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Therapeutic Insights into *Mirabilis Jalapa*: Linking Solvent Extraction to Pharmacological Activities, Toxicity Evaluation, Cytotoxicity, and Nanoformulation Advances

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ABSTRACT

Mirabilis jalapa, commonly known as "Four O'clock plant", is an ancient medicinal herb that claims a wide range of pharmacological properties such as antioxidant, anti-inflammatory, antibacterial, antidiabetic, and hepatoprotective, as well as potentially antiviral and anticancer properties in both *in vivo* and *in vitro* approaches. Due to its abundance of alkaloids, flavonoids, phenols, and glycosides and ethnobotanical, the plant is considered to possess these properties; however, it also shown a significant degree of activity across several different indications. In this regard, the current study focuses on its major importance concerning the polarity of the solvent used for extraction. Since aqueous extracts have demonstrated low activity, while maximum yields of bioactive fractions have been reported with methanol and ethanol. Some of the novel approaches include nanoformulation and green synthesis, which involve biogenic silver, zinc oxide, and polymeric nanoparticles to enhance solubility, stability, and selectivity, while decreasing toxicity toward normal cells. Among others, nanoparticles derived from ribosome-inactivating proteins (RIP) selectively exhibit excellent anticancer activity against breast cancer cells. The toxicological evaluation compliant with OECD guidelines predicts that *Mirabilis jalapa* extracts are broadly safe up to 2,000 mg/kg in rodents, and thus further confirming their therapeutic utility. This review consolidates the extraction processes, phytochemical screening, and pharmacological findings, as well as their applications in biotechnology systematically based on the research conducted on *Mirabilis jalapa*. Providing a foundation for further drug discovery and translational research derived from this multipurpose medicinal plant, this work combines cytotoxicity data, toxicity assessment, and nanotechnology-based formulations.

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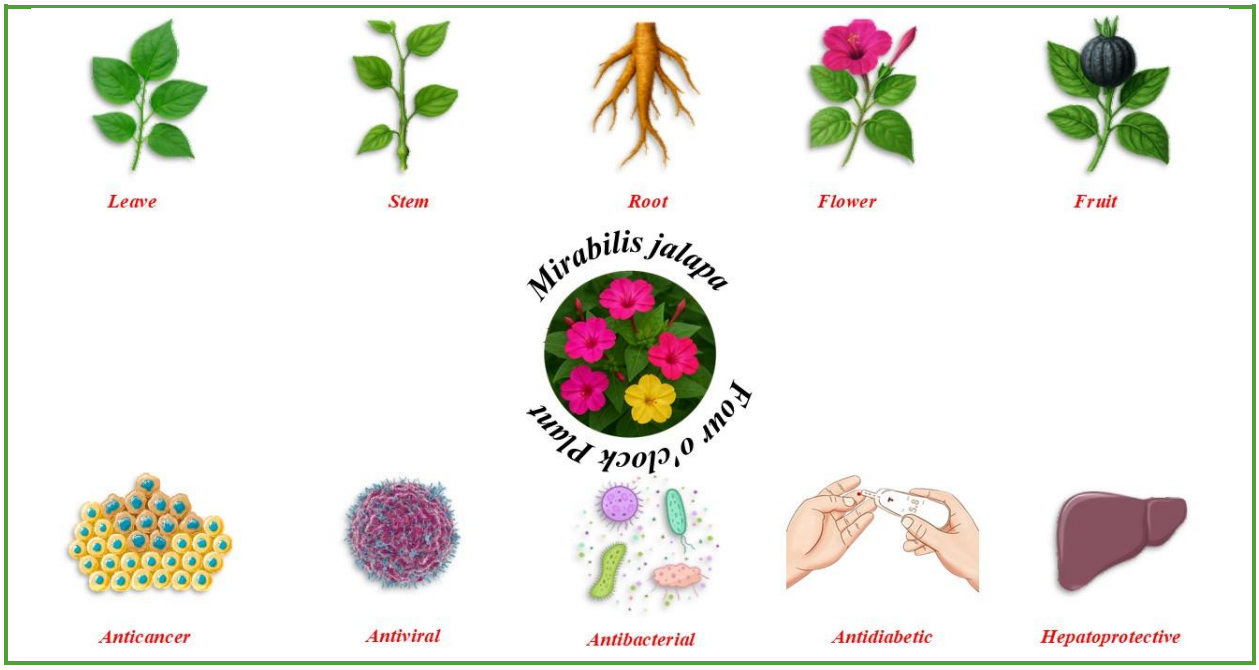
Graphical Abstract



Introduction

Over the years, a variety of herbal treatments have been accessible to keep people healthy at all times. The majority of the plants are used to cure a variety of serious illnesses as well as for preventative measures that protect people from dangerous substances and illnesses. The WHO survey estimates that between 70 and 80 percent of individuals in underdeveloped nations still use those medicinal plants for their medical needs [1]. Indigenous people and local communities have generated a significant portion of the global pharmaceutical industry based on therapeutic plants. For thousands of years, people have utilized herbs as conventional herbal medicine for wellness benefits. There are over 25,000 higher plant species on the planet. These plant species are typically employed for nutritional purposes, religious practices, and ultimately for therapeutic applications. The plant, employed for a multitude of purposes beyond mere sustenance, is also utilized in sacred spaces. Furthermore, it can be utilized for medicinal applications by transforming it into a green synthesized product for the treatment of ailments [2]. Some communities employ a little of these medicinal plants for therapeutic purposes. One of the most well-known among them is *Mirabilis jalapa*, which is commonly known as "four o'clock" herb, has been used traditionally for various ailments and has demonstrated specific pharmacological activities in preclinical studies, including anti-inflammatory, antioxidant, antimicrobial, antinociceptive, and immunomodulatory effects. These findings support further investigation of *Mirabilis jalapa* as a complementary therapeutic agent rather than a universal cure [3]. Due to the increasing cost of conventional pharmaceuticals, medicinal plants may present a more accessible alternative. Plant-based medicines continue to play a vital role in healthcare, particularly in communities that rely on traditional remedies. To date, phytochemical screening has identified a number of elements with diverse pharmacological effects, including alkaloids, flavonoids, phenolic compounds, steroids,

