for 5 min after cooling the samples, 2.5 mL of phosphate buffer saline was added. Turbidity was measured spectrophotometrically at 600 nm (24). The percentage inhibition of protein denaturation was calculated as follows:

Percentage Inhibition (%) =

Abs control - Abs Sample/Abs Control X 100.

Cytotoxicity Assay

The cytotoxic effect of the ethanol extract was evaluated on SH-SY5Y human neuroblastoma cells

using the MTT assay. Cell viability was calculated relative to untreated control cells (25).

RESULTS AND DISCUSSION

Extractive values and Organoleptic characteristics

The extractive values and physical characteristics of the different solvent extracts are presented in Table 1. Among the extracts, the ethanol extract exhibited the highest extractive value (12 %), indicating greater extraction efficiency than the other solvents.

Table 1: Organoleptic characteristics and Extractive values of *Mirabilis jalapa Linn* stem extracts

Name of the extracts	Consistency	Odour	Color	Extractive Value (%)
Benzene	Semi-solid	Characteristic	Light-green	4.2
Chloroform	Semi-solid	Characteristic	Green	4.7
Dichloromethane	Semi-solid	Characteristic	Green	5.6
Ethanol	Semi-solid	Pungent	Dark-Green	12.0
Aqueous	Semi-solid	Characteristic	Light-green	4.6

Preliminary Phytochemical Screening

The qualitative phytochemical screening of the successive solvent extracts of *Mirabilis jalapa Linn* stem revealed the presence of various bioactive constituents with varying intensities, as presented in Table 2. Among the extracts, the ethanol extract exhibited the richest phytochemical profile, showing a strong presence (+++) of alkaloids, carbohydrates, tannins, unsaturated hydrocarbons, and flavonoids, along with a moderate presence (++) of triterpenoids and a positive presence (+) of saponins. The aqueous extract also demonstrated a high abundance of alkaloids, carbohydrates, tannins, unsaturated hydrocarbons, and flavonoids. In contrast, the chloroform and dichloromethane extracts showed moderate to low levels of most phytoconstituents, while the petroleum ether extract exhibited the least number of phytochemical constituents. Overall, the findings indicate that ethanol was the most effective solvent for extracting a broad spectrum of phytochemicals from the stem of *Mirabilis jalapa Linn*, suggesting its suitability for further pharmacological investigations.

Table 2: Preliminary Phytochemical screening of *Mirabilis jalap Linn* stem

S.No	Chemical Test	AE MJ	EE MJ	CE MJ	DC MJ	BE MJ
1	**ALKALOIDS**					
	a)Dragandr off'sTest	+++	+++	++	+++	+
	b)Hager'sTest	+++	+++	++	+++	+
	c)Wagner'sTest	+++	+++	++	+++	+
	d)Mayer'sTes t	+++	+++	+	+++	+
2	**TRITERPENOIDS**					
	a)SalkowskyTest	+	++	+	+	+
	b)HirschornTest	+	++	+	+	+
	c)Lieberman-burchardTest	+	++	++	+	+
3	**SAPONINS**					
	a)FoamTest	+	+	-	-	-
	b)Lieberman-burchardTest	+	+	-	-	-
4	**CARBOHYDRATE**					
	a)Molisch'sTest	+++	+++	++	-	-
	b)Tollen'sTest	+++	++	++	-	-
	c)Fehling's test.	+++	+++	++	-	-
	d)Barfoed'sTest	+++	+++	++	-	-
5	**TANNINS**					
	a)Chromicacid test	+++	+++	-	-	-
	b)FerricChloride test	+++	+++	-	+	-
6	**UNSATURATED HYDROCARBON**					
	a)Bromine Test	+++	+++	-	+	-
	b)Potassium permanganate	+++	+++	-	+	-
7	**FLAVONOIDS**					
	a)Lead AcetateTest	+++	+++	++	+	+

(+++) -abundant presence, (++) - moderate presence, (+) - weak presence, and (−) -absence of the phytochemical constituent. AEMJ-

Aqueous extract of *Mirabilis jalapa*, EEMJ-Ethanol extract of *Mirabilis jalapa*, CEM J-Chloroform extract of *Mirabilis jalapa*, DCM J-Dichloromethane extract of *Mirabilis jalapa*, BEMJ- Benezene extract of *Mirabilis jalapa*,

