

# ***Mirabilis jalapa* Linn. : A folklore ayurvedic medicinal plant in Sri Lanka.**

## **Abstract**

*Mirabilis jalapa* Linn. (Nyctaginaceae), commonly referred to as the Four O'clock plant (Sinhala name: Hendirikka), is a popular ornamental plant grown for the beauty of its flowers and used in folklore remedies. This plant is used to treat a variety of disease conditions around the world. It is widely used for abdominal colic, aphrodisiac, boils, diarrhea, inflammations, genitourinary disorders, muscular pain, and other issues by people from various countries. This plant contains several phytochemical compounds isolated from its parts, such as alkaloids, brassicasterol, carbohydrates, flavonoids, glycosides, phytosterols (beta-sitosterol and stigmasterol), oleanolic acid, trigonelline, and ursolic acid. This plant has been studied for its anti-inflammatory, antimicrobial (antiviral, antibacterial, and antifungal), antioxidant & cytotoxic, anti-tumor, anti-nociceptive, antihistamine and immune modulatory, anti-tubercular induced hepatotoxic effect, antiparasitic, dermatological, and hypoglycemic and antihyperlipidemic properties. It is also used as a reductant in the production of gold nanoparticles. This research aims to review the literature on *Mirabilis Jalapa's* morphology, ethnomedicinal, phytochemical, and pharmacological properties.

**Keywords:** *Mirabilis jalapa*, Four-o-clock plant, ethnomedicine, Sri Lanka

## **1. Introduction**

Medicinal plants are a great alternative to finding new treatments and developing novel antimicrobials to combat many diseases[1,2,3,4,5,6]. *Mirabilis jalapa* (Sinhala name: Hendirikka), is commonly called the Marvel of Peru or Four-o-clock plant (refer to figures 1-7). This herbaceous bushy plant has a

perennial life span and can grow to a maximum height of 1 m[7]. *M. jalapa* is an autotrophic flowering plant. Flowers are trumpet-in shape and held either singly or in clusters of 3-7. The flowers measure up to 5 cm long and have multiple colors white, yellow, red, magenta, and pink, sometimes more than one color on the same plant. Bicolour flowers also can be rarely seen. These flowers are fragrant with a lemon scent which involves fauna pollination by attracting hummingbirds and butterflies. Flowers of *M. jalapa* are bisexual and have radial symmetry. These terminal flowers open in the late afternoon and stay open till the following day, giving it the name Four-o'clock plant[8]. Pollen grains of *M. jalapa* are spheroidal or oblique spheroidal in shape, and the diameter is about 125 to 140 micrometers, and thickness ranges from 10 to 15 micrometers[9]. Tubers are large and black carrot-shaped and grow to a foot or longer. These perennial tuberous roots can grow up to 18 kg in warmer climates. Underground tubers are the specialized storage organ of *M. jalapa* and prefer fertile loamy soil, moist soil, and well-drained soil[10].

*M. jalapa* is a branched herb that consists of numerous branches. Leaves are pointed, have an egg shape, and can be up to 9 cm long, with a broad end at the base (ovate), oblong, or triangular; the leaf tip is sharp, and the base cordate. The petiole, or leaf stem, is 4 cm long[11]. Seeds are small and brown or black in color. After developing as round, wrinkled, and greenish-yellow single-seeded fruits, they mature into spherical, wrinkled, and black[12].



Figure 1: The foliage on the shrub-like plants appear in medium green color[117].



Figure 2: Seeding of *Mirabilis jalapa* with first true leaves[117].



Figure 3: Different colors of the flower *Mirabilis jalapa*[117].



Flower buds, open flower, morning fading flower, and plant with many spent flowers[117].



Figure 4: The plant's wrinkled, dark-colored fruits and harvested[117].



Figure 5: Tuberous root system of *Mirabilis jalapa*[117, 118].



Figure 6: Well Grown plant of *Mirabilis jalapa*[119].

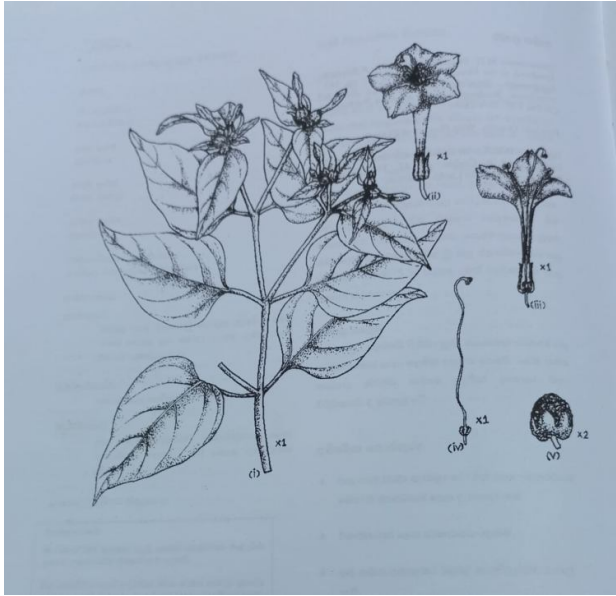


Figure 7: Schematic diagram of *Mirabilis jalapa*[120].

## 1.1 Synonyms and other names

The synonyms of *Mirabilis jalapa* are,

*M. dechotoma* Lin. (In Brazil), *M. dechotoma* Lin. and *M. longiflora* Linn. (In tropical America), *M. lindheimeri* Lin. and *M. odorata* Lin.[13].

Other scientific names of *Mirabilis jalapa*,

“*Jalapa congesta* Moench, *Jalapa officinalis* Garsult, *Mirabilis ambigul* Trautv., *Mirabilis pedunculata* Stokes, *Mirabilis planiflora* Trautv, *Mirabilis procera* Bertol., *Mirabilis pubescens* Zipp. ex Span, *Mirabilis suaveolens* Billb. ex Beurl., *Mirabilis xalapa* Noronha, *Nyctago hortnesis* Dum. Cours., *Nyctago jalapa*(L.) DC., *Nyctago versicolor* Salisb”[14].

## 1.2 Common names of *Mirabilis jalapa*

### 1.2.1 International common names

**English:** Four O’ clock plant, Beauty of night & Marvel of Peru USA)

**Spanish:** Buenas trades; dengue (Chile); Clavellina; Don diego de noche; Don Juan de noche; Falsa jalapa; Flor de not.

