Sustainable Development of Four O' Clock Flower-Based Natural Food Colourant with Green Nanoparticle-Intensified Stability and Biofunctionality

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Research Article

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Abstract

Use of artificial colouring agents in sweets and packed food has become a common scenario now a day, which pose serious consequences in consumers especially children. Various studies around the globe reported the serious health hazards of artificial food dyes which include organ damage of liver, kidney and lungs thereby affecting metabolic enzymes, serum creatine and urea levels and brain damage resulting in neurotoxicity and even behavioural deficits in children. Food products which use artificial dye like soft drinks, ice cream, pastries, biscuits, cake, cookies etc. are more or less a part of modern generation's daily food habit. Hence, it is imperative to search for alternative safe and healthy food additives of natural origin. In this direction, the current review focusses to exploit the feasibility of *Mirabilis jalapa* (four o'clock) flower extract as food colouring agent along with its possibility as a therapeutic agent. Phytochemical screening, Antioxidant activity evaluates the medicinally active substance and Optimization of the pigment. Therapeutic activity of the natural pigment will be determined by *in vitro* studies in animal cell lines. Nanoparticles of *M. jalapa* flower extract solution will be used for slow and sustained cellular delivery of the extract to study detailed physiological and biochemical interactions. Cellular delivery of iron nanocluster proven spectrophotometric analysis in the UV-visible range, SEM and XRD. The current investigation will proficient to propose prudent natural alternative for the currently used chemical food colouring agents in a cost-effective way, which can mark numerous consequential fitness matter due to life style modification in an effective manner.

1. Introduction

Food colour is a frequently-overlooked astounding character that assuredly influences flavor apprehension (Hutchings *et al*., 2003; Darko *et al*., 2014; Stich E. 2016). Fashioning the color and or the savour of vegetables appear today accessibly utilizing LED illumination as pre-or post-reaping ministrations with processed food and pigments staining food products are normally wobbling in strident circumstances (Matulka *et al*., 2014, Schoefs *et al*., 2016; Barthelat, F 2019). Therefore, to nourish or merely reinstate product tincture consistency, colouring negotiators are deliberately appended to subsistence products (European Commission and the Council, 2009; Reinhart A. 2014; Spence C. 2016; Stich E. *et al*.,2016). To pinnacle the significance of similar ailment, it is ample to recollect the cosmic succession of Alzheimer's disease: the aggregate of convalescent pinpointed was 35 million in 2010; the prognosis for 2030 is 65 million and 115 million in 2050 (Reinhart A, 2014; Scotter MJ,2015; Ishikawa *et al*., 2012).

The dyestuff firmness is determined by a constituent for instance enzymes, temperature, oxygen, and pH (Pedreno *et al*., 2001; Wang *et al*., 2017). Betalains have been auspiciously cast-off in mercantile food tinging performances for an integer of years (Goldman *et al*., 1996; Xu G *et al*.,2008; Mondragón Palomino, *et al*., 2013) and pursued to be a salient origin of red colour in the food manufacturing industry. In juxtaposition with terpenoids and dimensions, the antimicrobial pursuit of these snatches from the tuberous root of *M. jalapa* has been delineated (Pramanick DD,2015; Aher AN *et al*.,2016). The reciprocity of nanoparticles with profuse substances is immensely divergent from the association with

the metal of a prominent proportion range (Rozina R, 2016. Sadiq ME, 2018). The fruits with solitary seeds are spherical, wrinkled and black when matured (Wang Yi-Fen *et al*., 2002; Kakad SL *et al*., 2015). Respective constituents including stigmasterol, brassicasterol, and *Mirabilis* antiviral protein, rotenoids (*mirabijalone* A-D, *boeravinones* C and F) were secluded from the ethereal segments (Aoki *et al*., 2008) delineated that *four 'clock plant* had scores of biological pursuits for instance as antispasmodic, antibacterial, antiviral, antifungal and protein synthesis hindrance. *M. jalapa* is pre-owned in vegetal therapeutics as ministrations employ diarrhoea, malaria (Daniel, 2006; Liya, F.I *et al*.,2014).

Four 'clock plant be the possession to the family Nyctaginaceae which is universally known as the enchantress of the night, four o'clock, or marvel of Peru. *M. jalapa* is a perennial, herbaceous, bushy plant that reaches outstretch heights of for the most part 1 meter, infrequently up to 2 meters, in height (Atanassov AI,1986; Yi-Fen *et al*., 2002; Yakubu MT *et al*., 2019). It has opposite leaves, exceedingly substantial spectacular flowers, curvaceous, ellipsoidal fruits and conspicuous tuberous roots (Sarray DKA *et al*, 2015; Gogoi J *et al*., 2016). *Mirabilis* in Latin measures 'wonderful' and *Jalapa* is an admired name in Central and North America (Hanani E *et al*.,2017; Antonella Rosa et al., 2020). The explicit source of *Mirabilis jalapa* is unknown, but it is trusted to originate from parts of tropical America (The Wealth of India, Raw Materials, 1998; Gogoi J *et al*., 2016; Kai Lüersen et al.,2019). It conceivably is grown as an annual, principally in the temperate zone, the stems are sturdy, full, and quadrangular with many ramifications and rooting at the nodes (Acevedo-Rodríguez P *et al*. 2012; Kuznetsova V *et al*., 2019). A peculiar facet of *M. jalapa* is that flowers with contrasting colours grow simultaneously on the self-same plant (Damascena LS *et al*. 2009; Chou, S *et al*., 2016; Vangjeli, J *et al*., 2018). Four'O clock plant is the bulk foremost appliance for genetic engineers because they authorize the initiation of determined genes into plants (Maheswari, K. C *et al*., 2016; Acevedo-Rodríguez, P;2012). At the same time, this extensive strategy proffers minuscule prompt aspiration for the direction of polygenic traits because uniform if all the constituent genes determine a quantitative character could be recognized and secluded, a sizeable quota of DNA would have to be fetched and the discrete genes presumably would have to be intimated in a synchronized system (Hanani E *et al*.,2017).

The synonym of the study plant is; *M. dichotomy* Lin. Taxonomic classification Kingdom: Plantae; Subkingdom: Tracheobionta; Division: Angiosperms; Class: Dicotyledons; Subclass: Caryophylidae; Order: Caryophyllales; Family: Nyctaginaceae; Genus: *Mirabilis*; Species: Jalapa (Bruneton 2009; Lim; Singh *et al*., 2016).

Morphology

M. jalapa Linn. habitually grows 0.6 to 0.9 m towering and unbiased as outspread. Leaves are tapering; flowers customarily unfasten from behind afternoon onwards, hence the first of its familiar names. Flowers in groups of three, bracelets; adjoin the perianth; routinely yellow; crimson, white, inception Four'O clock. Gamophyllus, stamens five with disparate tendrils (Oviedo Prieto R *et al*., 2012; New York Botanical Garden, 2016). The solitary ovule within the superior ovary of the unilocular carpel is accompanied by a surrounding nectariferous circle near the ovary. The achene fruit is in close proximity,

enclosed by a persistent perianth that is leathery and ribbed. (PFAF, 2016; Khurian, 2003; PROTA, 2016). The self-congenial, the consummate flowers, one and all has 5 to 6 stamens and a unique- ovule ovary (PIER, 2016; Pramanick *et al*., 2015; USDA-NRCS, 2016). Fruits are rugged oviform and roots are protuberant tuberous. Pollen aetiology the configuration of the pollen grains of *M. jalapa* is orbicular, oblate circular, with a circumference extending from 125 to 140 µm and opacity of 10 to 15 µm (Royal Horticultural Society, 2016; Yakubu MT,2019; Xu G *et al*., 2008). Pollen hermaphrodism is customarily initiated in this species (white-pink, mixed and mixed radiated); infrequent giant, dimorphic aberrant, disfigure, and juncture grains have been perceived (Xiong Y *et al*., 2010; USDA-ARS, 2016).

Vernacular name Andhra Pradesh: Chandrakantha; Assamese: Godhuli gopal; Bengali: Sandhyamaloti; Brazil: Marvel; China: Xizao hua; France: Belle de nuit; Hebrew: Lilanit RavGonit; Indonesia: Bunga pukul empat; Japan: Oshiroi-bana; Karnataka: Sanje mallige; Kerala: Naalumani poovu; Maithili: Sanjhaa Phool; Netherlands: Nachtschone; Pakistan: Gul adnan, gul-e-abbas; Persian: Laleh abbasi; Sri Lanka: Hendirikka; Turkish: Akşam sefası (Malakar, M *et al*.2020; Uotila P,2011; Royal Horticultural Society, 2016).

Geographical Distribution

Mirabilis jalapa Linn. (Family Nyctaginaceae) was authentically botanically noted in 1753 even though it hitherto had long been administrated as a decorative plant all around the analeptic of the world (Vladimirov *et al*., 2015; Pramanick *et al*.,2018). This plant is naturalized all over world. In India, grows mainly in West Bengal, Manipur, and Western Himalayas (Baliousis, E. 2014; Chou *et al*.,2016). The flowers are frizzled in elucidated butter and are secured on the blister (Garcia-Mendoza *et al*., 2012; Velayos 2014). Skin emission and also has humectant possessions (Gutiérrez et al.,2014; Chou S, 2016). The leaf extract is taken orally for ministrations of Hepatitis (Mostaph *et al*.,2013; Sykes, W.R. 2016; Jaramillo Díaz *et al* 2017; Onana, J.M. 2011; Mohlenbrock, R.H. 2014; Sykes, W.R. 2016; Prakash, L *et al*.,2018;). The Stem and leaves are owned for decolouration (Jaramillo Díaz, P *et al*.,2017; Chang *et al*., 2014). Roots and flowers are cast-off medicinally in Ayurveda, Siddha and other conventional order of medicine for restoring copious positioning (Sykes, W.R. 2016; Chang *et al*., 2014; Vangjeli, J. 2017; Sykes, W.R, 2016; Bernal R., 2016; Velayos *et al*.,2014). The autochthonous of Shivalik Hills, Himachal Pradesh utilize the root tubers and flowers which are ingested as a relish for their nourishing worth (Gutiérrez, J *et al*., 2014; Balkrishna, A. 2018). The mush of the root tuber is put in to scrutinize the extension of mature tumours in the consanguine sector of Rajasthan (Urziceanu, M. *et al*. 2020; Rozina R, 2016; Zuloaga *et al*., 2008). The tuber is dispensed in a minuscule quota to rehabilitate piles (Sadiq ME,2018; Pramanick DD *et al*., 2015). Flowers are warned in food dyeing, cast-off to pigment food in world outspread (Vangjeli, J. 2017, Sumbal *et al* 2019).