triterpenes, glycosides, tannins, saponins, and lignin [4-6]. In prior assessments of plant-based activity, it has been documented that those plants containing constituents such as alkaloids exhibit Anti-SARS-CoV-2 activity when compared with commercially available compounds through an *in silico* approach [7]. Recent studies on plant *Mirabilis jalapa* indicate that the activity is due to the presence of some possible constituents, such as flavonoids and alkaloids. Remarkably, compounds such as lupeol and kaempferol, which have been isolated from the plant's floral preparations, have demonstrated potent antidiabetic actions *in vitro* by blocking the alpha-glucosidase and alpha-amylase enzymes [8]. Advanced analytical methods like gas chromatography-mass spectrometry (GC-MS) have identified important plant components such as myo-inositol and derivatives of decanoic acid, which are connected to a number of biological processes. These results demonstrate *Mirabilis jalapa* pharmacological adaptability, bolstering its traditional application and emphasizing its potential for creating new therapeutic medicines [9]. Previous investigations on this plant have shown that it possesses anti-inflammatory, anti-diabetic, and antioxidant properties [10,11]. The vernacular names of *Mirabilis jalapa* across major Indian languages are summarized in Table 1, highlighting its widespread traditional recognition and cultural relevance. The detailed taxonomical classification of *Mirabilis jalapa* is provided in Table 2**,** presenting its hierarchical position from kingdom to species for accurate botanical identification. The primary objectives of this review were to present the extraction processes employed to identify the phytochemicals present in *Mirabilis jalapa* and to examine the analytical techniques used to evaluate its pharmacological activities.

Pharmacognostic Profile of *Mirabilis jalapa*

Biological name: *Mirabilis jalapa*

Family: Nyctaginaceae

Synonym: Four O'clock plant, Beauty of Night, and Marvel of Peru.

Morphology

The leaves of *Mirabilis jalapa* (Figure 1) are pointed with a needle-like appearance and possess a petiole ranging from 1 to 4 cm in length. The leaves are ovate to subcordate in shape, measuring approximately 3.5–7.5 cm in width and 2–9 cm in length. They have an entire margin and an acuminate apex. The stem is erect, branched, cylindrical, or slightly pubescent and shows a characteristic swelling at the nodes.

Table 1. Vernacular names of *Mirabilis jalapa* in various Indian languages

Tamil	Andhimalligai
Telugu	Chandrakanta
Malayalam	Antmalari
Kannada	Chandramalligei
English	Four o'clock plant, marvel of peru
Hindi	Gul- abbas
Guajarati	Gubbaji
Punjabi	Gulabbas
Marathi	Gulbas
Oriya	Rangai
Sanskrit	Kirshnakeli

Table 2. Taxonomical classification of *Mirabilis jalapa*

Kingdom	Plantae
Sub- kingdom	Trancheobionta
Division	Angiosperm
Class	Dicotyledon
Sub-class	Caryophylidae
Order	Caryophyllales
Family	Nyctaginaceae
Genus	*Mirabilis*
Species	*jalapa*

Figure 1. Morphological photograph of (A) flower, (B) stem, (C) leaves of *Mirabilis jalapa*

The root is tuberous and swollen at the node, with a black or brown coloration. The flowers are borne in several clusters at the apex, each with a pedicel measuring 1-2 cm. The floral structure includes a single carpel, five stamens, and a single ovule. The fruit is coriaceous and ovoid in shape, single-seeded, with a spherical surface that becomes wrinkled and black upon maturation.

Habitat

Mirabilis jalapa is grown as an ornamental plant. since it is a herbaceous plant that is primarily found in waste areas and former homes and growing between 0.6 and 0.9 meters in height and width.

Phytochemical Screening

Different components of *Mirabilis jalapa* have been investigated for phytochemicals using various extraction techniques and solvents (Table 3). Leaves extracted by maceration in water showed the occurrence of alkaloids, flavonoids, phenols, tannins, and saponins [12].

Table 3. Phytochemical screening of *Mirabilis jalapa*

Plant parts used		Methods of extraction	Solvent	Presents of phytochemical	Ref.
Leaves		Maceration	Water	Alkaloids, flavonoids, phenol, tannin and saponins	[12]
Flower		Cold maceration	Water	Carbohydrates, proteins, free amino acids, and flavonoids	[13]
			Ethanol	Alkaloids, flavonoids, glycosides, tannin, saponins and lignin	
			Methanol	Alkaloids, carbohydrate, inulin, tannins, terpenoids, flavonoids, proteins, and free amino acids	
			Petroleum ether	Alkaloids, inulin, tannins, terpenoids, and lignin	
			Chloroform	Alkaloids, carbohydrate, glycosides, tannins, terpenoids and volatile oils	
Leaves		Maceration	Acetone Chloroform Ethanol Methanol	Alkaloids, flavonoids, phenol, glycosides, tannin, saponins, and lignin	[14]
Leaves		Maceration	Methanol	Alkaloids, flavonoids, phenol, glycosides, tannin, saponins, and lignin	[15]
Fresh	Leaves	Maceration	Methanol	Tannin, glycosides, alkaloids, alkaloids, terpenes, and flavonoids	[16]
	Steam			Tannin, glycosides, alkaloids, alkaloids, terpenes, flavonoids and saponins	
	Flower			Tannin, glycosides, alkaloids, alkaloids, terpenes, and flavonoids	
Dried	Leaves			Tannin, glycosides, alkaloids, alkaloids, terpenes, and flavonoids	
	Steam			Tannin, glycosides, alkaloids, alkaloids, terpenes, flavonoids, and saponins	
	Flower			Tannin, glycosides, anthraquinone, alkaloids, terpenes, and flavonoids	
Leaves		Percolation cum green synthesis	Solvent: Water and silver nitrate	Aqueous: tannins, flavonoid, alkaloid, and phenol Green synthesis: Saponin, and alkaloid	[17]