**French:** Belle de nuit; Merveille de pérou

**Chinese:** Zi mo li

**Portuguese:** Jalapa verdadeira; Jalapa-bastarda; Maravilhas-do-peru[14].

### **1.2.2. Local common names**

**Arabic:** Sheb al-leil

**Albania:** Leleakshami

**American Samoa:** Peteli

**Australia:** Common four O' clock plant

**Bahamas:** Morning rose

**Bangladesh:** Sandyamalati

**Benin:** Azehonzo

**Brazil:** Bonina; Maravilha; Munuminha

**Burundi:** Karifoma

**Congo:** Bende

**Congo democratic republic:** Kalofomo

**Cook island:** Tiara more; Ura ura

**Croatia:** Nocurak

**Cuba:** Suspiros

**Czech Republic:** Nocenka zahradni

**Denmark:** Vidunderblomst

**Dominican Republic:** Jalape; Jasmin

**Ethiopia:** Ababa diimaa

**Fiji:** Lalawavu

**Finland:** Ihmekukka

**French Gulana:** herbe de quatre heures

**French Polynesia:** Numera

**Germany:** Wunderblume

**Greece:** Deilino

**Haiti:** Belle de nuit blanche

**Hungary:** Nagy csodatolcser

**India:** Akashmuri; Andhi mandarai; Anthimalari; Antimantaram; Chandrakanth; Chandramalli; Godhuli; Gopal; Goolabbas; Gulbakshi; Indraganti; Krishnakeli; Meremdi; Mukak lei; Naalu mani poovu; Rangini; Saayankale; Sandya malati; Sanje mallige; Sham di sohnep

**Indonesia:** Bunga pukul empat

**Iran:** Ialeh abbasi

**Israel:** Lilanit rav-gonit

**Italy:** Bella di note

**Japan:** Oshiroibana

**Kiribati:** Marvel of te aouaua

**Korea:** Punkkot  
**Madagascar:** Belakariva; Folera  
**Malta:** Bajtar  
**Marshall Islands:** Eman aur; Eman awa; Emen aur  
**Mexico:** Artetito; Linda tarde; Tlaliqulin  
**Micronesia, Federated states of:** Apetin woun; Gaelun; Koluk elu  
**Namibia:** Vieruurtjie  
**Netherlands:** Wonder bloem  
**Norway:** Miraklelblom  
**Pakistan:** Gul adnan  
**Portugal:** Arrebique; Jalapa-falsa; Suspiros  
**Romania:** Barba imparatului  
**Russian Federation:** Nochnaya krasavitsa  
**Slovenia:** Nocna frajlica  
**South Africa:** Vieruurtile  
**Spain:** Arrebolera; Bella de nit; Bella de noche  
**Sri Lanka:** Hendirikka  
**Sweden:** Underblomma  
**Tonga:** Maravillas de indias  
**Turkey:** Aksam sefasi  
**Tuvalu:** Peteli  
**UK:** Garden jalap; Japanese wonder flower; Pearl of Egypt[14].

### **1.3 Taxonomic hierarchy**

**Domain:** Eukaryota  
**Kingdom:** Plantae  
**Subkingdom:** Viridiplantae- green plants  
**Infrakingdom:** Streptophyta-land plants  
**Superdivision:** Embryophyta  
**Division:** Tracheophyta- vascular plants  
**Subdivision:** Spermatophytina- spermatophytes (seed plants)  
**Class:** Mangoliopsida  
**Superorder:** Caryophyllanae  
**Order:** Caryophyllales  
**Family:** Nyctaginaceae-four O' clocks  
**Genus:** *Mirabilis*  
**Species:** *Mirabilis jalapa* Linn[15].

#### **1.4 Geographical distribution**

“*Mirabilis jalapa* is distributed worldwide in sites like waste grounds, old homes, and flower beds. It is also grown in Anthropogenic (man-made or disturbed environments), meadows, and fields. It is the most commonly grown ornamental *Mirabilis* species and comes in various colors”[13].

“The plant is native in Belize, El Salvador, Guatemala, Honduras, Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Nicaragua and introduced in to Alabama, Albania, Algeria, Angola, Argentina Northeast, Argentina Northwest, Arizona, Arkansas, Ascension, Assam, Austria, Azores, Bahamas, Baleares, Bangladesh, Benin, Bermuda, Bolivia, Botswana, Brazil North, Brazil Northeast, Brazil South, Brazil Southeast, Brazil West-Central, Bulgaria, Burundi, California, Cambodia, Cameroon, Canary Is., Cape Provinces, Cape Verde, Caroline Is., Cayman Is., Chagos Archipelago, China Southeast, Christmas I., Colombia, Comoros, Connecticut, Cook Is., Costa Rica, Cuba, Czechoslovakia, Desventurados Is., District of Columbia, Dominican Republic, East Aegean Is., East Himalaya, Easter Is., Ethiopia, Fiji, Florida, France, Free State, French Guiana, Galápagos, Gambia, Georgia, Germany, Gilbert Is., Great Britain, Greece, Guinea, Guinea-Bissau, Gulf of Guinea Is., Guyana, Haiti, Hawaii, Illinois, India, Indiana, Italy, Jamaica, Japan, Jawa, Juan Fernández Is., Kentucky, Kenya, Korea, Kriti, Kuwait, Laccadive Is., Laos, Leeward Is., Lesser Sunda Is., Libya, Louisiana, Madeira, Malawi, Marianas, Marquesas, Marshall Is., Maryland, Mauritius, Mississippi, Morocco, Mozambique, Namibia, Nansei-shoto, Nauru, Nepal, Nevada, New Caledonia, New Jersey, New Mexico, New South Wales, New York, Nicobar Is., Nigeria, Niue, Norfolk Is., North Carolina, Northern Provinces, Ohio, Oklahoma, Pakistan, Paraguay, Pennsylvania, Peru, Pitcairn Is., Puerto Rico, Queensland, Rhode I., Rodrigues, Romania, Rwanda, Réunion, Samoa, Saudi Arabia, Senegal, Seychelles, Sierra Leone, Society Is., Solomon Is., Somalia, South Carolina, South European Russia, Southwest Caribbean, Spain, Sri Lanka, St. Helena, Sudan, Suriname, Sweden, Tanzania, Tasmania, Texas, Thailand, Tokelau-Manihiki, Trans caucasus, Trinidad-Tobago, Tristan da Cunha, Tuamotu, Tubuai Is, Tunisia, Turkmenistan, Tuvalu, Uganda, Uruguay, Utah, Uzbekistan, Venezuela, Venezuelan Antilles, Vermont, Victoria, Vietnam, Virginia, West Himalaya, Windward Is., Yemen, Zambia, Zaïre and Zimbabwe”[16].