2. Materials and methods

Research Locale

State of Kerala counterbalanced a dimension of 38,863 km^2 with a citizenry density of 859 per km^2 and spread across 14 districts. Kerala reclines for about 580 km on the edge of the Malabar Coast, diversifying in width from abruptly 30 to 120 km (Vineethkumar, V *et al*., 2021; Mahamood, K.N *et al*., 2020). It is fringed by the states of Karnataka to the north and Tamil Nadu to the east and by the Arabian Sea to the south and west (Lalitha, M *et al*.,2021). On the eastern edge of the state, Anai Peak (8,842 feet,2,695 metres), the multistorey pinnacle in peninsular India, crests the Western Ghats (Alappat Linto *et al*., 2021; Khadke *et al*., 2018). The weather of Kerala is composed and stretched compact from condition to condition. All around the year, quotidian temperatures habitually stand up from the low 70s F (low 20s C) into the 80s F (27 to 32°C) (Ray *et al*., 2021; Arulbalaji, P *et al*., 2021). The state is straightly manifest to the southwest monsoon, which persuade from July through September, nevertheless it besides encounters rain from the reverse (northeast) monsoon, which blows in October and November. Precipitousness a stutter about 115 inches (3,000 mm) annually state-wide, with a little descend sustaining more than 200 inches (5,000 mm) (Nath et al., 2021; Achu, A. L *et al*., 2021).

Morphological characterization

Mirabilis jalapa Linn. routinely grows 0.6 to 0.9 m tall and equitable as outspread. Leaves are pointed; flowers normally open from late afternoon onwards, consequently the original of its common names (Yakubu MT et al., 2019; Vangjeli, J *et al*., 2017). Flowers in groups of three, blossoms having penda bracelets; surround the perianth; generally yellow; multihued and extended out during sundown (Kuznietsova V et al., 2019; Sein et al, 2016). Perianth are penda lobes, penda stamen disparate strings. Solitary pistil, mono locular, uni gynoecium adjoining the ovary (Sumbal et al 2019; Hanani E *et al*.,2017). Fruit caryopsis is covered with furrowed line, ribbed, tenacious perianth. The self-amicable, the absolute flowers, one and all have to numerous filament solitary ovary (Antonella Rosa et al., 2020; Maheswari, K. C *et al*., 2016). Fruits are coriaceous obovoid and roots are protruding tuberous. The single-seeded fruits are globular, wrinkled, and black beyond maturity, having commenced greenish-yellow (Khurian, 2003; Chou, S et al., 2016).

Mirabilis jalapa Flower pigment as a natural food dye

The nutriment industry is a multiplex and immense trading that consummate the world community's demand for nourishment. Colours are an unpreventable portrayal of every subsistence. Food pigment is any stain, shade or solidity that transmits complexion when it is adjoin to sustenance or drink (Journal from FDA.2012; Zou L *et al*., 2020). The inclusion of colourants to foods is conviction to have transpire in Egyptian metropolis as untimely as 1500BC when confectionary makers affix natural extracts and wine to refine product emergence (Meggos H 1995; Pramanick, D.D *et al*., 2018). Carotenoids, chlorophyllin, anthocyanins and betalain contain four principal classifications of plant dyestuff grown to tincture food outcomes (Rodriguez et al.,2016; Kumar S et al., 2017). *Mirabilis jalapa* (4'O clock plant) is a perennial herb that outstretches a high point of 50–100 cm from a tuberous root. A few of cultured mixed-breed can grow up to a meter in high point. This novice-friendly plant is cultivated globally for the allure of its flowers, which come in varying shades such as white, red, pink, or even multicolored, accompanied by a

delightful fragrance. (Diggs *et al*., 1999; Abraham J et al., 2020). The flowers of *Mirabilis jalapa* restrain Betalains which are a grade of red and yellow tyrosine-derived colourants. The essence purified from *Mirabilis jalapa* can be worned in the nutriment industry as a food dyeing envoy as it subdues the colorant Betalain (Yakubu MT et al., 2020; Sigurdson, G. T et al., 2017).

As food colour agent

Nature engenders a heterogeneity of dazzling stain and a integer of mercantile worth for staining food, these plant colourants are normally scrutinized to be innocuous, and deliverance the possibility of a little ailment like as atherosclerosis and cancer (Food and Drug Administration (FDA). 2021; Merinas-Amo *et al*., 2021). For this rationale, Betalains are extensively worned as an instinctive food colour medium (Anter, J *et al*., 2021; Mpountoukas, P et *al*., 2021). The betalain withdraw from the 4'O Clock plant flower is water-soluble and has antioxidant pursuit, the nanoparticles generate from this extract manifest a spacious diversity of immersion spectra in Uv-vis spectrophotometry (Picollo M *et al.*,2019; Gaikwad J *et al*., 2020).

Mirabilis jalapa -Betalain pigment

The term "betalain" originates from the Latin name of the common beet (Beta vulgaris), the source from which betalains were initially extracted. Betalain is commercially pre-owned as a innate dye (Das, A et al., 2020; Guerrero-Rubio, M.A et al., 2020). It sources beeturia (red urine) and red faeces in a little people who are impotent to interrupt it down. The engrossment of the nourishment industry in betalains has grown already they were recognized by *in vitro* procedure as antioxidants (Fu, Y *et al*., 2020; Sadowska-Bartosz *et al*., 2021), which authorized safeguard opposed to oxidation of truncated-thickness lipoproteins (Vieira Teixeira da Silva, D *et al*.,2019; Sawicki, T *et al*., 2018). The intense red colour of beets, bougainvillea, amaranth and 4'O clock plant sequel from the existence of betalain stain (Robinson T.,1963; Belhadj Slimen *et al*., 2017). The betalains are mass frequently perceptible in the petals of flowers still be allowed colour the fruits, leaves, stems and roots of plants that repress them (Grewal *et al*.,2017; Rodrigues, A.C.B *et al*., 2018). Derived from tyrosine, betalains are fragrant indole compounds that exist as glycosides, featuring both a sugar component and a pigmented segment. Their compound is advanced by luminosity (Polturak *et al*., 2017; Tian *et al*., 2020).

Mirabilis jalapa flower Sample collection and Extraction Methods

Four-o'clock besides called **marvel-of-Peru, or beauty-of-the-night**, (*Mirabilis jalapa*) attractive perennial plant, of the family Nyctaginaceae, indigenous to tropical America (Boyles, C *et al*., 2020; Kumar S et al., 2017). Four-o'clock is an expeditious-growing species up to one meter (three feet) tall, with oval leaves on short leafstalks (Kumorkiewicz-Jamro *et al*., 2020; Bao, X.W *et al*., 2019; Pramanick, D.D *et al*., 2018). The stems are distended at the juncture. The plant is called four o'clock for the reason that its flowers, from white and yellow to spectra of pink and red, are occasionally striped and speckled, open in the late afternoon and close by morning (Yakubu MT et al., 2019; Aher AN et al., 2016). The collection of fresh Four O'clock plants (Mirabilis jalapa Linn) took place in the local vicinity of Thodupuzha, situated in the

Idukki district of the Kerala state in India. The Specimen was authenticated by the Taxonomist Dr. Jomy Augustine, Head of the Department, Post Graduate and Research Department of Plant Sciences, Supported by FIST (DST, Govt. of India) & SARD (KSCSTE, Govt. of Kerala), St Thomas College, Pala, Kottayam District. -Kerala, India.

Fully developed blossoms are gathered and meticulously cleansed with double-distilled water.

The plant specimens underwent a meticulous rinsing procedure using purified water, followed by the determination of their fresh weight. Subsequently, the specimens underwent oven-drying at 50°C for a duration of 24 hours. The dried specimens were pulverized utilizing a Waring blender and preserved in hermetically sealed containers for subsequent analysis.The extraction of *Mirabilis jalapa* flower involved taking 10 grams of recently harvested flowers in a beaker, proceeded by extensive cleansing using tap water and subsequent rinsing with distilled water at least twice. The flowers were subsequently, finely sliced into small segments. The finely chopped flowers underwent a boiling process in 50 milliliters of purified water for a duration of 5 minutes. Following the boiling process, the flower pigment extract was permitted to reach a lower temperature and subsequently filtered. The flower pigment samples, after filtration, were gathered and preserved in airtight bottles at 4°C for subsequent analysis

Determination of total betalains: UV- VIS Spectrophotometric Analysis

The meticulous pigments underwent dilution with purified water and measurements were conducted at a wavelength of 535 nm. The quantification was expressed as mg betalains/100g, determined using the following equation determined by (Kuznietsova V et al., 2019; Kai Lüersen *et al*.,2019). Ultraviolet-visible (UV- VIS) spectrophotometer: The principle of UV-visible Spectrophotometer is mainly based on the beer's law and lamberts law Beer's law: states that the quota of luminescence absorbed by a substance is equivalent to the level of concentration for absorbing material. An α C Lambert's law: states that the quantity of luminosity absorbed by a substance is proportional to the opacity of absorbing material A α L. The UV/ visible spectrum of a material over a precise wavelength range comprises of immersion values estimated at respective wavelength points. In the UV-Visible spectrophotometer, the absorption of divergent natural nutriment stains such as red, yellow, orange, and green of the specimen was juxtaposed using copious nanometers 425, 480, 510, 520, 628 discretely.

HPLC analysis

The HPLC scanning was fetched (Palo Alto, CA, USA) by comprising a quaternary pump (Agilent Technologies 1260), a vacuum degasser, a variable wavelength detector and a 20 µl sample injector, column thermostat. The detection wavelength was 254 nm. The mobile phase consisted of 0.7M Sodium Acetate (pH = 5 ± 0.1) with a flow rate of 1ml/min. The column thermostat was maintained at 25^{o}C (Sathe PS, Dighe VV 2017, Gogoi J 2016).

HPLC conditions for TARTRAZINE

Chromatographic instrumentation and conditions

The explication performed by Analysis using High-Performance Liquid Chromatography comprising a quaternary pump (Agilent Technologies 1260), a vacuum degasser, a variable wavelength detector and a 20 µl sample injector, column thermostat. The separation was performed on the Zorbax Eclipse Plus C18 column. Column specification: 3.5µm, 4.6X100 mm, Agilent. The detection wavelength was 254 nm. The mobile phase consisted of 0.7M Sodium Acetate (pH = 5 ± 0.1) with a flow rate of 1ml/min. The column thermostat was maintained at 25^{o}C.

Preparation of Standard stock solutions

1mg of the standard (Tartrazine) was solubilized in a volume of 1 milliliter of the methanol phase and filtered through a 0.22 µm syringe filter and was five times serially diluted by two-fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg/mL) and 20 µl was injected to the column for analysis.

Preparation of Standard stock solutions

1mg of the specimen (*M. Jalapa* flower pigment) was dissolved in a volume of 1 milliliter of the methanol phase and filtered through a 0.22 µm syringe filter and 20 µl was injected into the column for analysis.

The analysis was monitored using open CDS EZ Chrom Workstation VL software.

Phytoconstituents Analysis

Phytoconstituents are compounds are produced by plants. they are present in flowers, fruits, vegetables, grains and other plant segments. The biologically active metabolites act as defensive mechanism averse to disease, and infections, protect cells from damage and reduce the risk of cancer. Alkaloids, tannins, terpenoids, glycosides, flavonoids, saponins and phenols of *Mirabilis jalapa* flower extracts are tested as per standard protocols (Ogbonna *et al*., 2016; Singh A *et al*., 2019). Phytochemical screening of *M. Jalapa* flower extracts water as solvents are carried out to identify the bioactive compounds using standard phytochemical methods described by (Hanani, 2017; Singh, 2019).