Quantitative Analysis

Table 3: Quantitative Phytochemical profile of different solvent extracts of *Mirabilis jalapa Linn.*

Name of extracts	Total Alkaloid Content (%)	Total flavonoids Content (mg QE/g)	Total Tannin Content (mg TA E/g)	Total Saponin Content (mg DE/g)
Aqueous	60.22	269.4	159.5	4.02
Ethanol	84.13	283.33	189.5	11.02
Chloroform	70.54	102.77	-	-
Dichloro methane	50.19	34.72	-	-
Benzene	30.16	12.50	-	-

(%) - percentage, (mg QE/g)- milligram Quercertin/gram, (mg TAE/g)- milligramTannic acid/gram, (mg DE/g)-milligram Diosgenin/gram

The quantitative phytochemical analysis presented in table 3 revealed considerable variation in the phytoconstituent content among the different solvent extracts of *Mirabilis jalapa Linn.* The ethanol extract showed the highest contents of alkaloids (84.13 %), flavonoids (283.33 mg QE/g), tannins (189.5 mg TAE/g), and saponins (11.02 mg DE/g), followed by the aqueous extract. Chloroform and dichloromethane extracts exhibited comparatively lower alkaloid and flavonoid contents, whereas tannins and saponins were not detected in these extracts. Overall, the findings indicate that ethanol was the most effective solvent for extracting the major quantified phytoconstituents from the stem of *M. jalapa Linn*.

Table 4: HPTLC Fingerprinting profile of *Mirabilis jalapa Linn*. stem extracts

S.no	Pl ant extract	No. Of Spots	Rf Values	HRf Values	Spray Reagents
1	BEMJ	1	0.083	8.3	
2	DEMJ	3	0.064 0.423 0.974	6.4 42.3 97.4	**DRAGAN DROFF'S REAGENT**
3	CEMJ	3	0.532 0.993 0.185	53.2 99.3 18.5	
4	EEMJ	2	0.416 0.974	41.6 97.4	
5	AEMJ	1	0.3846	38.4	

Rf - Retardation factor, HRf- High Retardation factor, AEMJ-Aqueous extract of Mirabilis jalapa, EEMJ-Ethanol extract of Mirabilis jalapa, CEM J-Chloroform extract of Mirabilis jalapa, DCM J-Dichloromethane extract of Mirabilis jalapa, BEMJ-Benzene extract of Mirabilis jalapa,

The HPTLC fingerprint profile of the successive solvent extracts of *Mirabilis jalapa Linn* stem revealed variations in the number of resolved phytochemical constituents, as indicated by the number of spots and their corresponding Rf values (Table 3 & Figure 1). The dichloromethane (DEMJ) and chloroform (CEMJ) extracts exhibited three well-resolved spots each, indicating the presence of a greater diversity of phytoconstituents. The ethanol extract (EEMJ) showed two distinct spots, while the aqueous (AEMJ) and petroleum ether (BEMJ) extracts displayed a single spot each, suggesting the presence of fewer detectable compounds under the selected chromatographic conditions. The variation in Rf and hRf values among the extracts reflects differences in the polarity and chemical composition of the phytoconstituents, confirming the effectiveness of HPTLC fingerprinting in distinguishing the chemical profiles of the successive solvent extracts of *Mirabilis jalapa Linn* stem.

Figure 1. Comparative TLC Fingerprinting of *Mirabilis jalapa Linn*. stem extracts