## **2. Ethnomedicinal information**

“*Mirabilis jalapa* has been discovered to have numerous distinct biological and medicinal properties. It is widely used as a medicinal plant in almost all folklore medicines worldwide to treat various diseases”[9]. Traditional medicine employed the entire plant and individual parts of *M. jalapa* to treat various human diseases[10].

### **2.1. Ethnomedicinal information of the whole plant**

“The whole plant is widely used to relieve muscular pain, diarrhea, and abdominal colic by people from different countries”[17]. “The decoction of the entire plant is taken orally to treat kidney diseases for diuresis”[18]. “The inhabitants of Tehsil kadal, Swat district, Pakistan, use juice of the whole plant for relief from pain and also to provide a cure for Typhoid”[19].

“The leaves and roots are used medicinally in Ayurveda, Siddha, and other traditional systems of medicine for curing various ailments”[20].

### **2.2. Ethnomedicinal information of the leaves**

“The leaves of *M. jalapa* are used in inflammation, boils, and purgative and emetic properties”[21]. The infusion or decoction of leaves is popularly used for analgesic purposes[22]. This plant is an excellent diuretic; it promotes increased urine production, giving the leaf juice to patients suffering from bladder inflammation and urinary retention. The leaf juice is directly applied to the skin to treat infections like rashes or boils to promote fast healing. It is also used to treat wounds and cuts on the skin[23]. “The infusion of the leaves was applied topically to reduce swelling in bone fractures or twisting”[18]. “Leaves are crushed and mixed with salt in sprain and bruises”[24]. “Leaves are fried in clarified butter and are fastened on the abscess, while boiled leaves are eaten to reduce body pains”[25]. “The paste of leaves is used in amenorrhea and dysmenorrhea in women[26]., skin eruptions, and has emollient properties”[27]. “Leaf juice is taken orally to treat Hepatitis”[28, 29]. “The juice is slightly warmed and used as a poultice for abscesses which helps in healing wounds. It is also used as an eye drop to soothe eye inflammation” [25]. “The decoction of the leaves is used for genitourinary system disorders and treating injuries”[30]. “Juice expressed from leaves is used in treating skin allergies by indigestion and earaches in children”[31]. The stems and leaves are used for pigmentation[32].

“The decoction of the roots and leaves can be used for treating pain and inflammation in arthritis”[33].

### **2.3. Ethnomedicinal information of the roots**

The root of *M. jalapa* has diuretic, purgative, wound-healing, aphrodisiac, anti-inflammatory, anti-tumor, laxative, and anti-poison properties[23]. Roots are good for syphilitic sores[34]. Root paste is applied for inflammation[35, 36]. Roots were used to treat the accumulation of pus and/or liquid in cavities, cellular tissues, and inflamed and enlarged lymph nodes [31]. The solution of tuber paste is given orally to treat snake bites[37]. As a laxative, 2-4 grams of root powder in water is abdicated for adults[38]. The root is a great aphrodisiac; consuming the root powder of this plant increases sexual vigor and stamina in men[23].

### **2.4. Ethnomedicinal information of the flowers**

“The flower of *M. jalapa* exhales a strong odor at night, which can drive away mosquitos”[39]. “Flowers are used in food coloring; an edible crimson dye is used to color cakes and jellies”[20].

### **2.5. The ethnomedicinal uses of different plant parts around the world.**

“Different parts of *M. jalapa* has reported to have medicinal uses in several regions of the world, such as Latin America, South Africa, Zaire, Madagascar, India, and Pakistan, where they are used as a laxative and to treat infections, inflammation, allergies, and painful conditions”[22, 40, 41, 42]. “*M. jalapa* is used as an analgesic in Madagascar and Mexico to treat several painful conditions, including intestinal pain and pain produced by scorpion and bee stings”[40, 41].” Mexican people use various decoctions and preparations of the plant for the treatment of dysentery”[41]. “*M. jalapa* leaves are used in traditional folk medicine in the South of Brazil to treat inflammatory and painful diseases and as a laxative”[43, 44]. “It is reported that the powdered seed is used as a cosmetic powder by the natives of Japan”[45]. “In Malagasy, the plant was used to treat intestinal pains”[46]. “In Latin America and South Africa, the roots of *M. jalapa* were traditionally used for their purgative, emetic & cathartic properties”[46]. “In China, this plant is widely distributed and commonly used with its roots and has been used as traditional Chinese medicine and ethnic drug to treat diabetes[47], constipation[48], and genitourinary system disorders and injuries”[30]. “The natives of Shivalik Hills, Himachal Pradesh,

use the root tubers consumed as a pickle for their nutritive value. The paste of the root tuber is applied to check the growth of old tumors in tribal areas of Rajasthan”[49]. “The tuber is administered in minute quantities to cure piles”[25]. “The fruit paste made with coconut oil is applied externally for the relief from headaches of folk and domestic animals at the Bhadra wildlife sanctuary area in Karnataka”[50].

### **3. Phytochemical screening**

#### **3.1. Aerial parts**

Beta-sitosterol, brassicasterol, flavonoids, oleanolic acid, stigmasterol, triterpenes, and ursolic acid are phytochemical components identified from the aerial parts of the plant[44].

#### **3.2. Flowers**

Many betaxanthins pigments (indicaxanthin, vulgaxanthin-1, miraxanthin-I, -II, -III, -IV, -V & -VI) have been identified in the flowers[51].

#### **3.3. Leaves**

The extract of *M. jalapa* leaves contains acids 7.0%, alcohols 12.1%, hydrocarbons 17.8%, ketones 18.0%, sterols 21.2%, arabinose, beta-sitosterol, galactose, oxy-methyl-anthraquinone and trigonelline[52]. Essential elements such as Cu 0.067, Cr 0.14, Fe 5.02, Mn 0.42, Pb 0.04, Zn 1.19 mg/kg are present in the plant leaves [53]. Flavonoids quercetin and C-glycosyl-flavonoid are identified in leaves[54]. N-hexacosanal, tetracosanoic acid and triclosan-12-one has been isolated from leaves, while alanine, citric acid, glycine, leucine, tartaric acid, tryptophan, and valine were detected by paper chromatography[47]. The leaf’s major carbohydrate has been reported as D-pinitol, an o-methyl inositol[55]. “Bioassay guided fractionation of the methanol extract of leaves and stems has led to the isolation of an active polyphenolic amide, N-trans-feruloyl-4’-o-methyldopamine”[56].