Phytoconstituents assessment achieved by solvent extracts and standardized procedures to identify the constituents of flower extracts.

a) Detection of Tannins

The test solution was individually heated in the presence 20 ml sterile water with the help of water bath for 5 minutes. Collected the filtrate kept in room temperature for attain the stability, add 10% of $FeCl_3$ perceived for precipitate emergence indicating the appearance of tannins.

b) Detection of Flavonoids

Shinoda Test: Extract received a small amount of liquid therapy consisting of a couple of droplets of NaOH solution. The

The development of a vivid yellow hue, transitioning to colourlessness upon the introduction of a mild acid, signifies the existence of flavonoids.

c) Detection of Steroids

Salkowski test: The text extract was subjected to the application of a small quantity of liquid concentrated Sulphuric acid. Red colours at the low layer and yellow colour at the lower layer suggested the existence of triterpenoid.

d) Detection of Saponin

To conduct the foam test, introduce a small quantity of the extract into a test tube along with a modest amount of water, then vigorously shake the mixture. The detection of foam emerging after a brief period suggests the existence of saponin in the sample.

e) Detection of Reducing Sugar

Conducting the Benedict test involves combining 2ml of Benedict reagent with 5 drops of the test solution, followed by a 5-minute boil in a water bath and subsequent cooling. The emergence of a red, yellow, or green precipitate signifies the existence of reducing sugar in the substance.

f) Detection of protein and amino acids: Test for Xanthoprotein

Upon the addition of Concentrated Nitric Acid to the extracts, the development of a yellow color serves as an indicator for the presence of proteins.

g) Detection of Phenol

The application of 3–4 drops of solution containing Ferric chloride to the extracts results in the development of a bluish-black color, signifying the presence of Phenol.

h) Detection of Glycosides

Dissolving the extracts in a blend of 1% ferric sulfate solution in 5% glacial acetic acid, followed by the addition of one or two drops of concentrated sulfuric acid, leads resulting in the creation of a blue color, indicating the presence of deoxy sugar.

Antioxidant Examination

The antioxidant potential of aqueous extracts from *M. Jalapa* flowers was assessed via DPPH Radical Antioxidant Activity, Folin-Ciocalteu methods, and Total Antioxidant models. (Mehidi A *et al*.,2017; Feixiang., 2020). The antioxidant activity of *Mirabilis jalapa* flower samples is examined using standard protocols.

Antioxidant activity (DPPH assay: free radical neutralization activity)

Preparation of standard solution: The development *of a* solution by dissolving 3 mg of ascorbic acid in 3 ml of distilled water in a 1:1 ratio. Subsequently, the solution was further diluted to concentrations of 20, 40, 60, 80, and 100 µg/ml through the addition of distilled water. (Teepica *et al*., 2012).

Preparation of test sample:

Initial solutions o samples were made by dissolving 10 milligrams of dried hydro methanolic extract of the experimental substance flower in 10 ml of methanol to give the concentration of 1mg/ml. Then different sample concentrations (20, 40, 60, 80, and 100 micrograms per millilitres) were formulated by diluting them in distilled water.

Preparation of DPPH solution (2, 2- diphenyl-1-picryl hydroxyl)

A DPPH solution was formulated by dissolving 3.4 mg of DPPH in 3 ml of methanol. Combine 5 ml of a recently prepared DPPH solution with 5 ml of both the extract solution and standard solution (20, 40, 60, 80, and 100 µg/ml), ensuring thorough mixing. Allow the amalgamated solution mixtures to stand in darkness for a duration of 30 minutes. The transition from the initial purple hue of DPPH to yellow signifies the efficacy of the extract in exhibiting scavenging activity. Measure the absorbance of the mixture at 517 nm employing a UV-Visible Spectrophotometer, with ascorbic acid serving as a positive control. Evaluate the antioxidant efficacy of the methanolic extract and the standard antioxidant ascorbic acid by examining their radical scavenging effects on the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, following the methodology outlined by the specified source. (Feixiang Li et al., 2020; Robinson, J. P *et al*., 2017). A decreased absorbance in the blend of reactants is indicative of heightened free radical scavenging activity. To assess the antioxidant potential, treatments such as DPPH were employed in the pigment *Mirabilis jalapa* flower extract.

The proportion of scavenging was denoted by using the formula given below:

$$\%\text{DPPH radical-scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of the test sample})}{\text{Absorbance of control}} * 100$$

Antioxidant assay by Folin-Ciocalteu methods.

Determine the total phenolic content in the extract by employing the Folin-Ciocalteu method, following the procedure outlined in the mentioned source. (Yang et al., 2019; Dholakiya *et al*., 2017). Combine each sample solution (1.0 ml) and the standard (gallic acid) with 5 ml of Folin-Ciocalteu (Sigma-Aldrich) and 4 ml of 7% w/v Sodium carbonate, shaking the mixture thoroughly. Allow the solution to stand in darkness at room temperature for 30 minutes, following which the absorbance is measured at 680 nm using a spectrophotometer. After a 30-minute incubation period in darkness at room temperature, the absorbance at 680 nm was determined using a spectrophotometer. Express the quantity of total

phenolics as gallic acid equivalent (GAE) in milligrams per gram of dry plant extract, employing the provided expression; C = c x V/m

Biosynthesis of Nanoscale Iron Particles

Prepare the stock solution through the solution of 1 millimolar ferrous sulfate (FeSO4; Merck, based in Chennai, India) and adjusting the volume to a volume of 250 milliliters using distilled water.Combine 5 milliliters of *Mirabilis jalapa* blossom essence with 100 milliliters of a 1mM FeSO4 solution, and permit the reaction to progress under ambient conditions.

Characterization of Nanoparticles

Identification and characterization of the fabricated nanoparticles were carried out using UV-Vis spectroscopy, XRD, and SEM analyses.

UV-visible spectroscopy

UV–Vis Spectroscopy involved repeated scans of optical absorbance ranging from 380 to 500 nm using a UV-Vis spectrophotometer (Model 118, Systronics, Mumbai, India) with a precision of 1 unit nm. These scans were conducted to analyze the diminishing pace of silver, gold, copper, and iron ions by the Mirabilis floral pigment extract. The reaction concoction was subjected to a 20-fold dilution and subsequently prepared for UV-Vis spectrophotometry. Deionized water was cast-off towards calibrate the reference level.

Intermittent observation of the reduction process for Fe2 + was conducted using a UV-Vis Spectrophotometer. The UV-Vis spectra of the reaction solution were analyzed within the wavelength range of 375–760 nm.

SEM-XRD Analysis

X-ray diffraction

X-ray powder diffraction (XRD) stands as a rapid analytical method primarily employed for identifying phases in crystalline materials, providing insights into unit cell dimensions. The material under examination is finely ground, homogenized, and its average bulk composition is subsequently determined. Widely recognized as a standard method for investigating crystal structures and atomic spacing, X-ray diffraction relies on the constructive interference of monochromatic X-rays with a crystalline sample. These X-rays are created by a cathode ray tube, filtered to engender monochromatic radiation, collimated to concentrate, and administered toward the sample. Constructive interference, leading to the generation of a diffracted ray, occurs during the interaction of incident rays with the sample when conditions adhere to Bragg's Law ($n\lambda = 2d \sin \theta$).

This law connects the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then determined, refined and counted

(Azizian-Shermeh O et al., 2017, Baruah D et al., 2018; Ren Y *et al.*, 2019). The successful formation of nanoscale particles in the *Mirabilis jalapa* blossom colorant extract was confirmed through SEM-XRD analysis. This analytical methodology was implemented at Cochin University of Science and Technology (CUSAT), Kerala, utilizing the JSM 6390 instrument with an acceleration voltage of 20 kV.

SEM offers perspectives into diverse facets of the sample, encompassing outer structure, chemical configuration, crystalline structure, and the composition of materials comprising the sample. This technique furnishes detailed high-definition images by scanning a focused electron beam across the surface and detecting secondary or backscattered electron signals. XRD examination of the nanoparticles was carried out to validate the existence of elemental metallic components signals and to provide quantitative configurational information. (Khandel, P 2018; Abhishek Bhattacherjee et al., 2018).

Anti-Bacterial Assay

The Antibacterial assay employs the Kirby-Bauer disk diffusion method, utilizing antibiotic discs to assess the susceptibility of bacteria to specific antibiotics and determine the extent of their impact. During this examination, the discs containing antibiotics are strategically placed on a agar plate previously introduced with bacteria, and the entire plate is subsequently subjected to incubation (Vudagandla Sreenivasulu *et al*.,2021; Rao TN *et al*., 2019).

The presence of a zone around the antibiotic disc devoid of bacterial growth indicates the effectiveness of the antibiotic in inhibiting bacterial growth or causing bacterial death.

Termed a "zone of inhibition," this area devoid of bacterial growth serves as a direct measure of the antibacterial effectiveness of the tested antibiotic (Liu N *et al*., 2019; Yousaf H *et al*., 2020). The dimensions of the observed zone of inhibition are contingent on various factors, including the efficacy of the antibiotic in halting bacterial growth and the distribution of the antibiotic within the agar medium, which varies in accordance with the molecular composition of the antibiotic (Adewumi *et al*., 2019; Aher et al., 2016).

Assessing in vitro cytotoxic effects using the MTT assay method:

The L929 (Fibroblast) cell line was initially acquired from the National Centre for Cell Sciences (NCCS) in Pune, India, and cultured in Dulbecco’s Modified Eagle's Medium (DMEM) sourced from Sigma Aldrich, USA. Culturing the cellular lineage involved utilizing a tissue culture flask with a surface area of 25 square centimeters containing DMEM enhanced with 10% fetal bovine serum (FBS), L-glutamine, bicarbonate of soda (Germany, Merck), and an antibiotic solution composed of Penicillin (100 units per milliliter), *Streptomycin* (100 micrograms per milliliter), and Amphotericin B (2.5µg/ml). The maintained cellular cultures were housed in a controlled environment with 5% carbon dioxide in a humidified incubation chamber at 37ºC (NBS Eppendorf, Germany).

Cell viability was determined via both direct observation under an Inverted phase-contrast microscope and subsequent application of the MTT assay method.

The process of seeding cells into 96-well plates involved:

A confluent monolayer of cells, aged two days, underwent trypsinization, and the resulting cells were resuspended in a 10% growth medium. A 100µl cell suspension, containing 5x103 cells per well, was then seeded into a 96-well cell culture plate and placed in a controlled atmosphere with 5% carbon dioxide in a humidified incubation chamber at 37ºC.

Formulating the stock solution of the compound involved:

The preparation of the sample included weighing 1mg and dissolving it in 1mL DMEM with the assistance of a cyclomixer. Subsequently, the sample solution underwent filtration using a 0.22 µm Millipore syringe filter for guarantee sterility.

Cytotoxicity Assessment:

After a 24-hour period, the nutrient medium was aspirated, and newly formulated compounds in DMEM underwent a fivefold serial dilution with twofold steps (100 micrograms, 50 micrograms, 25 micrograms, 12.5 micrograms, 6.25 micrograms in 500 microliters of DMEM). Subsequently, 100 microliters of each concentration were introduced in triplicate to their respective wells and placed in the incubator at 37ºC in a controlled environment with 5% carbon dioxide in a humidified incubation chamber. Untreated control cells were concurrently maintained for comparison.