For flowers, cold maceration in water resulted in carbohydrates, proteins, free amino acids, and flavonoids [13]. In addition, ethanol extracts contained alkaloids, flavonoids, glycosides, tannins, saponins, and lignin; methanol extracts contained alkaloids, carbohydrates, inulin, tannins, terpenoids, flavonoids, proteins, and free amino acids. Petroleum ether extracts contained alkaloids, inulin, tannins, terpenoids, and lignin, while chloroform extracts contained alkaloids, carbohydrates, glycosides, tannins, terpenoids, and volatile oils. Leaf maceration with acetone in another study revealed alkaloids, flavonoids, phenols, glycosides, tannins, saponins, and lignin [14], while the same phytochemicals were observed with chloroform, ethanol, and methanol. Methanol leaf extract alone also validated alkaloids, flavonoids, phenols, glycosides, tannins, saponins, and lignin [15]. Fresh maceration of leaves with methanol released tannins, glycosides, alkaloids, terpenes, and flavonoids [16]. Similarly, steam extractions of both leaves and flowers validated the presence of tannins, glycosides, alkaloids, terpenes, flavonoids, and saponins. Dried leaf and flower extracts also contained equivalent phytochemicals, with dried flowers, in particular, demonstrating the presence of anthraquinones. Additionally, a percolation and green synthesis method with water and silver nitrate ($AgNO_3$) proved aqueous extracts to have tannins, flavonoids, alkaloids, and phenols, and the green synthesis pathway exhibited saponins and alkaloids [17].

Pharmacological Activity

Antioxidant

The phytochemical profile of *Mirabilis jalapa* reveals the presence of key compounds such as phenolic compounds and flavonoids, which are responsible for its antioxidant activity [14,16,18]. Both phenolic compounds and flavonoids are known to exhibit strong antioxidant properties. These compounds are typically soluble in mid-polar solvents, which is why extraction methods focus on these types of solvents. It has been shown that mid-polar solvents like ethanol and methanol are effective for extracting tannins, flavonoids, and phenolic compounds from the plant, because those solvents are mid polar in nature and can be used for extracting a wide variety of constituents, which will be effective in extracting the potential constituents through green synthesis. The maceration technique, a method of soaking plant material in solvent, has been found to yield the highest concentration of these antioxidant-rich constituents. Among various parts of *Mirabilis jalapa*, the leaves are particularly noted for their significant antioxidant activity, as evaluated through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Table 4). A comparative analysis [18,19] indicates that the seeds exhibit a moderate antioxidant effect. The presence of these antioxidant properties in the plant could be indicative of its potential anticancer activity.

Anti-inflammatory activity

Since ancient times, inflammation has been associated with conditions such as pain, swelling, and edema. During inflammation, arachidonic acid is converted into inflammatory mediators such as prostaglandins (PGs), thromboxane (TXs), and leukotrienes (LTs). Phytoconstituents like phenolic compounds and tannins have been shown to be effective against enzymes like cyclooxygenase (COX), which play a key role in the production of these inflammatory mediators. *Mirabilis jalapa* is recognized as an excellent source of both phenolic compounds and tannins, which contribute to its anti-inflammatory effects.

Table 4. Antioxidant activity of *Mirabilis jalapa*

Plant parts used	Methods of extraction	Solvent	Test conducted (Antioxidant assay)	Outcome of the activity	Ref.
Leaves	Maceration method	70% Ethanol	DPPH assay	Strong antiradical activity in DPPH assay, indicating antioxidant properties	[18]
Seeds	Maceration method	80% Methanol	DPPH assay	Good antioxidant activity attributed to polyphenols present in the extract	[19]
Seeds	Ultrasonic extraction	Ethanol, Methanol, ethyl acetate, water	Ferric reducing antioxidant power (FRAP), DPPH, and OH radical scavenging	Ethanolic extract exhibited strong antioxidant activity.	[20]
Flower	Maceration method	90% Methanol	DPPH assay	Carbon tetrachloride and ethyl acetate fractions showed the highest DPPH scavenging potential; activity linked to polyphenols and flavonoids.	[21]
Leaves	Soxhlet extraction	Petroleum ether, chloroform, and methanol	DPPH assay	Crude methanolic and petroleum ether extracts showed mild antioxidant activity.	[22]
Tuber	Maceration method	Petroleum ether, dichloromethane, acetone, methanol, and water	DPPH assay	All extracts exhibited antioxidant activity, with the aqueous extract being most effective compared to methanol and dichloromethane.	[23]
Aerial parts & root	Soxhlet extraction	Methanol	$ABTS^{+}$ radical scavenging assay, DPPH assay	Methanolic extracts of aerial parts and roots showed notable antioxidant activity due to flavonoid content.	[24]
Stem, leaves, flower, roots, and seeds	Maceration method	Methanol, water	FRAP, ABTS, DPPH, Fe^{2+} chelation, and OH^{-} scavenging	Methanolic extracts of leaves and stems showed highest antioxidant activity, attributed to polyphenols and flavonoids.	[16]
Leaves	Maceration method	Petroleum ether, dichloromethane, acetone, and methanol	DPPH assay	Acetone and methanol fractions exhibited good antioxidant activity.	[15]

To evaluate the plant's anti-inflammatory properties, studies have used a variety of models, such as cotton pellet granuloma techniques and carrageenan-induced paw edema. These studies revealed that ethanolic and methanol extracts of *Mirabilis jalapa* exhibited optimal anti-inflammatory activity (Table 5).