#### **3.4. Roots**

Roots are the largest source of biological content in *M. jalapa*[9]. The compounds isolated from the roots using column chromatography were further confirmed by NMR and MS. Compounds such as, astragaloside III, astragaloside IV, astragaloside VI, beta- sitosterol, 7-beta-D-glucopyranoside, daucosterol, 3,4, -dihydroxybenzaldehyde, flazin, gingerglycolipid A, 4’-

hydroxy-2,3-dihydroflavone and p-hydroxybenzaldehyde[57]. Roots also contain boeravinone C, chrysophanol, glycerin monoecisate and stigmasterol[52]. Roots contain 3% resin, oxymethylanthraquinone and carbohydrates, which, on hydrolysis, yield galactose and arabinose[46]. “An anti-plant viral protein active against mechanical transmission of plant viruses has been isolated and purified from roots using ammonium sulfate precipitation and ion-exchange chromatography”[10]. Retinoids mirabijalone A, B, C[58], and D along with 9-o-methyl-4-hydroxy boeravinone-B, boeravinone-C and F and 1,2,3,4-tetrahydro-1-methylisoquinoline-7,8-diol has been isolated from roots[10, 59]. (2,5, di-oxo-imadazoline-4-yl)-urea, beta-sitosterol, and glycerin monoecisate have been isolated from the plant’s 75% ethanolic root extract [60].

### **3.5. Seeds**

Seeds have been found to show high protein content ( $11.0 \pm 0.75$ g/100 seeds). Amino acid analysis of the total protein isolates showed that it consisted of 17 amino acids of which 9 are essential[61]. Some amino acids are arginine, aspartic acid, glutamic acid, histidine, glycine, threonine, and tyrosine. D-glucan, a polysaccharide from seed cotyledons, contains 38 glycosyl units.  $\beta$ -sitosterol,  $\beta$ -amyrin have been isolated from seeds[62]. 2 new antimicrobial peptides named Mj-AMP-1 & Mj-AMP-2 have also been isolated from the seeds[63]. The analysis of fatty acid constituents of the seed oil has shown that they include palmitic acid 18.3%, oleic acid 55.3%, linoleic acid 11.5%, and linolenic acid 14.9%[64]. A fatty acid, 8-hydroxyoctadeca-*cis*-11,14-dienoic acid has been isolated from the seed oil[52].

### **3.6. Stem**

Aqueous and methanolic extracts of stems have shown the presence of alkaloids, carbohydrates, flavonoids, tannins, and unsaturated hydrocarbons[56]. The following essential elements have been found(mg/kg) in stems. They are Cr 0.13, Cu 0.58, Fe 4.88, Mn 0.72, Pb 0.13 and Zn 1.74 mg/kg[53].

## **4. Bioactivity**

### **4.1. Antibacterial effect**

- “Antibacterial activity of ethanolic extract of red color flower of *M. jalapa* has been examined *in vitro* against *Staphylococcus aureus*. This research demonstrates that the plant has a strong antibacterial activity and is active against many microorganisms”[65].
- *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* have been included in a study to observe the antibacterial properties of *M. jalapa*. The plant extract has shown the highest inhibition against *B. subtilis*, with almost 47% of inhibition[66].
- The ethanolic extract of the plant’s leaves has been involved in a study along with the agar disc-diffusion method to screen the antibacterial effect of the plant. The study used *E. coli*, *S. typhi*, *Staphylococcus aureus*, *Bacillus cereus*, and *Klebsiella pneumoniae* as the control organisms. Results revealed that the plant extract possesses antibacterial activity. Thus, the plant is a good source of agents for biocontrol and chemotherapy[67].
- A study has been conducted on the antimicrobial effect using the ethanolic extracts of *Mirabilis jalapa* leaves against Gram-positive and Gram-negative bacteria. The inhibition zones have resulted as 11.1 mm for *S. aureus*, 13.5 mm for *S. typhi*, 15.0 mm for *E. coli*, and 15.5 mm for *P. aeruginosa*[53].
- A study has been carried out to observe the antibacterial activity of *M. jalapa* using aqueous & methanolic seed powder extracts and their combinations (1:2 mixture of aqueous and methanol extract) at a dilution of 40, 4, 0.4, & 0.04 mg/ml. The antibacterial activity has been tested against *S. aureus*, *Streptococcus pyogenes*, *E. coli*, *Enterobacter* spp., *Vibrio cholerae*, *Shigella flexneri* & *S. typhi*. The aqueous extract has produced a good growth inhibition against all the tested bacteria except *Enterobacter* spp., while the methanol extract has shown inhibition against all but *S. aureus*. The combination of extracts has shown good inhibition against all the bacteria to a dilution of 4 mg/ml and inhibition of *Staphylococcus aureus* at 0.4 mg/ml[40].
- Studies have demonstrated that the alcoholic extract of leaves shows antibacterial effects against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *S. aureus* & *S. typhi*[41, 68].
- N-trans-feruloyl 4'-O-methyldopamine, a polyphenolic compound isolated from the methanolic extract of *M. jalapa*, has been shown to have

moderate activity against multi-drug resistant bacteria (MRD) *S. aureus*[56].