Cytotoxicity Evaluation through Direct Microscopic Examination:

After full day of therapy, the complete plate underwent examination employing a reversed phase-contrast tissue biology magnifier (Olympus CKX41 with Optika Pro5 CCD) camera), and scrutiny at the microscopic level was conducted documented through image recording. Any identifiable alterations in cell morphology, including cell rounding, shrinkage, as as well as the presence of granules and the formation of vacuoles in the cytoplasm, were regarded as potential indicators of cytotoxicity.

MTT-based Cell Viability Assessment Method:

The MTT reagent (Sigma, M-5655) weighing fifteen milligrams was solubilized in 3 milliliter of PBS and exposed to filter sterilization for complete sterilization. Following a 24-hour incubation period, the substances in the wells were aspirated, and 30 µl of the reconstituted MTT mixture was utilized uniformly added to both test and wells for cell control. Subsequently, the dish was gently agitated and then placed in a 5% CO2 incubator with controlled humidity at 37ºC for a period of 4 hours. Following the designated after the incubation duration, the supernatant was carefully aspirated, and 100 µl of the Solvent for MTT Dissolution, composed of Dimethyl sulfoxide (DMSO) introduced. The wells underwent gentle mixing through pipetting up and down to facilitate the solubilization of formazan crystals. Subsequently, absorbance measurements were determined using a microplate reader at a frequency of 540 nm, as outlined by Mazumder, K*et al*.,2020.

The calculation of growth suppression percentage was calculated utilizing the formula:

$$\% \text{ Of viability} = \frac{\text{Average Optical Density (OD) of Samples x } 100}{\text{Average Optical Density (OD) of the control cohort}}$$

3. Results and Discussions

Perpetual herbage, concerning 2feet-4feet elevated, the stalk herbaceous, elliptical, bifurcate, upright, much subdivided, frequently reddish, with engorged convergence, pubescent. Leaves paradoxical, simple, leaf edge elliptical to oviform-trilateral, cavernous green, concerning 10.9–6.9 x 5.1–2.8 cm, the prong aciculated, the fringe whole, the brinks complete, the bottom cordate, couple the veneer smooths; petioles concerning 3.4–1.3 cm protracted and 0.3- 0.4mm broad, juvenile, exstipulate. Blossoming terminal, cymose, simple-corymb, concerning 5.5–3.5 cm, tenacious, ebracteate, bracteolate, pedunculate. Flowers funnel-sculpt, yellow, purple, white, or multi-colored and orifice in the late afternoon, regarding every one flower, understand a receptacle, integrate, actinomorphic, pentamerous, hypogynous. Perianth tepal (5) sympetalous, funnel-shaped, abundant coloured Stamen 5, habitually exercised, sis analogous throughout the ovary, tendril disparate, fibrous, dithecous, protective fistulae. Ovary 1-celled, singlecarpellary, one ovule, rudimental placentation, supercilious, styles filariform, exercised, stigma capitellate regard with stipitate papillae, a melliferous circle adjoining the ovary. Fruit bulbous, with reference black, corrugated, tenacious perianth. Figure 1 *Mirabilis jalapa* morphological characters, plant Seed-episperm defender to the pericarp, regarding 0.6 – 0.5 cm, embryo recurved. Blossoming and fruiting time – August to December.

Table 1
Mirabilis jalapa absorbance by UV Visible Spectrophotometer Statistical data representation of Standard and Sample.

Absorbance	Mean Value	Correlation value(n = 10)	Regression equations
Standard (X)	0.787	0.999	X = 1.552 Y + 1.122
Sample (Y)	0.725		Y = 0.921 X − 0.007
*1% level of significance			

In the current work, the calibre and samples are recognized by UV-Visible Spectrophotometer. Based on the wavelength of calibres and samples immersion differentiated, naturalistically represented and statistical illustration of correlation by S.P.S.S. Software and Regression was analyzed. *Mirabilis jalapa* absorbance by UV Visible Spectrophotometer Statistical data representation of Standard and Sample mentioned in Table 1 and Fig. 2.

HPLC Analysis Conditions Mirabilis jalapa flower pigment

The outcome of the investigation authorized the recognition of betalain in the trial of *Mirabilis jalapa* herb. The HPLC chromatogram of *Mirabilis jalapa* flowers is specified in Figs. 3 and 4. Nine phenolic composites were detected in *Mirabilis jalapa*: hydroxycinnamic acids, flavonoids, isoflavonoids and coumarins. The quantifiable gratified of betalain composites as determined by the HPLC technique in *Mirabilis jalapa* raw substance is dispensed in Fig. 4. The content of colourant composite was present in *Mirabilis* jalapa flowers, as much as 262.55039 pp. The acquired sequel will be convenient in the enlargement of standard control systems for *Mirabilis* jalapa herb and concoct of natural food dye devising on its basis. The outcome of the current survey expressed that the aqueous extract of *Mirabilis jalapa* flowers evinced significant antimicrobial activity in the case of gram-positive, gram-negative, and fungal species. The review also outlined HPTLC observation of betalain from flower extract. The overall examination, thus, accentuates the ability of *Mirabilis jalapa* as an imperishable origin of a novel broad spectrum of natural food dye effects. This may be because of the existence of dynamic integrant betalain.

Table 2
Phytoconstituents Analysis of *Mirabilis jalapa*

Phytochemicals	***S1***
Protein	***+***
Phenol	***-***
Alkaloids	**+**
carbohydrate	**+**
Tannin	**+**
Terpenoids	**+**
Glycosides	**+**
Flavonoid	**+**
Steroid	
Saponin	**+**

Prefatory phytochemical conceals divulged the appearance of flavonoids, phenols, tannins and reducing sugars in the studied test flower, represented in Table 2 manifesting its collaboration in the medicinal sector. The anti-bacterial identity of the test flower, will be committed by the antimicrobial analysis. Screening and actual gauging of the test flowers could provide an attainable substitute that may be coupled with feasible and environmentally sustainable.

Antioxidant activity (DPPH assay: free radical scavenging activity

Table 3
Determination of free radical scavenging activity of *Mirabilis jalapa* pigments using DPPH.

Sl.no	*Sample concentration in mg/ml*	*Percentage Of inhibition standard*	*Percentage Of inhibition of sample (aqueous extract)*
1	*0.20*	*44.01*	*43.95*
2	*0.40*	*73.38*	*71.59*
3	0.60	89.23	87.03
4	0.80	93.79	95.86
5	1.00	95.75	94.94

Table 4: Determination of antioxidant activity by Folin-Ciocalteu Method

Sl.no	The volume of standard solution (mg/ml)	OD at 680 nm standard solution(mg/ml)	OD at 680 nm of sample solution(mg/ml) (aqueous extract)
1	*0.20*	*0.68*	*0.56*
2	*0.40*	*0.85*	*0.72*
3	0.60	0.97	0.76
4	0.80	1.02	0.87
5	1.00	1.43	0.92

The decline in saturation of DPPH radical was created by antioxidants, on account of the advance of the reaction uniting antioxidant fragment and radical, which affects the scavenging of the radical by hydrogen contribution data mentioned in figure-5 and 6. The current outcomes propose that the trailed plant extracts have modest to vigorous antioxidant pursuits elevated correlation was indicated between pigment attentiveness and antioxidant dimensions. It was perceived that flower stains with multistorey antioxidant possessions move to be an incandescently tinged dye, data is represented in Table 3 and table 4. The *Mirabilis jalapa* manifested brisk scavenging pursuit than the other flowers like *Clitorea, Rosa indica* and *Hibiscus rosa Sinensis* (Vankar and Srivastava, 2010). It has been implacable that the antioxidant activity of plant by-products is mostly because of the radical scavenging effect of phenolic compounds such as flavonoids, polyphenols and tannins (Srivastava et. al., 2007). The antioxidant effect of phenolic composites is mostly due to oxidation-reduction possessions, which can take part in a salient part in gripping and counterbalancing free radicals, decreasing singlet and triplet oxygen, or deteriorating peroxides. For flavonoids, the prime strand dictates radical-scavenging potentialities. The ortho-dihydroxy structure on the B ring, which has the most electron contributing possessions and confers higher firmness to the radical form and plays a part in electron delocalization. The 2, 3-double bond with a 4-oxo function in the C ring, which is accountable for electron outsourcing from the B ring.

The 3-glycosylation decreases their effect when collated with corresponding aglycones. Antioxidant pursuit of *Mirabilis jalapa* flower by Folin - Ciocalteu method was delineated in Fig. 10 and Table 6.

Iron Nanoparticles: Unveiling Nature's Biogenic Synthesis Pathways

Bio-organized Iron nanoparticles from the flower of *Mirabilis jalapa* plant. The stain (1/100 dilutions) was put on to 1mM Ferric sulphate mix and stayed to respond to occurred A colour switch was discerned from dark red to pale green. This happened as an outcome of the depletion of iron ions exist in the suspension. The Ultra Violet Absorption profile active of green delivery of nanoparticles of silver procedure are given out in Fig. 7 and Table 5.

Table 5
UV Absorption spectrum of Iron nanoparticles formed form *Mirabilis jalapa flower* pigment during different time of incubation.

Time	***380nm***	***430nm***	***560nm***	***680nm***
30[1]	***1.219***	***1.210***	***0.959***	***0.740***
60[1]	***1.273***	***1.281***	***0.964***	***0.773***
90[1]	**1.311**	**1.320**	**0.983**	**0.789**
120[1]	**1.482**	**1.372**	**0.989**	**0.843**
150[1]	**1.551**	**1.455**	**0.993**	**0.886**
Blank	**0.000**	**0.000**	**0.000**	**0.000**

Characterization of Nanoparticles

UV-visible spectroscopy

UV –Vis Spectroscopy the periodic scans of the ocular retention between 380 and 500nm with a UV- Vis spectrophotometer (Model 118, Systronics, Mumbai, India) at an intension of 1 nm were performed to perused the depletion rate of Silver, Gold, copper and iron ions by *Mirabilis flower* pigment extract. The riposte mixture was adulterated 20 times and worned for UV-Vis spectrophotometry. Deionized water was cast-off to calibrate the baseline. The reduction of Fe^{2+} was observed intermittently by using a UV-Vis The spectrophotometer was employed to assess the UV-Vis of nanoparticles of silver reaction solution within the range of 380–680 nm. The alteration in color, transitioning from red to pink, brick red, blue, and green, resulted from the lowering of aurum, cuprum, and iron found in the solution, respectively. The control treatment, devoid of argentum, aurum, cuprum and iron, exhibited no color alteration when subjected to identical incubation conditions. The synthesized silver nanoparticles underwent characterization through UV-VIS spectrometry. The *Mirabilis jalapa* flower exhibited a maximum peak for silver nanoparticles at 430nm. Over time, the strength of the peak at 380nm increased until the reduction

process was completed. Biosynthesized gold nanoparticles the topmost peak was determined to be 380nm for *Mirabilis jalapa* flower. The potency of the peak at 380nm was expanded with time until the reduction was accomplished. Bio-delivered copper nanoparticles the highest peak was established to be 380nm for *Mirabilis jalapa* flower. The vigour of the peak at 380nm showed an upward trend over time until the reduction concluded. Bio-produced Iron nanoparticles the largest peak was reported to be 680nm for *Mirabilis jalapa* flower. The peak strength at 680nm was expanded with time until the reduction was finalized.