Table 5. Anti-inflammatory activity of *Mirabilis jalaba*

Plant parts used	Methods of extraction	Solvent	Evaluation of anti-inflammatory activity	Outcome of the activity	Ref.
Leaves	Soxhlet extraction	Alcoholic extract and petroleum ether fractions	Carrageenan-induced paw edema model; cotton pellet granuloma model	Total alcoholic extract (300 mg/kg) and successive petroleum ether fraction (200 mg/kg) showed anti-inflammatory activity in carrageenan-induced paw edema; in cotton pellet granuloma, TAE (300 mg/kg) and SPE (200 mg/kg) inhibited granuloma formation.	[25]
Flower	Cold maceration and infusion	Ethanol and water	Bovine serum albumin (BSA) assay; Egg albumin denaturation; HRBC membrane stabilization method	Both aqueous and ethanolic extracts showed activity in bsa assay; aqueous extract showed higher protein denaturation than ethanol. In egg albumin denaturation, aqueous extract showed 73.20% inhibition (close to standard 74.66%); both extracts exhibited good membrane stabilization.	[26]
Leaves	Cold maceration	Aqueous	Carrageenan-induced paw edema; formalin-induced paw edema.	Aqueous extract showed anti-inflammatory activity in the carrageenan model, likely via cyclooxygenase inhibition; it also exhibited inhibitory effects on formalin-induced paw edema.	[27]
Seeds	Maceration	80% Methanol	Colorimetric methods	Methanolic extract exhibited anti-inflammatory potential associated with phenolic content.	[18]

Among the different parts of the plant, the leaves are considered to have the highest concentration of these active constituents, making them particularly effective in combating inflammation. However, the seeds of the plant also demonstrate anti-inflammatory activity, which has been confirmed using a colorimetric method [18].

Antidiabetic activity

In terms of controlling blood glucose levels, the aerial portions of *Mirabilis jalapa* have demonstrated encouraging results (Table 6). This activity was evaluated in an animal model using male albino mice, where streptozotocin was used to induce diabetes. Glibenclamide and metformin were used as standard drugs in the oral glucose tolerance test [28]. In addition to this, *Mirabilis jalapa* also exhibited activity in inhibiting alpha-amylase and alpha-glucosidase enzymes, as analyzed [26]. Both alcoholic and hydroalcoholic extracts demonstrated better results in terms of blood glucose regulation. This outcome can be attributed to the presence of potent bioactive constituents in the extracts.

Hepatoprotective activity

In some cases, the use of pharmacologically active drugs in the treatment of chronic diseases can lead to toxic effects on liver cells.

Table 6. Antidiabetic activity on *Mirabilis jalapa*

Plant parts used	Solvent	Method of analysis	Induced agents	Standard	Outcome of the diabetic activity	Ref.
Root	70% Ethanol	Animal model (male albino mice); Oral glucose tolerance test (OGTT); Measurement of serum insulin levels	Streptozotocin freshly prepared in 0.1 mol/L citrate buffer (pH 4.5)	Glibenclamide and Metformin	Single administration of ethanolic extract of *Mirabilis jalapa* root (EEM) did not significantly affect blood glucose; however, continuous and repeated dosing reduced blood glucose in diabetic mice, indicating potential use for type 2 diabetes.	[28]
Flower	Ethanol and water	*α*-Amylase inhibition assay; *α*-Glucosidase inhibition assay	(*In vitro* enzyme inhibition study)	Acarbose	Ethanolic extract showed maximum α-amylase inhibition (38.77% at 100 µg/mL); aqueous extract showed 38.36% inhibition at 50 µg/mL. Methanol extract produced maximum α-glucosidase inhibition (26.82% at 50 µg/mL). Overall inhibition was lower compared to the standard.	[26]
Aerial parts	Hydro-alcoholic	Animal model (male albino mice); Oral glucose tolerance test (OGTT)	Streptozotocin freshly prepared in 0.1 mol/L citrate buffer (pH 4.5)	Metformin	Hydroalcoholic extract significantly reduced blood glucose levels following a single dose administration.	[29]

However, *Mirabilis jalapa* leaf extract has demonstrated not only significant pharmacological actions, but also hepatoprotective effects (Table 7). This was confirmed through biochemical parameter analysis conducted by [30].

Antibacterial activity

The research on *Mirabilis jalapa* clearly demonstrates that the leaf part of the plant has promising antibacterial effects, particularly when methanol is used as the extraction solvent. As seen in Table 8, the extract demonstrated efficacy against both Gram-positive and Gram-negative bacteria.

Among the various pathogens tested, the methanolic extract from the leaves showed the most effective antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *K. pneumoniae*, using the disc diffusion method. However, some pathogens, including *S. pyogenes*, *Enterococcus*, and *P. aeruginosa*, showed resistance to the plant

extract [31]. Phytochemical screening confirmed the presence of tannins in the plant extract, which are likely responsible for the observed antibacterial activity. Interestingly, the aqueous extract was found to be ineffective against most of the tested pathogens. This suggests that isolated compounds from *Mirabilis jalapa* could serve as novel biomolecules, potentially overcoming bacterial resistance to standard drugs currently available on the market.