- The antibacterial activity of the aqueous, ethanol, methanol, chloroform & petroleum ether leaves extracts of *Mirabilis jalapa* has been involved in a study to screen their antibacterial activity. *E. coli.*, *S. aureus*, *Streptococcus pneumoniae*, *B. cereus*, *Enterococcus faecalis*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, *S. typhi*, and *Shigella dysenteriae* have been used as control organisms. The aqueous, chloroform and petroleum ether extracts have displayed minimal inhibition. In contrast, all the ethanolic & methanolic extracts have shown good antibacterial activity against the selected pathogens (inhibition zones are 11-15 mm against all the tested bacteria, except 8 mm for *Streptococcus pneumoniae*)[69].
- The antimicrobial effect of acetone, chloroform, ethanol & methanol extracts of the leaves of *M. jalapa* have been investigated in a study against *Bacillus subtilis*, *E. coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. The methanol extract exhibits the largest growth inhibition zone at 500 micrograms/disc against *Staphylococcus aureus*. The methanol extract has shown the lowest MIC of 39 micrograms/ml against *Staphylococcus aureus*[70].
- The aerial parts of *M. jalapa* have been used in a study to prepare a methanolic extract to observe the antibacterial activity using the agar well diffusion method. The pathogenic strains of *Staphylococcus aureus*, *Bacillus* spp., *Pseudomonas* spp., have been involved for the study. The methanolic extract has displayed antibacterial activity against microorganisms[71].
- Methanol, acetone, diethyl ether and chloroform extracts of *Mirabilis jalapa* varieties have been tested for the antibacterial activity against Gram negative (*Pseudomonas aeruginosa* and *E. coli*) and Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*). The methanol and acetone extracts have shown more potent inhibitory activity when compared to the other two extracts. However, the leaf methanolic extract of the white flowered variety has resulted in the highest antibacterial activity at 500mg/ml, followed by the methanolic leaves extracts of pink, yellow, orange flowered activities, respectively[72].
- A study has been carried out on the antimicrobial activities of various extracts of *Mirabilis jalapa* tubers. The organisms chosen for the study

are Gram positives *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus epidermidis*, *Micrococcus luteus* and *Enterococcus faecalis*. *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were taken for Gram negatives. Results exhibited that the water extract was the most effective with a MIC of < 200 micrograms/ml against *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*[73].

- Aqueous and methanol extracts of *Mirabilis jalapa* seeds have been used in a study to screen antimicrobial activity against Gram negative and Gram-positive bacteria, isolated respectively from infected wounds and diarrhoeic feces. The disc diffusion method was used in the study. The study suggests that further investigations are required to identify the active principles in seeds of *Mirabilis jalapa*[40].
- An investigation of the antibacterial effects of aqueous and alcoholic extracts of *Mirabilis jalapa* leaves has been investigated. The study reveals that 0.5mg/ml of concentration is an effective inhibitor for the growth of *Staphylococcus aureus*, *E. coli* and *Proteus mirabilis*[74].
- The methanolic extract of *Mirabilis jalapa* aerial parts has been examined for the antibacterial potential against Gram positive and Gram-negative organisms. According to the study results, the extract displays significant activity against all the tested microorganisms with MIC of 1 mg/ml[71, 116].
- The petroleum ether, benzene, chloroform, ethyl alcohol and methanol extracts of *Mirabilis jalapa* leaves have been used in a study to determine the antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus epidermidis*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. All the extracts except petroleum ether have resulted in possessing potent antibacterial effects. The methanol extract shows a stronger and broader spectrum of microbial activity compared to the other extracts. It has carried inhibition zones of 25, 22, 21, 21, 20, 22 mm, against *Staphylococcus aureus*, *Streptococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *E. coli*, respectively[75].
- The ethanolic leaf extract of *Mirabilis jalapa* has been examined for its antibacterial activity against *Salmonella typhi* and *Bacillus cereus*. Zones of growth inhibition for the extract at a concentration of 20

micrograms/ml were  $34.33 \pm 1.70$  mm and  $51.33 \pm 1.88$  mm against *Salmonella typhi* and *Bacillus cereus*, respectively while the zones of growth inhibition of separated bioactive fraction at 3mg/ml were  $40.33 \pm 1.33$  mm and  $40.67 \pm 1.70$  mm, against the same pathogens, respectively[65].

- Ethanol, ethyl acetate, chloroform, formaldehyde and distilled water extracts of *Mirabilis jalapa* leaves have been included in a study to test the antimicrobial activity against Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Pseudomonas aeruginosa* and *E. coli*). The study reveals that the ethanolic leaf extract shows the most potent activity against *Staphylococcus aureus* (36 mm), *Bacillus subtilis* (28 mm), *Pseudomonas aeruginosa* (27 mm), *E. coli* (24 mm), followed by water extract against *Bacillus subtilis* and *Staphylococcus aureus* (19 and 16 mm, respectively); formaldehyde extract against *E. coli* (19 mm); and chloroform extract against *Pseudomonas aeruginosa* (18 mm)[76].
- A study has been done on the aqueous and ethanolic extracts of the leaves of white-flowered *Mirabilis jalapa* for the screening of antibacterial effect against *Staphylococcus aureus*, *Salmonella typhi*, *E. coli*, *Vibrio cholerae* and *Bacillus subtilis*. The ethanolic extract has shown the highest (with reference to the activities of tetracycline which was considered as the standard 100%) inhibition against *Salmonella typhi* (54.74%) than *Staphylococcus aureus* (54%), *Vibrio cholerae* (51.95%), *E. coli* (51.08%) and *Bacillus subtilis* (50%). The aqueous extract has not been shown to possess antibacterial activity[77].
- Tubers of *Mirabilis jalapa* were used to prepare petroleum ether, acetone, water, methanol, and dichloromethane extracts. These extracts have been used in agar diffusion methods against 8 strains of bacteria. Named as; *Staphylococcus aureus*, *Streptococcus epidermidis*, *Bacillus cereus*, *Micrococcus luteus*, *Enterococcus faecalis*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. All the extracts have shown moderate antibacterial activity[78].
- Two fractions of the crude extract of *Mirabilis jalapa* radix (triterpenoids & flavone) have shown antimicrobial activity when tested against *Staphylococcus aureus*[79].
- The leaf extracts of aqueous, acetone, and ethanol were used to investigate antibacterial activity *in vitro* against biofilm and Extended-spectrum beta-lactam producing uropathogenic *E. coli* (UPEC). The most

potent antibacterial activity against all tested biofilm producing UPEC strains was shown by the ethanolic extract whereas, it has only inhibited 2 of the 4 ESBL producing UPEC strains[80].

- “Antibacterial activity of the acetone, ethyl acetate, petroleum ether and ethanol extracts of *Mirabilis jalapa* leaves has been tested against biofilm-producing uropathogenic *E.coli* (UPEC 1, 17, 57 and 82). An inhibition zone of 22, 20 and 17 mm, has been exhibited by the acetone extract against biofilm producing UPEC 1,17 and 82 strains. The petroleum ether extract has exhibited a zone of growth inhibition of 18 and 15mm, respectively against biofilm producing strains UPEC 1 and 17. The ethyl acetate extract has exhibited a zone of inhibition of 20, 19 and 21mm, respectively against biofilm producing strains UPE , 17 and 82”[81].