SEM-XRD Analysis

The SEM-XRD analysis proved the effective formation of iron nanoparticles in all the samples. The SEM imaging system with various resolutions of green cellular delivery of iron nanoparticles was shown in Figs. 8, 9 proportionately.

The Diffracted peaks' broadening might encompass statistics about the sample nanostructure; it can as well own to enumerate unit cell dimensions. The conversion comprises Bragg’s law:

n 2*d* sin

dhkl n /2 sin

In which the h, k, and l account for Miller indices for the peak which assists us to perceive the diffraction planes of one and all nanoparticles. The Silver nanoparticles have FCC lattice which reveals a peak at 2 = 27.075^0 in the XRD scanning. The Gold nanoparticles have FCC lattice which exhibits an XRD peak at 2 = 27.091^0. The Copper nanoparticles as well have an FCC lattice which manifests the XRD peak at 2 = 32.327^0.. .. The Iron nanoparticle has a BCC lattice with an XRD peak at 2 = 23.1930.

Table 6 Zone of inhibition against various bacteria (*Escherichia coli, Salmonella typhi, Staphylococcus species, Klebsiella species, Bacillus species,)* using Iron nanoparticles produced by *Mirabilis jalapa* flower pigment 1/100 dilution.

Microorganisms	***Control***	***Sample***	***Measure of zone of inhibition in mm***				
			10µl	***20µl***	***30µl***	***40µl***	***50µl***
Escherichia coli	***20***	***21***	***23***	***27***	***28***	***29***	***33***
Salmonella typhi	**19**	**18**	**24**	**26**	**28**	**29**	**35**
Bacillus species	**19**	**18**	**25**	**26**	**28**	**31**	**32**
Staphylococcus aureus	**21**	**20**	**21**	**24**	**26**	**28**	**31**
Klebsiella species	**18**	**19**	**22**	**26**	**28**	**30**	**34**

Iron nanoparticles from *Mirabilis jalapa* samples of 1/100 dilution reported a zone of inhibition ranging between 30 to 33mm in size against *Escherichia coli*, 30-35mm in size against *Salmonella typhi, 32-33mm in size against Bacillus species*, 31-36mm in size against *Staphylococcus aureus, 31*-35mm in size against *Klebsiella species.* The computation of five discrete volumes of nanoparticles solution in the disc of the agar plate is assisted to recognize the disparity in the dimensions of the zone of inhibition. The disk containing 50 µl of *Mirabilis jalapa* flower extract exhibited a greater inhibitory impact on bacteria mentioned in Fig. 9 and Table 6.

In vitro assessment of cytotoxic effects against L929 (Fibroblast) cells was determined using the MTT assay.

Figure 10

Evaluating in vitro cytotoxic effects through the MTT assay on L929 (Fibroblast) cells (a) untreated control (b) 6. 25 µg/ml (c) 12.5 µg/ml (d) 25 µg/ml (e) 50 µg/ml and (f) 100 µg/ml.

Table 7

Investigation of in vitro cytotoxicity using the MTT assay against L929 (Fibroblast) cells.

Sample Concentration (µg/mL)	OD value I	OD value II	OD value III	Average OD	Percentage Viability
Control	1.1264	1.1206	1.1284	1.1251	100.00
6.25	1.0985	1.0932	1.0983	1.0967	97.47
12.5	1.0536	1.0484	1.0465	1.0495	93.28
25	0.9954	0.9929	0.9832	0.9905	88.04
50	0.8314	0.8293	0.8115	0.8241	73.24
100	0.7747	0.7744	0.7631	0.7707	68.50

Cytotoxicity Evaluation:

The data received from the MTT assay is analyzed to evaluate the cytotoxicity of the *Mirabilis jalapa* sample. Initially, the blank control absorbance value is deducted from all other data points to establish a baseline.

Subsequent to this, the THF solvent control value is standardized to 100% viability, given the absence of any growth-inhibiting compound in this control scenario.

Subsequent to the initial absorbance adjustments, all data values are converted to their respective percentages of viability relative to the control. Following a 24-hour period, the growth medium is aspirated, and newly prepared compounds in DMEM undergo a five-fold serial dilution by two-fold steps

(100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of DMEM). Subsequently, 100µl from each concentration is added in triplicate to the designated wells, and the plates are then incubated at 37ºC in a humidified 5% CO2 incubator. Non-treated control cells were also maintained. *Invitro* cytotoxic effect against L929 (Fibroblast) cells by MTT Assay was depicted in Fig. 48 and table 18 of untreated control,6. 25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml subsequently. 12.5 µg/ml exhibited the highest 97.47 percentage viability and 100 µg/ml revealed the least 68.50 percentage viability.

Cytotoxicity Assay by Direct Microscopic observation

Following a 24-hour treatment period, a thorough examination of the entire plate was conducted using an inverted phase-contrast tissue culture microscope, specifically the Olympus CKX41 equipped with an Optika Pro5 CCD camera. Microscopic investigations were meticulously documented through image recording. Any observable alterations in cell morphology, such as cell rounding, shrinkage, or the presence of granulation and vacuolization in the cytoplasm, were regarded as indicative of cytotoxicity. The findings of this assessment are visually represented in Fig. 10,11 and table 7, illustrating the impact of *Mirabilis jalapa* exposure on the cytotoxicity of L929 (Fibroblast) cells.

4. Conclusions

The growing inclination among consumers for safer and healthier choices food options has driven the increasing desire for organic food colorants. *Mirabilis jalapa*, commonly known as the four o'clock flower, possesses vibrant and strikingly appealing pigments in its flowers. This comprehensive research review is focused on investigating the viability of *Mirabilis jalapa* flower extract as a natural food colorant, assessing its color stability, safety, and suitability for integration into diverse food systems. Recent investigations also delve into the therapeutic potential of *Mirabilis jalapa* flower extract, building upon its traditional use in numerous cultures for its alleged therapeutic properties. This analysis will explore the bioactive constituent present in the flower extract and evaluate their pharmacological pursuit, including antioxidant, antimicrobial, and anticancer effects. Furthermore, the research will delve into the cardinal mechanisms of action, safety profile, and prospective applications of *Mirabilis jalapa* flower extract in the field of medicine. This research study, to scrutinized the effect of different sucrose standard concentrations on the viability of *Mirabilis jalapa* protoplasts to optimize the conditions for their successful culture and manipulation. Efficient cellular delivery of bioactive composite to target cells is a pivotal challenge in modern medicine. Nanoparticle-based delivery systems have manifested great potential in enhancing the cellular uptake and restorative efficacy of natural products. Iron Nanoparticles have emerged as promising carriers for delivering drugs and natural compounds due to their distinctive properties. This research thesis also focuses on the green and sustainable approach of nanoparticles using *Mirabilis jalapa* flower extract and probes their potential as a cellular delivery platform for bioactive compounds. The extract successfully transmitted vibrant and visually attractive colors to a food product, nourishing its color potency during processing and employing consumer acceptability. The findings may pave the way for future research and development of nanomedicine applications, with significant imputation for targeted therapy and personalized medicine.

Declarations

CRediT authorship contribution statement Rathi C R: Methodology, Validation, Investigation, Writing - Original Draft, Visualization. Sajeshkumar N K: Methodology, Investigation. Ali Ubeyitogullari: Conceptualization, Methodology, Validation, Visualization, Writing - Review & Editing, Supervision, Project administration, Funding acquisition, Resources. Prem Jose Vazhacharickal: Methodology, Validation, Visualization, Writing - Review & Editing, Supervision. Jiby John Mathew: Conceptualization, Methodology, Validation, Writing - Review & Editing.

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Figures

Figure 1

Mirabilis jalapa plant (a-arrangements of leaves and flower, b- arrangements of leaves and buds, c- tubers-buds and flower stages, e- L.S of flower, f- T.S of the ovary, g-Fruit buds, h- mature fruits, i- seeds).