Antibacterial Activity

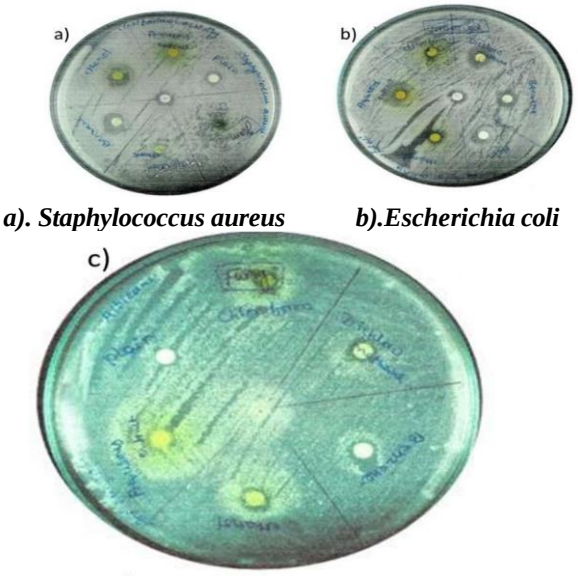
The antibacterial activity of the successive solvent extracts of *Mirabilis jalapa Linn* stem against selected Gram-positive and Gram-negative bacteria is presented in Table 4 and figure 2. The results demonstrated that the antibacterial activity varied depending on the extraction solvent and the test microorganism. Among the extracts, the ethanol extract (EEMJ) exhibited the highest activity against the Gram-positive bacterium *Staphylococcus aureus* (20.66 ± 0.33 mm), whereas no extract showed inhibitory activity against *Bacillus subtilis*. Against Gram-negative bacteria, the petroleum ether extract (BEMJ) produced the largest zone of inhibition against *Pseudomonas aeruginosa* (31.00 mm), which was comparable to the standard ampicillin (30.00 mm). The dichloromethane extract (DEMJ) exhibited the greatest activity against *Escherichia coli* (20.66 ± 0.33 mm), followed by the aqueous extract (AEMJ) (16.66 ± 0.33 mm), while the chloroform extract (CEMJ) showed comparatively lower antibacterial activity. No zone of inhibition was observed for the DMSO control, confirming that the solvent had no inhibitory effect.

Qualitative phytochemical screening revealed that the ethanol extract possessed the richest phytochemical profile, containing alkaloids, carbohydrates, tannins, unsaturated hydrocarbons, flavonoids, triterpenoids, and saponins. Flavonoids are known to disrupt bacterial cell membranes and inhibit nucleic acid synthesis, while tannins exert antimicrobial activity by precipitating microbial proteins and inhibiting essential enzymes, thereby suppressing bacterial growth (26-27) (Cushnie and Lamb, 2005; Scalbert, 1991). Alkaloids interfere with bacterial DNA replication and protein synthesis, whereas triterpenoids and saponins damage the integrity of the microbial cell membrane, resulting in leakage of intracellular constituents and cell death (Cowan, 1999; Daglia, 2012) (28-29). The The superior antibacterial activity exhibited by the ethanol extract may therefore be attributed to the synergistic action of these bioactive phytoconstituents. Similar correlations between phytochemical richness and enhanced antibacterial activity have been reported for several medicinal plant extracts (Okwu and Okwu, 2004) (30).

Table 5: Comparative Antibacterial activity of *Mirabilis jalapa Linn.* stem extracts against selected bacterial pathogens

S. No	Plant Extracts	Gram positive Organism	Gram negative organism
		Diameter of zone of inhibition (mm)	Diameter of zone of inhibition (mm)
		SA	BS	EC	PA
1	Control (DMSO)	-	-	-	-
2	BEMJ	14.33±0.33	-	13.33±0.33	31
3	DEMJ	-	-	20.66±0.33	27
4	CEMJ	-	-	8.66±0.66	16
5	EEMJ	20.66±0.33	-	12.33±0.33	28
6	AEMJ		-	16.66±0.33	20
7	Standard (Ampicillin)	28.33±0.33	-	14.66±0.66	30

SA-*Staphylococcus aureus,* BS-*Bacillus subtilis,* EC-*Escherichia coli,* PA- *Pseudomonas aeruginosa.*



a). Staphylococcus aureus ***b).Escherichia coli***

c). Aspergillus niger

Figure 2. a,b) Antibacterial and c) Antifungal activity of *Mirabilis jalapa Linn.* stem extracts against selected bacterial and fungus strains

Anti fungal Activity

The benzene extract demonstrated antifungal activity against *Candida albicans* and *Aspergillus niger* with inhibition zones of 15.33 ± 0.33 mm and 12.33 ± 0.33

mm, respectively. The antifungal effect may be associated with the presence of saponins and flavonoids capable of disrupting fungal cell membranes (Table 5 and figure 2).

Table 6: Comparative Antifungal activity of *Mirabilis jalapa Linn.* stem extracts against *Candida albicans* and *Aspergillus niger*

S.No	Plant extracts	Diameter of zone of inhibition in mm	
		Candid albicans	***Aspergillus niger***
1	Control (DMSO)	-	-
2	BEMJ	15.33±0.33	12.33±0.33
3	DEMJ	11.33±0.33	-
4	CEMJ	13.66±0.66	-
5	EEMJ	12.33±0.33	-
6	AEMJ	9.33±0.33	-
7	Standard (Flucanozole)	16.33±0.33	-

Anti-inflammatory Activity

The ethanol extract exhibited concentration-dependent inhibition of protein denaturation. The percentage inhibition increased from 43.86 % at 50 µg/mL to 88.07 % at 250 µg/mL. The activity was comparable to that of the standard drug at higher concentrations. The anti-inflammatory effect may be attributed to triterpenoids, flavonoids, and alkaloids, which are known to inhibit inflammatory mediators and stabilize proteins against denaturation (31).