#### **4.2. Antifungal activity**

- The isoflavone and dehydroretinol identified in the plant cell culture of *Mirabilis jalapa* is found to possess antifungal activity with IC50 of 25 and 48 micrograms/ml, respectively, against *Candida albicans* DSY1024[82].
- “Agar diffusion method has observed the antifungal activity of *Mirabilis jalapa* against *Aspergillus niger*, *Fusarium solani*, *Fusarium oxysporum*, and *Fusarium granulation*. Two phenolic compounds isolated from *Mirabilis jalapa* have shown antifungal activity against *Candida ablicans*”[74]. “The methanol extracts of *Mirabilis jalapa* have shown to have the potential inhibitory effect against *Aspergillus niger* and *Deadalea flavida* while not affecting *Candida albicans*”[83].
- The antimicrobial activity of ethanol, ethyl acetate, chloroform, formaldehyde, and distilled water extracts of *Mirabilis jalapa* leaves were involved in the study. *Candida albicans* has been used as the control organism. The results show that the ethanolic leaf extract has the most potent antibacterial activity against the pathogen (29 mm), followed by the chloroform extract (14 mm)[76].
- Two antimicrobial peptides named Mj-AMP1 and Mj-AMP2 have been isolated from *Mirabilis jalapa* seeds. The seeds have shown a broad spectrum of antifungal activity. These 2 peptides were found to be active against 13 plant pathogenic fungi. The concentration required for 50%

inhibition of fungal growth has varied from 6-300 micrograms/ml for the peptide Mj-AMP1, while it was 0.5-20 micrograms/ml for Mj-AMP2[63].

- “Tubers of the plant have been used in a study to make extracts of petroleum ether, acetone, water, methanol, and dichloromethane extracts. Agar well diffusion method has been followed for the screening of antifungal activity against *Aspergillus niger*, *Fusarium solani*, *Fusarium oxysporum* and *Fusarium granulation*. The study results showed that the water extracts have fungal toxicity”[73].
- *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus* have been chosen for a study to observe the antifungal effect using the acetone, chloroform, ethanol, and methanol extracts of *Mirabilis jalapa* leaves. Methanol extract has exhibited the highest inhibition of fungal radial mycelial growth (97.8% at 500 microgram/ml medium) against *Aspergillus flavus*. The methanol extract has shown the lowest MIC against *Aspergillus flavus* (45 micrograms/ml)[70].
- The antimicrobial effect of ethanolic extract of *Mirabilis jalapa* leaves has been used in a study against 2 fungi which has resulted in inhibition zones of 35 mm and 30 mm for *Penicillium notatum* and *Rhizopus stolonifer*, respectively[53].

### 4.3. Antiviral activity

- *Mirabilis jalapa* has been found to have a high concentration of ribosome-inactivating proteins, which are antiviral proteins. The leaf suspension-cultured extract and its function with 90% saturated ammonium sulfate solution demonstrated anti-plant viral activity comparable to the original plant's roots and leaves[68].
- “1,2,3,4-tetrahydro-1-methylisoquinoline-7,8-diol compound, isolated from the root of *Mirabilis jalapa* has shown a 48% inhibition against HIV-1 reverse transcriptase at 210 micrograms/ml”[84].
- Leaves of *Mirabilis jalapa* have been used to prepare an ethanolic extract which was involved in the screening of antiviral activity against HSV-1 and VSV (Vesicular stomatitis viruses) by simplified plaque reduction assay. Results have demonstrated pharmacological activities to a certain extent[85].
- A protein isolated from the roots and leaves of *Mirabilis jalapa* demonstrated antiviral activity against potato virus X, potato virus Y,

potato leaf roll virus, and potato spindle tuber viroid that was comparable to that of the original plant's roots and leaves[86, 87, 88, 89, 90].

“The purified protein has been shown to inhibit the mechanical transmission of tomato mosaic virus (TMV) in tobacco, tomato, pepper plants and cucumber green mottle virus in cucumber plants”[91].

#### **4.4. Anti-inflammatory activity**

- “A study on the anti-inflammatory activity of *Mirabilis jalapa* hydroethanolic flower extracts was conducted. This has been tested in rats with formaldehyde and Complete Freund's adjuvant (CFA) induced arthritis. The extract significantly reduced paw edema in both models (P<0.001). CFA rats' body weight, hematological, and antioxidant changes have been restored to normal”[92].
- The alcoholic, aqueous, and petroleum ether extracts prepared from *Mirabilis jalapa* leaves were used in a study to determine the plant's anti-inflammatory activity. Carrageenan-induced paw edema, formalin-induced paw edema, and cotton pellet-induced granuloma methods were used on Wistar albino rat models, respectively. According to the findings, all extracts have the anti-inflammatory potential[47].
- The anti-inflammatory activity was tested in Wistar albino rats using Carrageenan and formalin-induced paw edema models. *Mirabilis jalapa* leaf aqueous extract was used. In the carrageenan-induced paw edema model, anti-inflammatory activity was dose-dependent. The aqueous extract inhibited paw edema by 37.5% and 54.0% on the fourth hour at 200 and 400 mg/kg doses, respectively. In contrast, the formalin-induced paw edema models showed a similar pattern of paw edema. As a result, the findings show that the aqueous extract of the leaves has promising anti-inflammatory properties[93].
- “The anti-inflammatory potential of *Mirabilis jalapa* Linn. flowers and *abelmoschus esculentus* leaves were tested *in vitro* using heat-induced denaturation and membrane stabilization methods. It has been revealed that *Mirabilis jalapa* flower extract exhibits anti-inflammatory activity at a concentration of 0.1g/ml than the leaves of *Abelmoschus esculentus*, comparable to that of the standard aspirin” [94].
- *Mirabilis jalapa* leaves were used in a study to produce total alcoholic extract and petroleum ether fractions. “Carrageenan-induced rat paw

edema and cotton pellet-induced granuloma models were used to test for anti-inflammatory activity. The results show that both test samples inhibit the increase in fibroblasts and the synthesis of collagen and mucopolysaccharides during the formation of granuloma tissue in chronic inflammation. As a result, these findings provide strong evidence to support the folklore claim of the drug's use as an anti-inflammatory agent"[95].