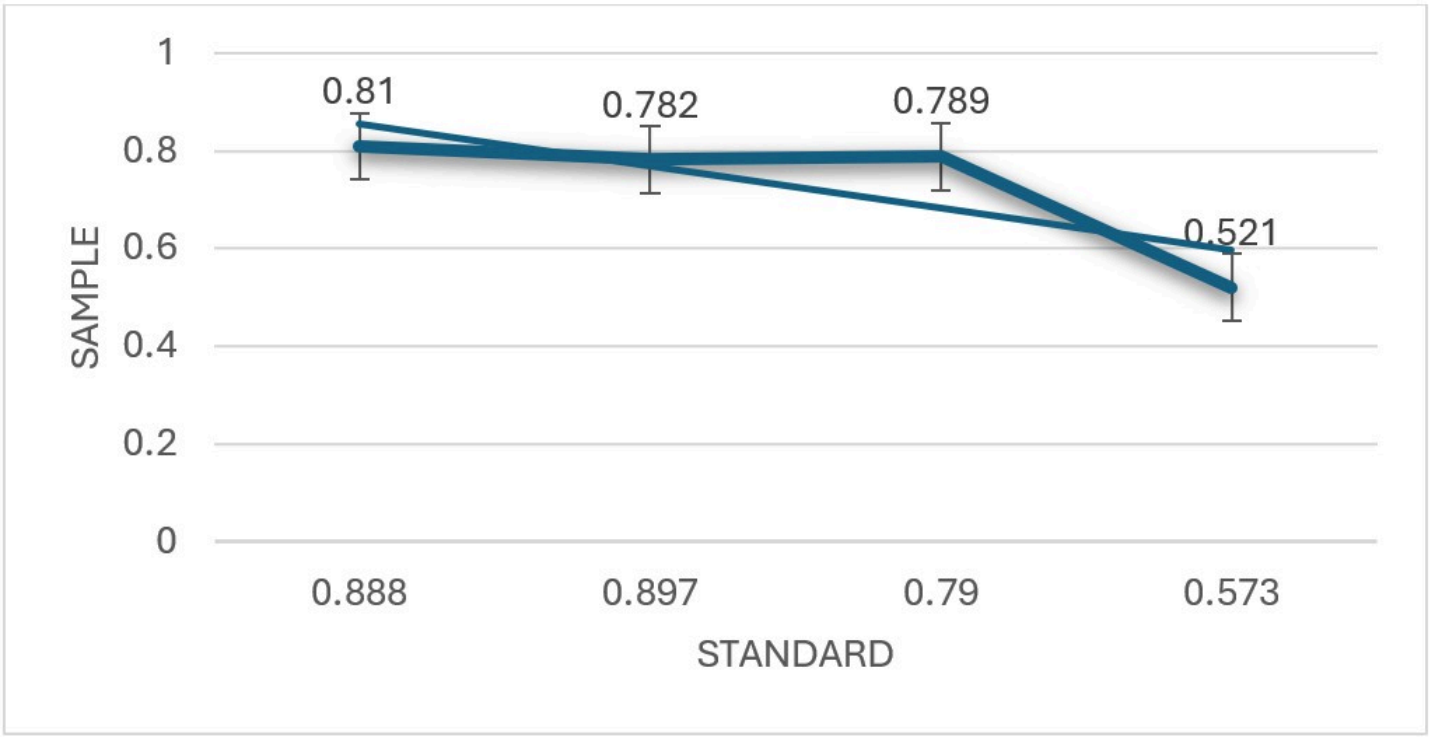


Figure 2

Graphical representation of the correlation between standard and sample

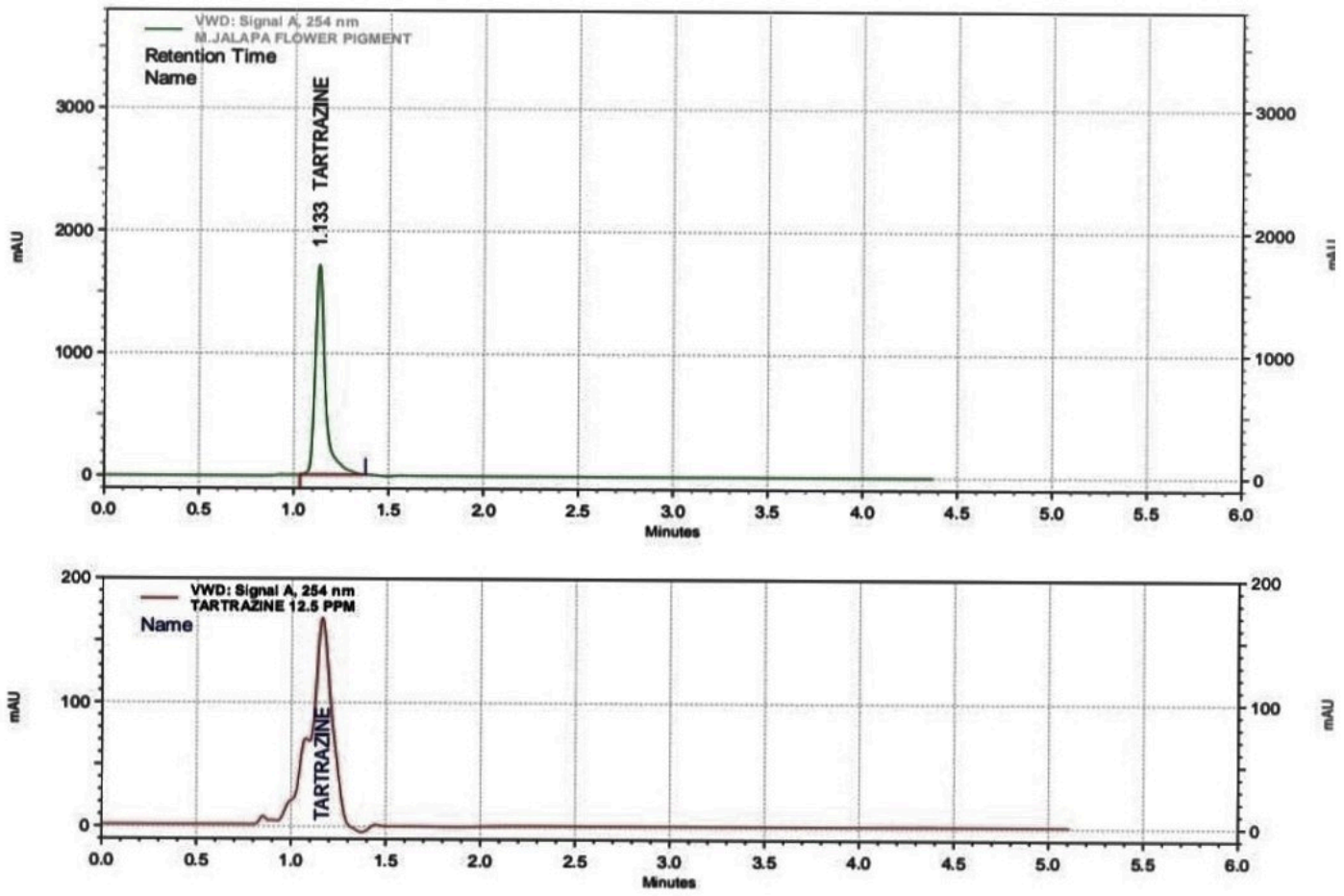
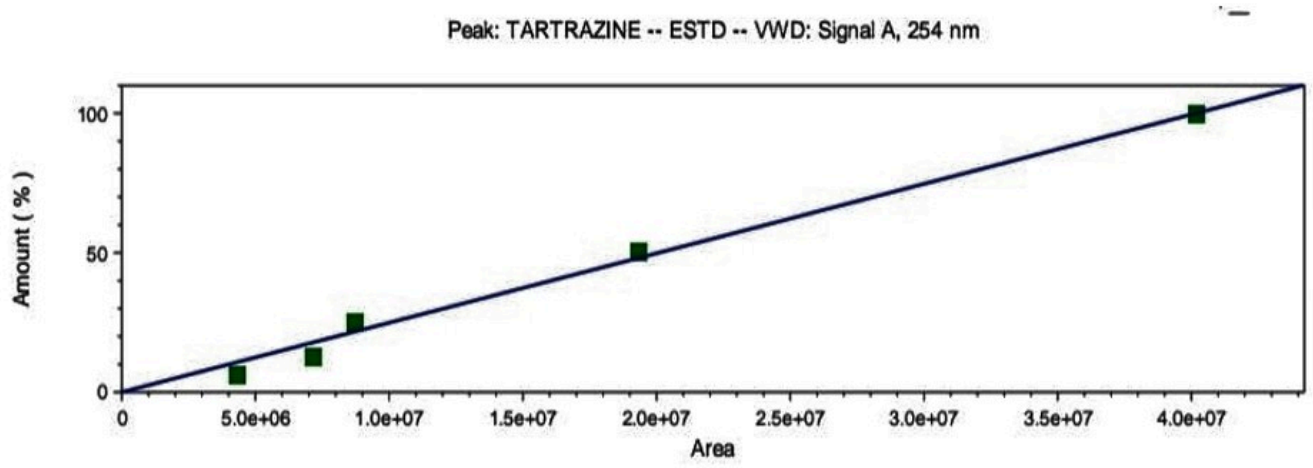


Figure 3

Area % Report of *Mirabilis jalapa* flower pigment HPLC conditions.



VWD: Signal A, 254 nm Results

Name	Retention Time	Area	Area %	Height	CONC. (PPM)
TARTRAZINE	1.133	105403335	100.00	28674520	**262.55039**
Totals		105403335	100.00	28674520	

Figure 4

Retention time and concentration of *Mirabilis jalapa* flower pigment HPLC conditions.

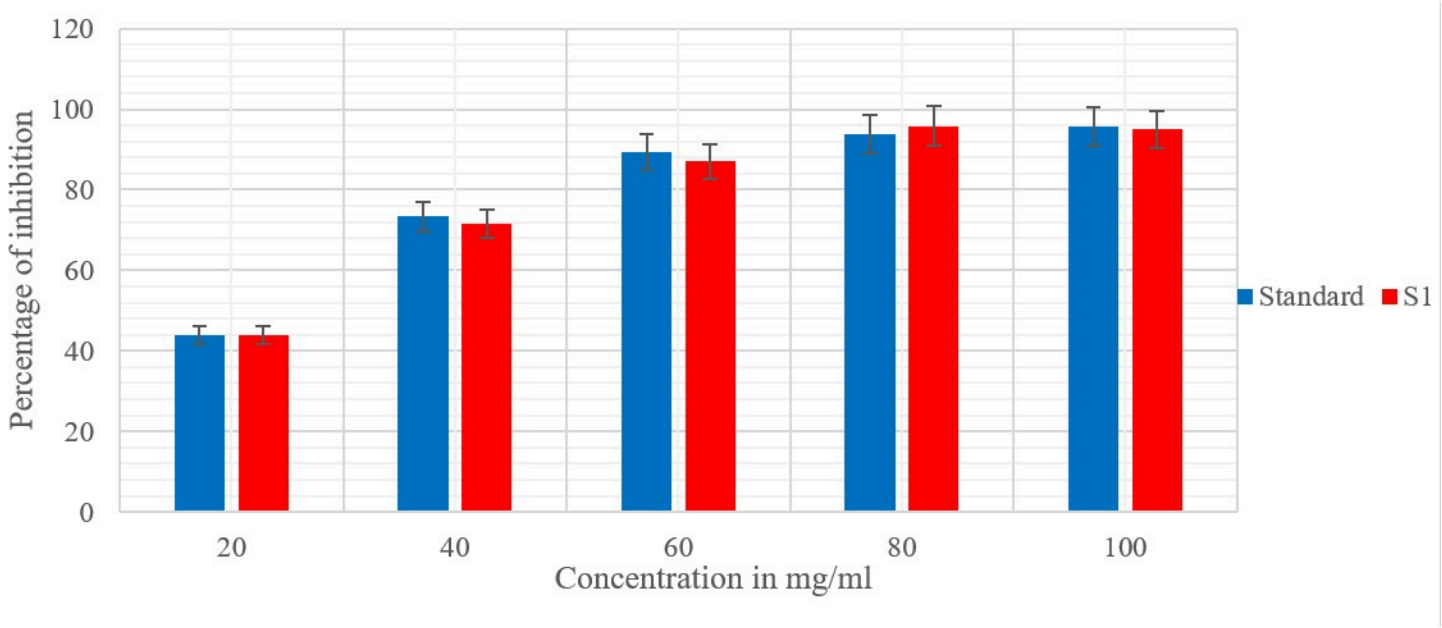


Figure 5

Determination of free radical scavenging activity of *Mirabilis jalapa* pigments using DPPH.