Table 7. Hepatoprotective activity on *Mirabilis jalapa*

Plant parts used	Methods of extraction	Solvent	Method of analysis	Evaluation of hepatoprotective	Outcome of the activity	Ref.
Leaves	Soxhlet extraction	Ethanol	Animal models (albino rat) hepatotoxicity was induced: isoniazid, rifampicin, pyrazinamide and ethambutol standard: Silymarin	Biochemical parameters serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alanine aminotransferase (ALP), total bilirubin and total cholesterol levels	Ethanolic extract lowered bilirubin level to normal, also showed hepatoprotective activity at 500mg/kg	[30]

Table 8. Antibacterial activity in *Mirabilis jalapa*

Plant parts used	Methods of extraction	Solvent	Method of analysis	G +ve	G -ve	Outcome of the activity	Ref.
Leaves	Maceration	Aqueous	Agar well diffusion, MIC	*S. pyogenes*, *B. subtilis*, *Enterococcus*, *and S. aureus*	*K. pneumoniae*, *P. aeruginosa*, and *E. coli*	Effective against *B. subtilis*, *K. pneumoniae*, *S. aureus*, *E. coli*; resistant: *Enterococcus*, *S. pyogenes*, and *P. aeruginosa*	[31]
Leaves	Cold maceration & Soxhlet	Water (cold), petroleum	Disc diffusion, mic, and microdilution assay	*S. aureus*, *S. epidermidis*, and *B. subtilis*	*E. coli*, *P. aeruginosa*, and *K. pneumoniae*	All extracts active except petroleum ether	[32]
Leaves	Soxhlet and cold maceration	petroleum ether, chloroform,	Cup plate, MIC	*S. aureus* and *B. subtilis*	*E. coli* and *P. aeruginosa*	Ethanolic extract most active; no activity in aqueous extract	[33]
Seeds	Infusion	Aqueous, Methanol and totum	Disc diffusion	*S. aureus* and, *S. pyogenes*	*E. coli*, *Enterobacter*, *V. cholerae*, *S. flexneri*, and *S. typhi*	Methanol and totum extracts active against all; aqueous ineffective against *Enterobacter*	[34]

Plant parts used	Methods of extraction	Solvent	Method of analysis	G +ve	G -ve	Outcome of the activity	Ref.
Leaves	Maceration	Ethanol, methanol, diethyl	Agar well and kirby–bauer	*S. aureus*	*E. coli*, *P. aeruginosa*, and *K. pneumoniae*	Good antibacterial activity; methanol and Ethanol most effective	[35]
Flower	Maceration	Methanol	Disc diffusion	*B. subtilis*, *B. cereus*, *S. aureus*, and *Sarcina lutea*	*S. dysenteriae*, *S. typhi*, *V. parahemolyticus*, *V. mimicus*, *E. coli*	Methanolic extract active against all tested pathogens	[21]
Fresh tubers	Maceration	Petroleum ether, Dichloromet	Agar diffusion	*S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus*, and *E. faecalis*	*E. coli*, *P. aeruginosa*, and *K. pneumoniae*	Water extract most effective; petroleum ether active against all, especially *P. aeruginosa*	[23]
Stem, Leaves, Flower. Root	Maceration	Methanol and water	Diffusion methods	*S. aureus*	*E. coli*, *P. aeruginosa*, and *K. pneumoniae*	Combined methanolic extract active on all; flower methanol extracts inactive	[16]
Leaves	Maceration	water, ethanol, methanol	Agar diffusion and mic	*S. aureus*, *B. subtilis*	*S. typhi*, *E. coli*, *V. cholerae*	Active against all listed pathogens; aqueous methanolic had no action; Aqueous ethanolic showed good activity	[36]
Flower	Gold extraction	Water, methanol, chloroform, ethanol, and petroleum ether	Disc diffusion	*S. aureus*, *S. pneumoniae*, *B. cereus*, *Lactobacillus acidophilus*, and *E. faecalis*	*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi*, and *S. dysenteriae*	Aq. extract effective against *E. coli* & *S. aureus*; ethanol & methanol effective for *E. coli*, *B. cereus*, *P. aeruginosa*; Chloroform/Pet ether high inhibition	[13]
Leaves	Maceration	Acetone, chloroform, ethanol, and	Disc diffusion and mic	*B. subtilis* and *S. aureus*	*E. coli* and *K. pneumoniae*	Methanolic extract highest inhibition of *S. aureus*; acetone max zone for *E. coli*, *B. subtilis*, *S. pneumoniae*, *S. aureus*	[14]

Plant parts used	Methods of extraction	Solvent	Method of analysis	G +ve	G -ve	Outcome of the activity	Ref.
Leaves	Soxhlet extraction	Alcoholic and aqueous	Cup plate and disc diffusion	*B. subtilis* and *S. aureus*	*E. coli*, *K. pneumoniae*	Good activity against all; best against *S. aureus*, comparable to standard at 30 µg/mL	[37]
Leaves	Percolation	Aqueous and agnp extract	Agar well diffusion	*Bacillus* sp.	*E. coli*, *Salmonella* sp., *Proteus* sp., and *K. pneumoniae*	AgNPs showed strong antibacterial activity, especially at 20 µL	[17]

Antiviral activity

Mirabilis jalapa is reported to contain antiviral protein molecules, specifically ribosomal inactivating proteins. These protein molecules are found in abundance in both the roots and fresh leaves, as shown in Table 9. Extracts obtained from the leaves and roots have demonstrated significant activity against the Tomato Spotted Wilt Virus [38] and have also inhibited the Groundnut Bud Necrosis Virus in cowpea plants [39,40]. Recent investigations further confirm that non-polar solvent-based extracts from the root contain a high concentration of ribosomal inactivating proteins, which contribute to its antiviral activity in both plant as well as animal.

Antistress activity

Mirabilis jalapa has also demonstrated anti-stress activity, as analyzed by [41,42]. The extraction was performed on the leaf part using a mid-polar solvent like methanol, as shown in Table 10. Superoxide dismutase (SOD) and catalase (CAT) enzymatic tests were used to assess the anti-stress activity.

Diuretic activity

Through combined maceration extraction of the leaf part, *Mirabilis jalapa* exhibited a diuretic effect in an animal model, as tested by the Lipschitz test. This resulted in increased urinary output in the tested animals. The extraction was performed using mid-polar solvents like ethanol and methanol, which contain the active constituents believed to be responsible for this diuretic action, as shown in Table 11.