- A study was conducted to evaluate the anti-inflammatory activity of *Mirabilis jalapa* flower aqueous and alcoholic extracts. The study included *in vitro* models such as bovine serum albumin denaturation, egg albumin denaturation, and HRBC membrane stabilization methods. The extracts were tested at 50 to 300 micrograms/ml concentrations. Both extracts inhibited protein denaturation in a dose-dependent manner. According to the results, ethanolic extracts inhibited BSA, egg albumin denaturation, and HRBC membrane stabilization methods with the highest inhibition of 70.14%, 61.66%, and 55.23%, respectively. The highest percentage of inhibition observed in aqueous extract was 72.33%, 73%, and 59.35%, respectively. As a result, the study found that extracts have significant anti-inflammatory activity[96].

#### **4.5. Antioxidant and cytotoxic activity**

- The ferric reducing antioxidant power (FRAP) assay was used in a study to screen the total antioxidant capacity of *Mirabilis jalapa* leaf acetone, ethyl acetate, petroleum ether, and ethanol extracts. According to the study findings, ethanol extract has a higher antioxidant capacity than other extracts[81].
- The study found that methanolic extracts of *Mirabilis jalapa* aerial parts and roots have high antioxidant activity. The study aims to identify the bioactive components responsible for antioxidant activity as well as the mechanisms of action. According to the findings, the extract's total flavonoid content was discovered to be an active compound responsible for antioxidant activity, which could serve as a free radical inhibitor[97].
- *Mirabilis jalapa* aerial parts and roots were used in a study to make methanolic extract. The ABTS and DPPH free radical scavenging assays were used in the study. The study reveals the plant's enormous potential for future research to identify the bioactive components responsible for

antioxidant activity and elucidate their tentative mechanisms of action[98].

- The antioxidant activity of petroleum ether, chloroform, and methanol extracts of *Mirabilis jalapa* leaves and bark was determined using the DPPH test. Compared to ascorbic acid (IC<sub>50</sub> 70.985 micrograms/ml), the methanol extract of the plant's bark demonstrated antioxidant activity with an IC<sub>50</sub> value of 598.02 micrograms/ml[99].
- The seed epicarp ethyl acetate extract demonstrated a high free radical scavenging rate, with IC<sub>50</sub> values of 6.62 and 3.49 mg dry powder weight/ml. Assay methods used included the DPPH and OH assays[59].
- The antioxidant properties of *Mirabilis jalapa* tuber extracts in petroleum ether, acetone, water, methanol, and dichloromethane were investigated. The study used DPPH radical-scavenging activity, TBA assay for lipid peroxidation, and linoleic acid assay for  $\beta$ -carotene bleaching. According to the findings, water extracts have the highest antioxidant and free radical scavenging activity, followed by methanol and dichloromethane extracts. The study reveals that this is due to the high content of flavonoids and  $\beta$ -sitosterol, which were discovered for the first-time using LC/MS and GC/MS, respectively[73].
- A study for the *in vitro* examination of antioxidant activity has used the reducing power assay method and hydrogen peroxide scavenging activity. *Mirabilis jalapa* aerial parts were used to prepare a methanolic extract for this. According to the study, this antioxidant activity is due to flavonoids, polyphenols such as phenolic compounds, and tannins[71].
- Microtitration cytotoxicity assay has been used in a study to screen for cytotoxicity using the HeLa cell line. The ethanolic extracts of *Mirabilis jalapa* leaves were used for this. To a certain extent, the findings of this preliminary study have demonstrated anticancer activity[85].

#### **4.6. Antiparasitic activity**

- Using animal models, researchers have investigated the antimalarial effects of *Momordica charantia* L. and *Mirabilis jalapa* leaf extracts (*in vivo*). This study aims to look into the effect of plant extracts on malaria in a 4-day suppressive test. Oral induction of 50, 100, and 200 mg/kg methanolic plant extracts has been used in the study. The study's findings show that *Mirabilis jalapa* has the highest chemo suppression of

parasitemia at the lowest tested dose of 50 mg/kg body weight of mice when compared to the reference drug, chloroquine, which has the highest chemo suppression of parasitemia (100%) when administered orally at 20 mg/kg. As a result of this research, *Mirabilis jalapa* can be used to treat malaria[100].

- The larvicidal activity of *Mirabilis jalapa* leaf extracts in ethanol, benzene, methanol, and ethyl acetate was investigated against larvae of 3 mosquitoes that act as important vectors (*Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*). Methanolic leaf extracts had the highest larvicidal activity against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*, with LC50 values of 84.53, 64.58, 57.55 ppm, and LC90 values of 159.25, 120.28, and 104.20 ppm, respectively. The death rate is positively related to the extract concentration [101].
- The effect of aqueous and alcoholic extracts of *Mirabilis jalapa* roots on the spontaneous movements of the whole worm and the nerve-muscle preparation of *Setaris cervi* (cattle filarial parasite) and the survival of microfilariae has been investigated *in vitro*. The alcoholic extract has inhibited spontaneous movements of the whole worm and nerve-muscle preparation of *Setaris cervi*, according to research. The aqueous extract has inhibited the nerve-muscle preparation of *Setaris cervi*'s spontaneous movement. The alcoholic extract's effect on the whole worm was characterized by an increase in concentration amplitude followed by reversible paralysis. In contrast, neither extract had an initial stimulatory effect on nerve-muscle preparation[102].
- *Mirabilis jalapa* flower methanolic extracts have been chosen for a study to examine the antispasmodic effect *in vitro*. The study used rabbit jejunum, thoracic aorta, and guinea pig ileum. The flower extract (1-1000 mg/ml) has inhibited gut smooth muscle contractility (IC50  $18 \pm 0.7$  micrograms/ml), whereas it has stimulated rabbit aortic muscle concentration (EC50  $11.60 \pm 0.26$  micrograms/ml) in a concentration-dependent manner[103].
- The antihelmintic activity of *Mirabilis jalapa* aerial part extracts (20%, 40%, 60%, and 80%) was investigated using *Pheretima posthuma* as test worms. According to the study, the *Mirabilis jalapa* ethanolic extract has caused paralysis in 12.6 minutes and death in 13.5 minutes [71].