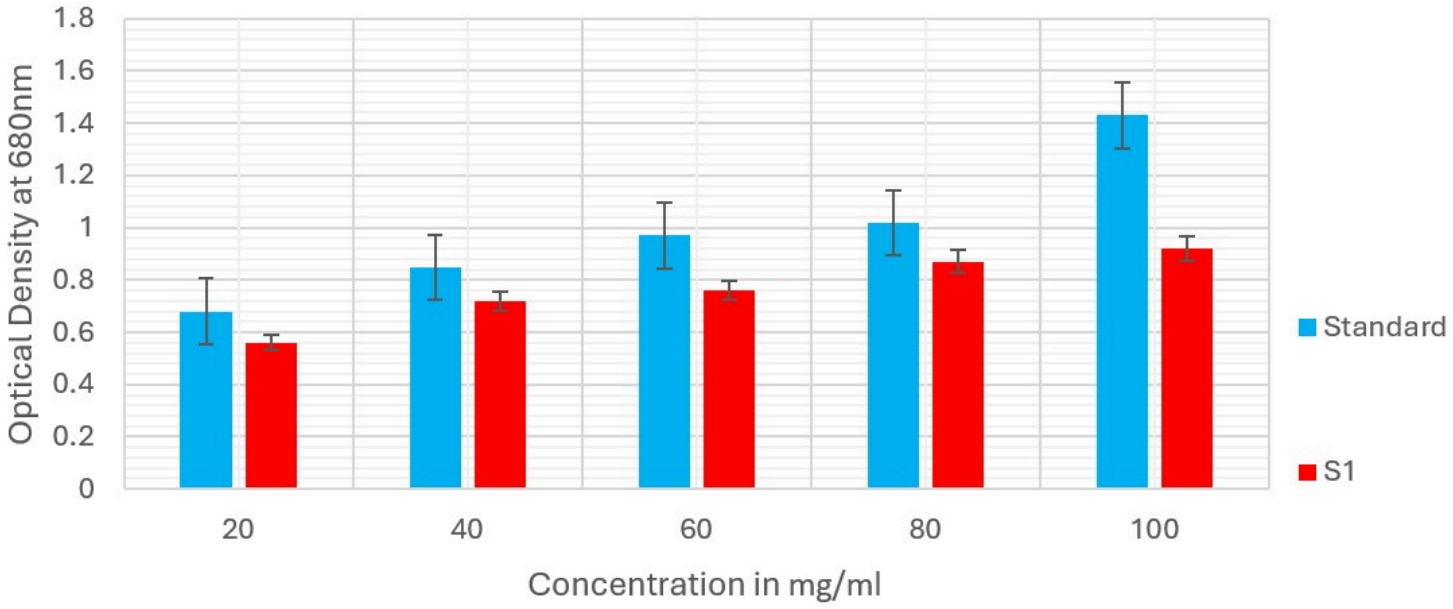


Figure 6

Antioxidant activity of *Mirabilis jalapa* flower by Folin - Ciocalteu Method

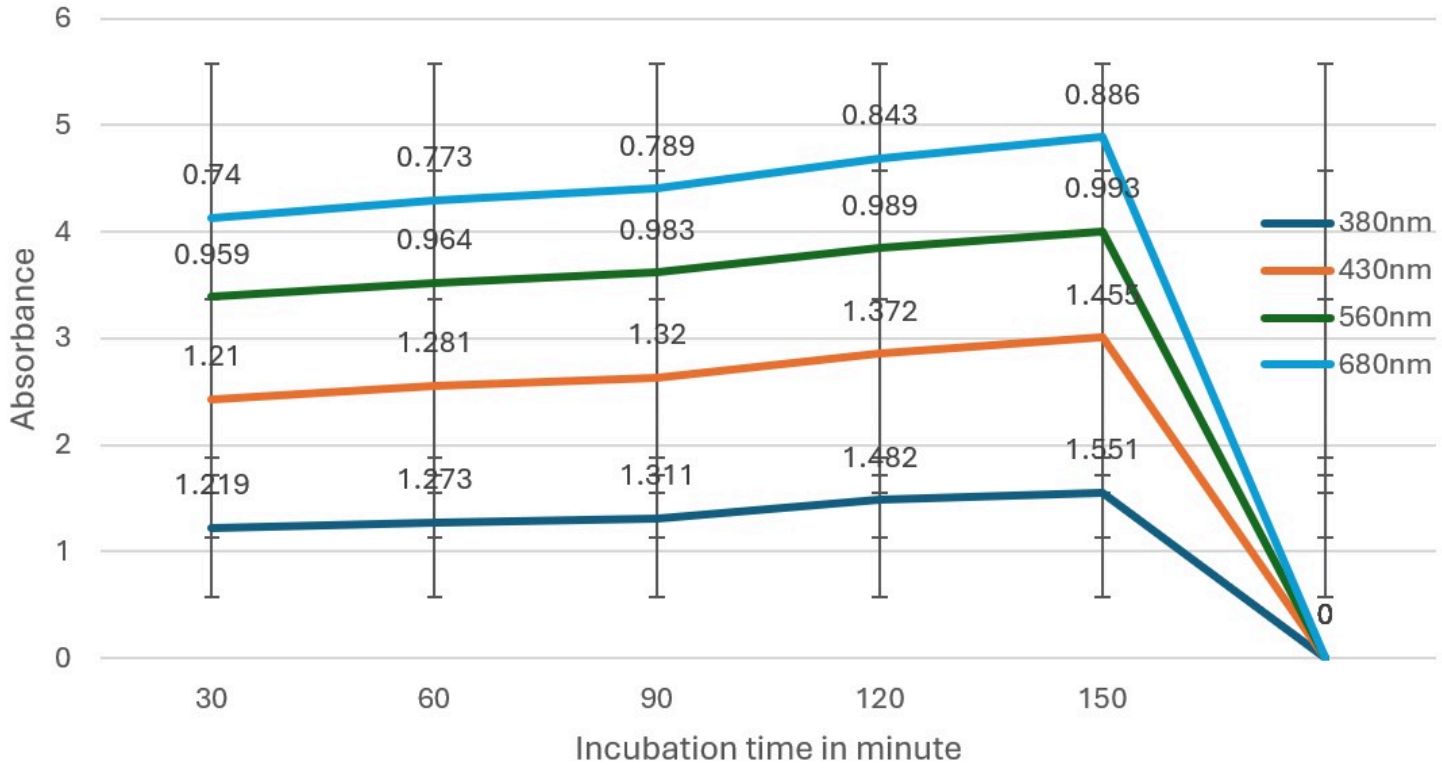


Figure 7

UV Absorption spectrum of Iron nanoparticles formed form *Mirabilis jalapa flower* pigment during different time of incubation.

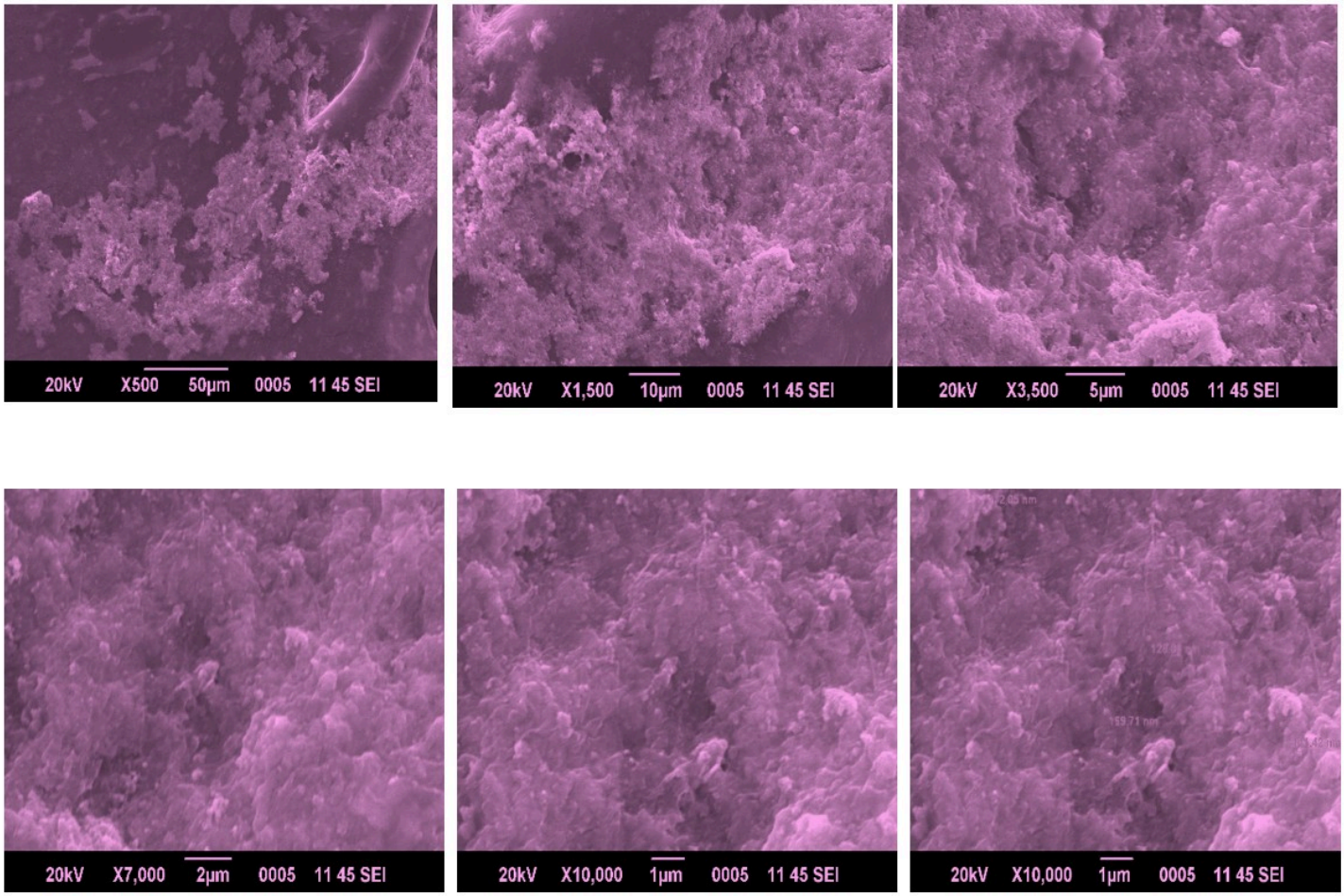


Figure 8

Iron nanoparticles formation of *Mirabilis jalapa* flower extract under SEM imaging system with various resolutions.