Anti-malarial activity

Mirabilis jalapa has also demonstrated antimalarial activity against the *Plasmodium berghei* pathogen.

The extraction was performed on the leafy part of the plant using the maceration method. As reported in the (Table 12), the methanolic leaf extract showed effectiveness against the tested pathogen [43].

Table 9. Antiviral activity in *Mirabilis jalapa*

Plant parts used	Methods of extraction	Solvent	Method of analysis	Evaluation of anti-viral activity	Outcome of the activity	Ref.
Fresh leaves and roots	Maceration	Cold phosphate buffer, 0.1% 2-mercaptoethanol.	TSWV by bioassay-extract spray and reduction in lesion	Cow pea	Pre inoculation spray (24 hrs period to virus innoculation) of root extract is highly effective against TSWV.	[38]
Roots	Maceration	Ethyl acetate	Groundnut bud necrosis virus necrosis on the Leaves and stems of tomato	Cow pea cultivar var	Mirabilis contains an antiviral protein protect the plant against GBNV under greenhouse conditions. Pre inoculation spray of MJ root extract reduced the lesion in the cowpea plant.	[39]
Root	Ice-cold extraction	Buffer (25 mM $NaPO_4$, pH 7.0, with 250 mM NaCl, 10 mM EDTA, 10 mM thiourea, 5 mM dithiothreitol [DTT], 1 mM phenyl methyl sulfonyl fluoride, and 1.5% polyvinylpolypyrolidone	Potato virus X, Potato virus Y, Potato spindle virus	*Gomphrena globosa*	The root and leaf extract has strong inhibitory action against PVX virus.	[40]

Table 10. Antistress activity in *Mirabilis jalapa*

Plant parts used	Methods of extraction	Solvent	Method of analysis	Evaluation of anti-stress activity	Outcome of the activity	Ref.
Leaves	Maceration	Methanol and water	Oxidative stress analysis enzyme collection	Drosophila melanogaster SOD & CAT enzymatic assays	The plant extract revealed the decreased level of ROS, thereby reducing the antioxidant enzyme activity	[41]

Table 11. Diuretic activity in *Mirabilis jalapa*

Plant parts used	Methods of extraction	Solvent	Method of analysis	Evaluation of diuretic	Outcome of the activity	Ref.
Leaves	Combined maceration	80% Ethanol	animal model (male albino rat) Oral route	Lipschitz test Std: furosemide	Ethanolic leaf extract of *Mirabilis jalapa* increase urine output and decrease urinary concentration of electrolytes. The presence of flavonoid facilitated the release of pgs right be the reason for the diuretic action in plant.	[42]

Table 12. Anti-malarial activity in *Mirabilis jalapa*

Plant parts used	Methods of extraction	Solvent	Method of analysis	Malarial parasite	Outcome of the activity	Ref.
Leaves	Maceration method	70% methanol	Animal model (Swiss albino mice)	Plasmodium berghei	Methanolic leaf extract are effective against Plasmodium berghei. The presence of flavonoid facilitated the release of PGs right be the reason for the diuretic action in plant.	[43]

Antifungal activity

Phytochemical investigations of *Mirabilis jalapa* have revealed the presence of several potent active constituents, including alkaloids, glycosides, and flavonoids. These compounds are known to be highly effective against microbes. In antifungal analyses, *Mirabilis jalapa* showed activity against pathogens such as *A. niger and C. albicans*. The extractions were carried out using both mid-polar and non-polar solvents, as shown in the (Table 13). Most of the activities were assessed using methods like agar well diffusion, minimum inhibitory concentration (MIC) determination, and cup plate diffusion. It was also noted that extracts obtained with petroleum ether exhibited minimal activity against the tested pathogens, as confirmed by the microdilution assay [32].

Comparison of Solvent Efficiency in Each Pharmacological Activity

From the extracted research data in *Mirabilis jalapa*, it is clearly evident that ethanolic and methanolic extracts have consistently reported the most promising action across pharmacological categories because solvents can extract a wide range of mid-polar phytoconstituents, such as flavonoids and phenolic substances While comparing the extracting efficacy, aqueous extract showed lower activity when compared to the mid polar solvents. However, its green-synthesized nanoparticles exhibited better activity when compared to normal extracts.

The biological activity of the flavonoids, alkaloids, terpenoids, and phenolics in the aqueous extracts is comparatively lower because the active metabolites responsible for the activity are poorly soluble in water. However, while comparing result outcome of each activity, methanolic and ethanolic extracts are effective and aqueous extracts were in most cases less preferable. Mid polar solvents such as methanol and ethanol effectively extract a broader number of bioactive materials, and provide improved biological activity shown in Table 14, comparison of solvent efficiency in each pharmacological activity. The observed variations in activity between investigations can be explained by this solvent-dependent variance.

Nanoformulations and Green Synthesis Studies of *Mirabilis jalapa*

Unlike normal extraction techniques, some novel approaches like nanoparticles of *Mirabilis jalapa* extracts with biopolymers are used which exhibit various biological activities. For example, alginate–chitosan nanoparticles carrying ribosome-inactivating proteins (with or without anti-EpCAM antibodies) have been lethal to normal cells, yet effectively destroy breast cancer cells [44-46]. Additionally, strong antioxidant, antibacterial, and antileishmanial activity is observed in zinc oxide, silver, and