#### **4.7. Hypoglycemic and hypolipidemic activity**

- *Mirabilis jalapa* root ethanolic extracts were tested for hypoglycemic and hypolipidemic activity in normal and streptozotocin-induced diabetic mice. The repeated administration of extract has been shown to lower blood glucose levels, improve insulin sensitivity index, lower serum total cholesterol, lower triglycerides, and increase glycogen content in the liver and skeletal muscles after and before diabetes induction[47].
- On streptozotocin-induced diabetic mice, the antihyperglycemic effect of *Mirabilis jalapa* hydroethanolic leaf extract has been investigated. At concentrations of 200 and 400 g/kg, the extract has significantly reduced the levels of glucose, urea, creatinine, aspartate transaminase, alanine transaminase, and alkaline phosphatase in the tested animals[104].

#### **4.8. Antinociceptive and analgesic activity**

- Hydroethanolic extracts of *Mirabilis jalapa* leaves and stems were used in a study to investigate the antinociceptive activity. Assay methods include an acetic acid-induced writhing mouse model and a thermal pain model using a tail-flick hot water bath. According to the findings, the plant has antinociceptive properties[105].
- Mice were given ethyl acetate fractions of *Mirabilis jalapa* leaves (10 mg/kg orally) to test the plant's antinociceptive effects. The study has used Complete Freund's Adjuvant- CFA, surgical incision paw edema, and partial sciatic nerve ligation as pain model methods. The extraction has significantly reduced the pain caused by CFA, paw edema, and partial sciatic nerve ligation. As a result, the plant's antinociceptive property has been confirmed[106].
- It has been studied that the ethanolic leaves extract of *Mirabilis jalapa* has analgesic and muscle relaxant activity on Swiss albino mice[107].

#### **4.9. Antihistamine activity and immune modulatory effect**

- The antihistamine activity of *Mirabilis jalapa* root extracts extracted in ethanol: acetone (1:1) was studied. This has been demonstrated in mice using clonidine-induced mast cell granulation and guinea pig tracheal chain preparation. According to the study findings, this plant's folklore use in the treatment of allergic disease and asthma is justified[108].

- An *in vivo* study of the immune modulatory activity of *Mirabilis jalapa* ethanolic tuber extracts has been included in mice. Assay methods used include haemagglutination antibody titer, delayed-type hypersensitivity, neutrophil adhesion test, and carbon clearance test. When compared to the control group, oral administration of the extract has significantly increased antibody titer, phagocytic index, neutrophil adhesion, and positive hypersensitivity response in mice[109].

#### **4.10. Anti-tubercular drug-induced hepatotoxicity**

- *Mirabilis jalapa's* antitubercular activity has been studied. *Mirabilis jalapa* Linn. leaves were found to have a protective effect against hepatotoxicity caused by antitubercular medications[110].

#### **4.11. Anti-cancer**

- The cytotoxic property of petroleum ether, chloroform, and methanol extracts of *Mirabilis jalapa* leaves and bark has been determined using the brine shrimp lethality bioassay technique. When compared to vincristine sulfate (LC50 value: 0.33 g/ml), the petroleum ether extract of the bark has demonstrated significant cytotoxic activity with an LC50 value of 8.12 g/ml[99].
- A protein from *Mirabilis jalapa* has been tested for anticancer activity against various cell lines. It has exhibited cytotoxicity against T47D and SiHa cell lines but has been less cytotoxic to mononuclear cells. It demonstrates more specific cytotoxic activity against cancer cell lines like MACF-7, A549, and HCT 116 than normal cell lines (Vero). It also had strong apoptotic properties[72, 111, 112, 113].
- A protein fraction with ribosome-inactivating protein (RIP) properties isolated from the leaves of *Mirabilis jalapa* has exhibited cytotoxicity against T47D and SiHa cell lines. The LC50 values for T47D and SiHa cell lines were 0.36 g/ml and 5.6 g/ml, respectively. It was not toxic to normal cells (LC50 of 21.04 g/ml). When compared to normal mononuclear cells, it has produced more cytotoxic activity against breast and cervical cancer cells (58-fold and 4-fold, respectively)[111].

#### **4.12. Dermatological effect**

- The effects of a hydro methanolic extract of the tuberous root of *Mirabilis jalapa*, as well as its terpenoid and flavonoid fractions, on skin wound healing in rats has been investigated using an excision wound model. The results have shown that flavonoid caused a significant decrease ( $P < 0.05$ ) in antioxidant enzyme levels in the wound healing process, whereas terpenoid fraction has caused a significant increase ( $P < 0.05$ ) in growth factor expression levels, but regeneration and remodeling stages were delayed due to the formation of a thicker ulcer layer. There have been no hair follicle-like blood capillaries, which could lead to the formation of a hypertrophic scar of the wound. The authors concluded that terpenoid fractions prolonged the proliferation phase and had a tendency to convert the wound into a hypertrophic wound[114].
- The wound healing activity of the ethanolic extract of the leaves of *Mirabilis jalapa* (1000 mg/kg Body weight) has been investigated using the excision wound model, incision wound model, and dead space wound models in experimentally induced diabetic rats. *Mirabilis jalapa* extract has shown significant wound contraction from the 9th to the 18th day, significantly increase in wound breaking strength, a significantly increase in dry tissue weight, and increased hydroxyproline content compared to normal control and diabetic control animals[115].

#### **4.13. Reducing agent for the production of gold nanoparticles.**

Nanoparticles are created using a variety of non-environmentally friendly chemical methods. Aqueous extract of ethanolic *Mirabilis jalapa* Linn. flowers have been used to reductively prepare gold nanoparticles from auric chloride. The flower extract has served as a reducing agent as well as a cage for the gold nanoparticles. The controlled reduction of the Au  $3+$  ion to Au  $0$  resulted in the production of gold nanoparticles. FT-IR and UV-Visible spectroscopy have been used to confirm the formation of gold nanoparticles[25].

### **5. Conclusion**

*Mirabilis Jalapa* Linn is a well-known herbal plant. It is commonly grown for medicinal and ornamental purposes. It exhibits a wide range of biological activities that aid in the utilization of this plant's medicinal benefits. However, there is still a great need for more research on this

species, and scientists must pay close attention to it if it is to reach its full potential.

### **COMPETING INTERESTS**

The authors have declared that no competing interests exist.

### **AUTHORS' CONTRIBUTIONS**

This work was conducted in collaboration among all authors. SPNNS, CBR developed the conceptualization of the current work. SPNNS, CBR and AKC contributed to the writing review and editing of the manuscript. Author CBR supervised the project. All authors read and approved the final manuscript.

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