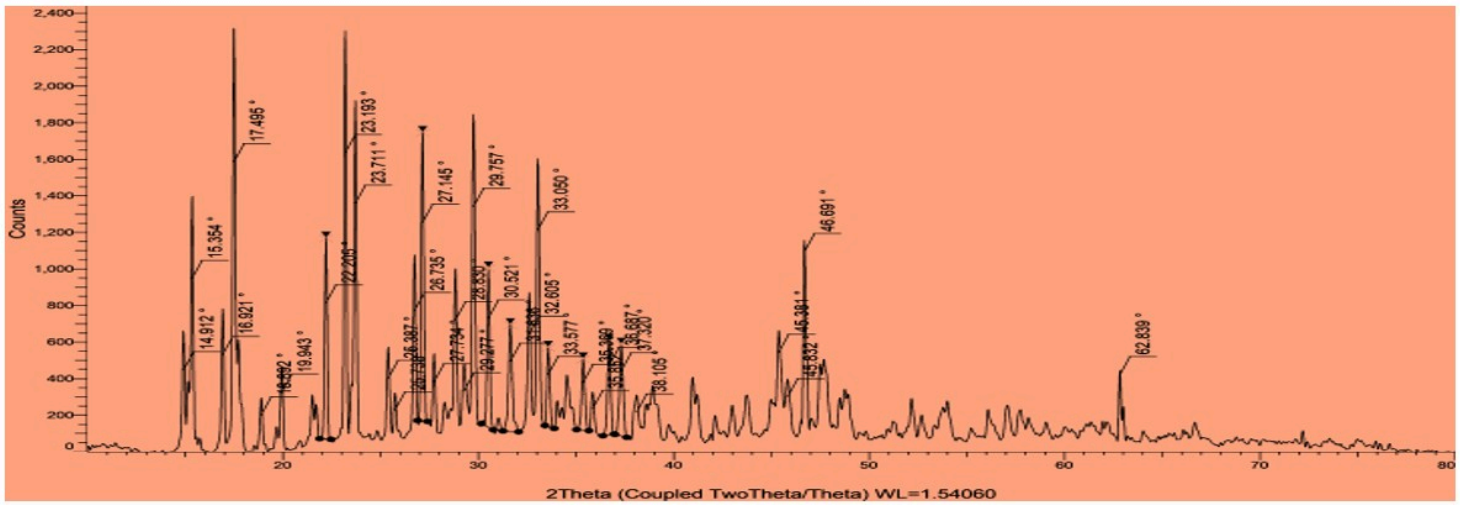


Figure 9

X-Ray Diffractogram Fe (Coupled TwoTheta/Theta) **Peak List #1-Angle Net Intensity d Value Gross Intensity Rel. Intensity-**62.839 ° 422.530 1.47766 Å 555.969 25.8%, 46.691 ° 1094.94 1.94385 Å 1228.38 66.9%, 45.832 ° 301.644 1.97827 Å 435.084 18.4%, 45.381 ° 543.942 1.99685 Å 677.383 33.2%, 38.105 ° 217.531 2.35975 Å 350.972 13.3%, 37.320 ° 443.738 2.40753 Å 577.180 27.1%, 36.687 ° 471.929 2.44766 Å 605.370 28.8%, 35.852 ° 236.607 2.50271 Å 370.049 14.5%, 35.369 ° 380.432 2.53576 Å 513.873 23.2%, 33.577 ° 423.331 2.66690 Å 556.773 25.9%, 33.050 ° 1214.12 2.70815 Å 1347.56 74.2%, 32.605 ° 643.747 2.74411 Å 777.190 39.3%, 31.636 ° 495.197 2.82594 Å 628.639 30.3%, 30.521 ° 732.043. .2.92662 Å 865.485 44.7%, 29.757 ° 1342.03 2.99998 Å 1475.48 82.0% **. Area List #1 - Scan Int. Right Int. Obs. Max d (Obs. Max) Gross Int. Net Height -** 200702A-03(Fe).raw (Smooth) #1 21.883 ° 22.455 ° 1.22903 1.15391 22.205 ° 4.00028 Å 22.5667 19.0631, 200702A-03(Fe).raw (Smooth) #1 26.914 ° 27.405 ° 2.95402 2.86536 27.145 ° 3.28241 Å 32.6071 27.3781, 200702A-03(Fe).raw (Smooth) #1 31.270 ° 32.068 ° 1.95107 1.87352 31.638 ° 2.82578 Å 14.3399 10.1078, 200702A-03(Fe).raw (Smooth) #1 36.383 ° 36.956 ° 1.49922 1.67353 36.686 ° 2.44766 Å 12.8727 8.96453, 200702A-03(Fe).raw (Smooth) #1 36.997 ° 37.610 ° 1.62038 1.35821 37.320 ° 2.40755 Å 12.4773 8.67834, 200702A-03(Fe).raw (Smooth) #1 30.166 ° 30.779 ° 2.65633 2.07618 30.520 ° 2.92663 Å 19.7267 15.0890, 200702A-03(Fe).raw (Smooth) #1 33.418 ° 33.888 ° 2.48493 2.19154 33.578 ° 2.66680 Å 12.2028 7.50120, 200702A-03(Fe).raw (Smooth) #1 35.033 ° 35.667 ° 2.09074 1.99573 35.370 ° 2.53570 Å 11.0917 6.73469 **- FWHM Chord Mid. d (Chord Mid.) I. Breadth Gravity C. d (Gravity C.) Raw Area Net Area C. Size K Instr. Width -** 0.150 22.204 ° 4.00037 Å 0.158 22.201 ° 4.00087 Å 5.0251 3.0161 600.1 Å 0.890 0.050, 0.156 27.146 ° 3.28232 Å 0.162 27.148 ° 3.28210 Å 6.9928 4.4274 582.8 Å 0.890 0.050, 0.200 31.640 ° 2.82557 Å 0.230 31.647 ° 2.82499 Å 5.6934 2.3203 459.8 Å 0.890 0.050, 0.181 36.690 ° 2.44744 Å 0.195 36.689 ° 2.44748 Å 3.9824 1.7473 512.7 Å 0.890 0.050, 0.201 37.326 ° 2.40721 Å 0.223 37.322 ° 2.40745 Å 4.2709 1.9357 464.5 Å 0.890 0.050, 0.161 30.522 ° 2.92651 Å 0.172 30.520 ° 2.92666 Å 5.4723 2.5990 570.0 Å 0.890 0.050, 0.167 33.583 ° 2.66643 Å 0.174 33.593 ° 2.66561 Å 3.4947 1.3050 550.5 Å 0.890 0.050, 0.179 35.372 ° 2.53555 Å 0.197

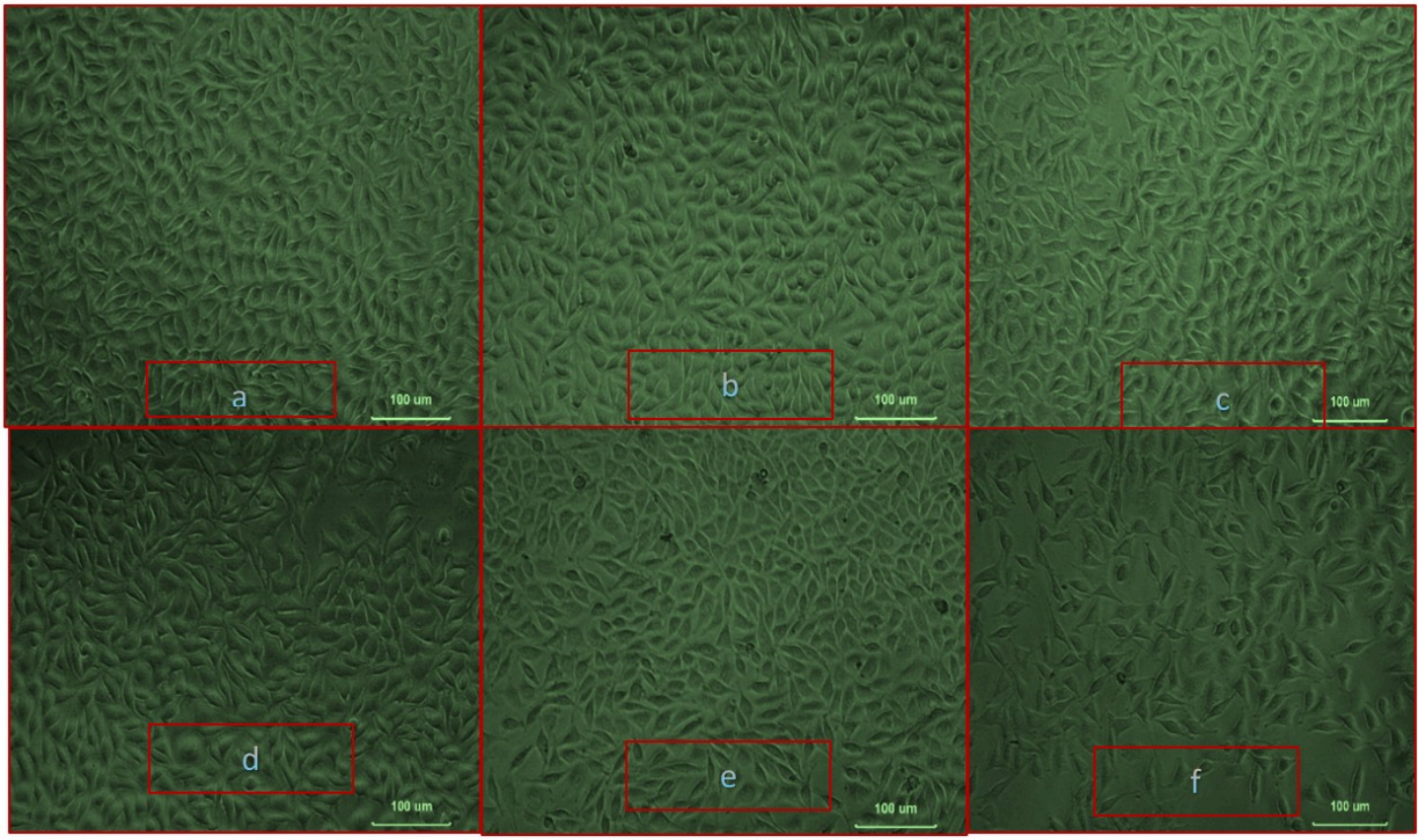


Figure 10

Evaluating in vitro cytotoxic effects through the MTT assay on L929 (Fibroblast) cells (a) untreated control (b) 6. 25 µg/ml (c) 12.5 µg/ml (d) 25 µg/ml (e) 50 µg/ml and (f) 100 µg/ml.

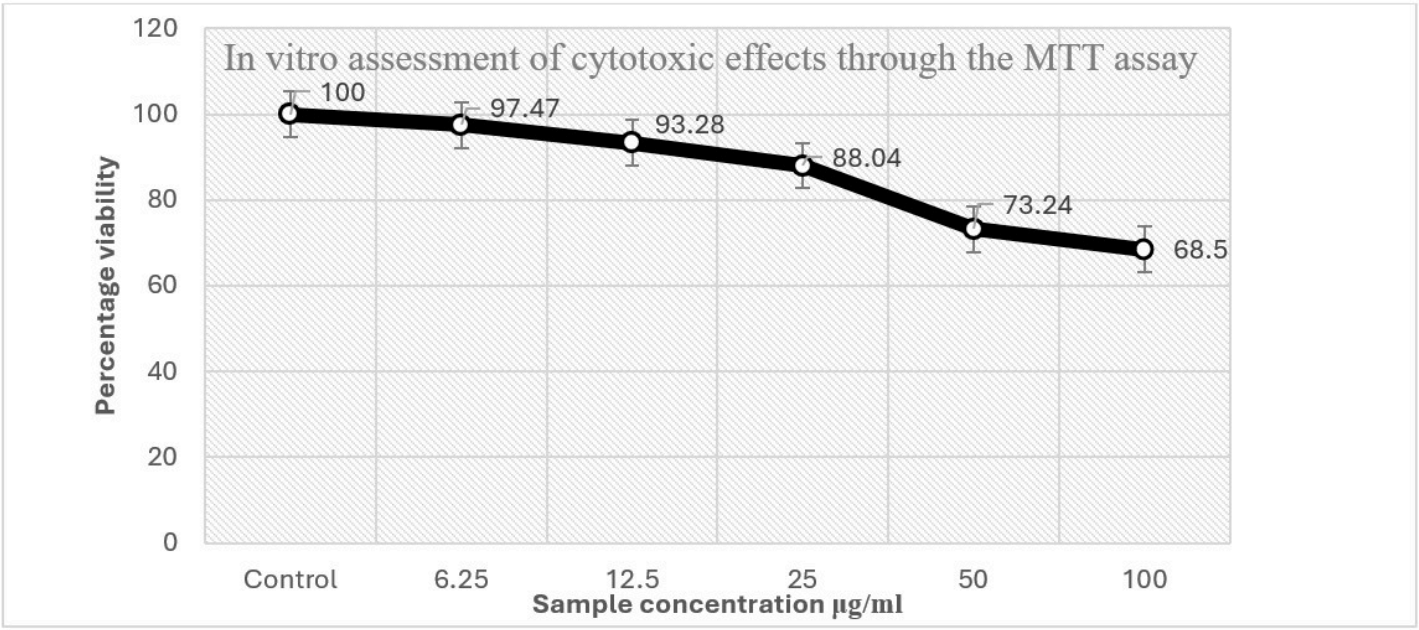


Figure 11

Creating a visual representation to showcase the evaluation of cytotoxicity in L929 (Fibroblast) cells resulting from exposure to *Mirabilis jalapa*, offers a comprehensive insight into the impact of *Mirabilis jalapa* on cellular viability.