Table 13. Antifungal activity in *Mirabilis jalapa*

Plant parts used	Methods of extraction	Solvent	Method of analysis	Antifungal activity	Outcome of the activity	Ref.
Leaves	Soxhlet extraction; Maceration	Petroleum ether, chloroform, ethyl acetate, ethanol; Aqueous (maceration)	Cup plate diffusion method	*A. niger*, *C. albicans*	Ethanolic and petroleum ether extracts showed strong activity against *C. albicans* and *A. niger*, with petroleum ether exhibiting moderate activity and the aqueous extract showing low activity	[33]
Flower, Leaf, Root, Seed	Maceration	Methanol	Maceration; spore culture assay	*A. niger*, *F. oxysporum*	Extracts exhibited MIC against both fungi. Pink and multicolour varieties showed better activity against *A. niger*. White and multicolour varieties were effective against *F. oxysporum*. Complete inhibition of sporulation observed at 14 mg/mL (*A. niger*) and 13 mg/mL (*F. oxysporum*) for white variety.	[44]
Leaves	Maceration	Methanol	Agar well diffusion; mic	*A. niger*, *Penicillium* sp., *Fusarium* sp.	Methanolic leaf extract showed high antifungal activity against all three fungal cultures.	[45]
Leaves	Soxhlet extraction; Maceration	Petroleum ether, benzene, chloroform, ethanol, methanol, water	Microdilution assay; disc diffusion assay	*C. albicans*	Benzene, chloroform, aqueous, ethanol, and methanol extracts showed antifungal activity; no activity in petroleum ether extract.	[32]

Table 14. Comparison of solvent efficiency in each pharmacological activity

Activity	Most active solvent	Key constituents extracted	Outcome on solvent
Antioxidant	Methanol, Ethanol	Flavonoids, Phenolics	Mid-polar solvents consistently best
Anti-inflammatory		Phenolics, Tannins	aqueous weak
Antidiabetic	Ethanol, Hydro alcohol	Alkaloids, Flavonoids	Comparable to std drugs *in vivo*
Antibacterial	Methanol	Tannins, Flavonoids	Aqueous mostly inactive
Antifungal	Methanol, Ethanol	Glycosides, Alkaloids	petroleum ether weak
Hepatoprotective	Ethanol	Polyphenols	Good results in isoniazid-induced toxicity
Antiviral	Non-polar (Ethyl acetate)	Ribosome inactivating proteins	Works in plant models

bimetallic ZnO/Ag nanoparticles produced by leaf extract mediation [47]. Equally, biogenic silver nanoparticles are prepared from leaf extracts have shown efficacy against harmful *Vibrio* species and antioxidant potential with little toxicity to healthy cells [48]. From characterization of silver nanoparticles green synthesized from plant leaves, they are also said to be effective against Gram positive and Gram-negative bacteria [49]. Likewise, synthesis of colloidal silver nanoparticles from root extracts yielded small-sized particles and exhibited most promising antibacterial activity against *E. coli* and *S. aureus*, as shown in Table 15. Nanoformulations and green synthesis studies of *Mirabilis jalapa* [50].

Toxicity Profile of *Mirabilis jalapa*

The toxicity study of *Mirabilis jalapa* has showed negative results until today for the conducted test. Following the OECD guidelines of 423 and 407, acute and sub-acute analyses were performed using Wister rats, it showed as innoxious for the ethanolic extract of leaf part of *Mirabilis jalapa* [51]. Additionally, the toxicity studies were conducted under Food and Drug Administration regulation No. 10 of 2022 in albino mice at doses of of 300 mg/kg of body weight and 2,000 mg/kg of body weight for Group I and Group II respectively [52]. Based on the observations, it is concluded that at the dose of 300 mg/kg, the animals did not show any toxic outcomes; however, at 2,000 mg/kg, a reduction I the animal's activity was observed. From the results the maximum toxicity dose for mice is 2,000 mg/kg but in Wister rats, no toxic symptoms were observed even at the 2,000 mg/kg of test dose, as shown in Table 16.

In vitro*- Cytotoxic Study of *Mirabilis jalapa

From the review various extracts and fractions of *Mirabilis jalapa* have been known to exhibit anticancer and cytotoxic activities in different experimental assays. The antiproliferative effect of methanol seed extracts against prostate (DU-145, 49.3% inhibition), lung (HCC95, 45.4%), and breast (MDA-MB-231, 38.2% and 600MPE, 24.1%) cancer cell lines suggests moderate to mild cytotoxicity.

The ethanolic leaf extract and its respective alkaloid fraction were known for their dose-dependent cytotoxicity against HepG2 liver carcinoma cells that were tested, whereas the flower alkaloids indicaxanthin and miraxanthin-V, although low in cytotoxicity (IC_{50} > 30 ppm), seemed to improve the uptake of iron in liver cells, thus giving the impression of safety towards normal cells. Chitosan-alginate nanoparticles loaded with ribosome-inactivating proteins (RIP) possessed high cytotoxicity on T47D breast cancer cells, with IC_{50} ≈ 13-15 µg/mL, but less toxicity on Vero normal cells: IC_{50} ≈ 28-34 µg/mL, thus indicating selectivity. In a bioassay screening, petroleum ether bark extract showed strong cytotoxicity with LC_{50} values of about 8.12 µg/mL against Brine shrimp presented in Table 17, *in vitro*- cytotoxic study of *Mirabilis jalapa*.

Dosage Standardization in *Mirabilis jalapa*

Standardised dosing protocols have not yet been established in ongoing studies of *Mirabilis jalapa*. Most of the studies report amounts of crude extracts in mg/kg bw (*in vivo*) or µg/mL (*in vitro*), with no normalization to the actual content of bioactive constituents in these extracts. Because of this, studies cannot be directly compared, and preclinical evidence may not find easly translate to possible clinical