Table 7: Anti-inflammatory activity of *Mirabilis jalap* stem extracts

Concentration µg/mL	Absorbance at 660 nm		Percentage inhibition (%)	
	Test	Standard	Test	Standard
50	0.390	0.315	43.86	55.23
100	0.292	0.281	58.22	60.03
150	0.210	0.212	71.40	70.03
200	0.165	0.137	78.90	81.78
250	0.122	0.083	88.07	90.07
Control	0.713	0.00		

Anti oxidant activity

As shown in Table 7, the antioxidant activity of *Mirabilis jalapa Linn*. stem extract demonstrated a concentration-dependent increase in radical scavenging activity in both DPPH and nitric oxide assays. The percentage inhibition increased from 12.12 % to 61.30 % for DPPH and from 13.10% to 69.10% for nitric oxide at concentrations of 100–500 µg/mL. The standard BHT showed slightly higher inhibition, reaching 72.62 % at 500 µg/mL. These findings indicated that the extract possesses appreciable antioxidant and free-radical scavenging potential(32).

Table 8: DPPH scavenging and Nitric oxide scavenging assay

Anti-oxidant Activity	Analyti-cal solute-ions	Percentage inhibition				
		100 (µg/mL)	**200 (µg/mL)**	**300 (µg/mL)**	**400 (µg/mL)**	**500 (µg/mL)**
DPPH	Sample	12.12 ± 0.50	27.30 ± 0.50	41.30 ± 0.55	53.25 ± 0.45	61.30 ± 0.45
Nitric Oxide Scav-enging	Sample	13.10 ± 0.50	30.50 ± 0.90	43.85 ± 0.18	56.90 ± 0.90	69.10 ± 0.30
	Std (BHT)	14.32 ± 0.50	32.24 ± 0.45	44.25 ± 0.50	58.55 ± 0.60	72.62 ± 0.12

DPPH-2,2-Diphenyl-1-picrylhydrazyl. BHT-Butylated Hydroxytoluene. The experiment was conducted in triplicates (n=3)

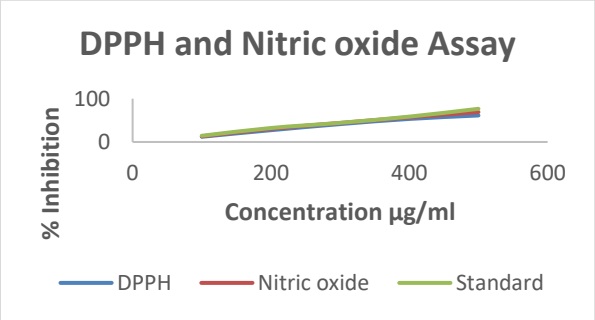


Figure 3: Comparative Antioxidant activity of *Mirabilis jalapa Linn.* stem extracts against DPPH and Nitric oxide radicals

Figure 3 shows the concentration-dependent antioxidant activity of *Mirabilis jalapa Linn.* stem extract in DPPH and nitric oxide scavenging assays. The percentage inhibition increased progressively with increasing concentration from 100 to 500 µg/mL, indicating a dose-dependent radical scavenging effect. Nitric oxide scavenging activity was higher than DPPH activity at the tested concentrations, while the standard BHT exhibited the highest inhibition. These findings demonstrated the significant antioxidant potential of the *M. jalapa* stem extract.

Cytotoxic Activity

The ethanol extract exhibited significant cytotoxic activity against SH-SY5Y neuroblastoma cells. Cell viability decreased from 87.78 % at 25 µg/mL to 27.91% at 600 µg/mL (Table 7 & figure 4). The estimated IC_{50} value was approximately 190 µg/mL, indicating substantial antiproliferative activity. The observed cytotoxicity may be associated with alkaloids and triterpenoids present in the extract. The results indicated that *Mirabilis jalapa* stem possesses significant biological activities and may serve as a promising source of therapeutic phytochemicals (33).