Table 15. Nanoformulations and green synthesis studies of *Mirabilis jalapa*

Activity	Carrier / Nanoparticle used	Key constituents / Agents	Outcome of the study	Ref.
Anticancer	Alginate–chitosan nanoparticles (± anti-EpCAM antibodies)	Ribosome-inactivating protein (rip-mj)	Selective cytotoxicity against T47D breast cancer cells (IC_{50} ≈ 13.3–14.9 µg/mL); spared normal Vero cells	[46]
Antioxidant & Antimicrobial	Monometallic ZnO NPs, Ag NPs, and Bimetallic ZnO/Ag NPs (leaf extract mediated)	Polyphenols, flavonoids, and terpenoids	Strong antioxidant activity (DPPH, FRAP); antibacterial and antileishmanial properties; particle size 12–67 nm	[47]
Anti-virucidal and Antioxidant	biogenic silver nanoparticles (bAgNPs) synthesized from aqueous leaf extract	Polyphenols, terpenoids	Potent activity against *Vibrio parahaemolyticus* and *V. harveyi* (MIC 31–93 µg/mL); strong antioxidant effects; low cytotoxicity on Vero cells (IC_{50} ~293 µg/mL)	[48]
Antimicrobial	Silver nanoparticles (leaf extract mediated)	Flavonoids, alkaloids, and tannins	Green, eco-friendly synthesis confirmed by UV–VIS, XRD, SEM, EDX; nanoparticles showed antibacterial activity against Gram-positive and Gram-negative strains	[49]
Antibacterial	Silver nanoparticles (root extract mediated)	Root phytochemicals (alkaloids, phenolics)	Synthesized AgNPs (~13–15 nm); activity against *E. coli* (MIC 2.9×10^{-4} g/mL) and *S. aureus* (2.54×10^{-4} g/mL)	[50]

Table 16. Toxicity profile of *Mirabilis jalapa*

Study type	Extract / Plant part	Dose tested	Animal model	Outcome /Observation	Ref.
Acute & sub-acute toxicity	Ethanolic leaf extract	Single dose: 2000 mg/kg; Sub-acute (28 days): 200 & 400 mg/kg	Albino wistar rats	No mortality; no behavioral, biochemical, or histopathological changes	[51]
Acute toxicity limit test	Ethanolic flower extract	Group I: 300 mg/kg; Group II: 2,000 mg/kg (single dose)	Mice	No mortality; no behavioral changes at 300 mg/kg. "Practically nontoxic" (LD_{50} > 2,000 mg/kg)	[52]

Table 17. *Invitro*- cytotoxic study of *Mirabilis jalapa*

Extract / Plant part	Cell line tested	IC_{50} / LC_{50} value	Outcome	Ref.
Methanol seed extract	DU-145 (prostate cancer)	% inhibition: 49.3% (at test conc.)	Significant antiproliferative activity	[19]
	HCC95 (lung cancer)	% Inhibition: 45.4%		
	MDA-MB-231 (breast cancer)	% Inhibition: 38.2%	Moderate cytotoxicity	
	600MPE (breast cancer)	% Inhibition: 24.1%	Mild cytotoxic effect	
Ethanolic leaf extract & alkaloid fraction	HepG2 (liver carcinoma)	IC_{50} (value not disclosed in abstract)	Both crude extract and alkaloid fraction showed dose-dependent cytotoxicity	[53]
Alkaloid fraction (flowers, indicaxanthin, and miraxanthin-V)	HepG2 (iron-deficient liver cells)	IC_{50} > 30 ppm (low cytotoxicity)	Promoted iron uptake; safe to normal cells	[54]
RIP-protein nanoparticles (chitosan–alginate)	T47D (breast cancer)	IC_{50} = 13.3 µg/mL (conjugated), 14.9 µg/mL (non-conjugated)	High selectivity and cytotoxicity against breast cancer cells	[46]
RIP-protein nanoparticles	Vero (normal cells)	IC_{50} = 27.8–33.6 µg/mL	Lower toxicity in normal cells (selectivity)	
Petroleum ether bark extract	Brine shrimp lethality assay (*Artemia salina*)	LC_{50} ≈ 8.12 µg/mL	High cytotoxic potential in screening bioassay	[55]

applications. For example, the ethanolic extract of leaves has shown anti-inflammatory activity at 300 mg/kg of ethanolic extract [25]; it also reported that comparable doses of ethanolic Mirabilis root extracts revealed significant antidiabetic effects in mice [28]. On the other hand, acute toxicity studies conducted by indicated that up to 2,000 mg/kg of ethanolic flower extracts is safe in mice, indicating a wide safety margin [52]. Since the most common ingredients that are responsible for pharmacological activity *in Mirabilis jalapa* are phenolic compounds. In analytical studies by have quantified phenolic compounds (*e.g.,* kaempferol, quercetin derivatives) via HPLC, suggesting normalization is possible based on markers [56].

In vitro vs. *in vivo* Inconsistencies of *Mirabilis jalapa*

Though *Mirabilis jalapa* has been reported in several *in vitro* assays to have strong antioxidant, antimicrobial, and cytotoxic activities, its translation into *in vivo* models is limited and often inconsistent. Extracts that appear very potent *in vitro* often act very weakly or not at all *in vivo* due to issues of bioavailability, metabolic stability, and systemic distribution. Strong radical scavenging activity may be observed *in vitro* for the phenolic- and flavonoid-rich extracts, but rapid metabolism and poor absorption will limit their efficacy *in vivo*. Such differences underscore the urgency for a well-coordinated set of studies on pharmacokinetics

and pharmacodynamics comprising ADME profiling, allowing for a correlation to be made between *in vitro* potency and *in vivo* efficacy, clarifying therapeutic relevance, and permitting rational dose optimization for the subsequent preclinical and clinical application.

Conclusion

The current review emphasizes the rich pharmacological potential of *Mirabilis jalapa* due to its varied phytochemical constituents, including flavonoids, phenolic compounds, alkaloids, terpenoids, and tannins. These specific compounds are primarily responsible for the antioxidant, anti-inflammatory, antidiabetic, antimicrobial, hepatoprotective, and other pharmacological activities. Interestingly, flavonoids and phenolics are significant contributors to a range of pharmacological effects. Although promising bioactivity has been reported, detailed isolation, structural elucidation, and mechanistic studies of these constituents remain under investigated. Further detailed investigations are essential to comprehensively explore their therapeutic potential and facilitate the development of new plant-derived drug candidates.

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