Table 9 : Cytotoxic activity *Mirabilis jalap* stem

Concentration	OD1	OD2	OD3	Average	% CV	SD
Control	1.489	1.502	1.546	1.512	100	0.0299
25	1.356	1.325	1.325	1.327	87.78	0.0271
50	1.102	1.123	1.123	1.153	76.28	0.0721
100	0.963	0.963	0.963	0.936	61.91	0.0319
200	0.756	0.715	0.715	0.724	47.89	0.0282
400	0.563	0.514	0.514	0.559	36.98	0.0436
600	0.412	0.399	0.399	0.422	27.91	0.0299

OD-Optical Density, %CV- Percentage coefficient of variation, SD- Standard Deviation

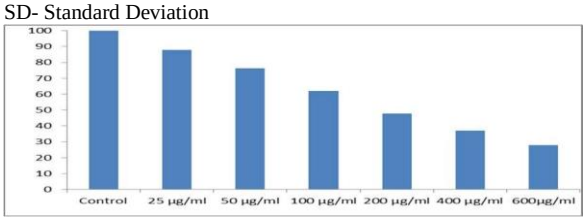
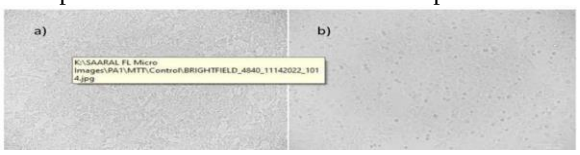


Figure 4: Effect of the ethanol extract of *Mirabilis jalapa Linn* stem on the viability of SH-SY5Y human neuroblastoma cells.

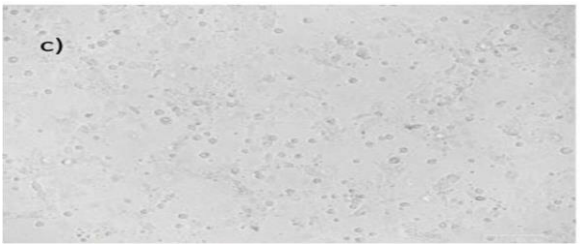
The half maximal inhibitory concentration (IC_{50}) values were calculated and the optimum doses were analysed at different time period.

Inhibitory of cell proliferation (%) = **(Mean OD of the Control – Mean OD of the Sample/ Mean OD of the Control) X 100**

The IC_{50} values were determined from the ethanol drug extract dose responsive curve where inhibition of 50 % cytotoxicity compared to control cells. All experiments were performed at least three times in triplicate.



a).Human Neuroblastoma cell line - Control
b).Treated Cell- Low dose



c). Treated Cell- High dose

Figure 5: Dose-Dependent Cytotoxic effects of *Mirabilis jalapa Linn.* stem extract on Human Neuroblastoma cells

Figure 5 showed the morphological changes observed in human neuroblastoma cells following treatment with *Mirabilis jalapa Linn.* stem extract. The control cells (a) exhibited normal cellular morphology, whereas low-dose treatment (b) produced noticeable morphological alterations. At the high dose (c), more pronounced changes in cell morphology and reduced cell density were observed, indicating a dose-

dependent cytotoxic effect of the extract on the human neuroblastoma cell line.

CONCLUSION

The present investigation revealed that the stem extracts of *Mirabilis jalapa* Linn. possess a diverse array of bioactive phytoconstituents, including alkaloids, flavonoids, triterpenoids, tannins, saponins, and carbohydrates. Quantitative phytochemical estimation further demonstrated appreciable levels of major constituents, with the ethanol extract showing the highest content of alkaloids, flavonoids, tannins, and saponins. The extracts exhibited promising antibacterial and antifungal activities, along with significant anti-inflammatory activity as demonstrated by inhibition of protein denaturation. Furthermore, the extracts showed concentration-dependent antioxidant activity in DPPH and nitric oxide scavenging assays, indicating their potential to neutralize free radicals. The ethanol extract also demonstrated notable cytotoxic activity against SH-SY5Y human neuroblastoma cells, with increasing cytotoxic effects at higher concentrations. Collectively, these findings highlight the pharmacological potential of *M. jalapa* stem and support its traditional medicinal applications, suggesting that it could serve as a valuable natural source of bioactive compounds for future pharmaceutical development. Further studies involving the isolation, structural characterization, and mechanistic evaluation of the active constituents are warranted to establish their therapeutic potential.

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