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Chromatographic and Spectroscopic Analysis of Flower Pigments in *Mirabilis Jalapa*

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ABSTRACT

The growing popularity of natural colorants derived from plant-based sources can be attributed to customer's increased health awareness. Pigment extraction is one of the most crucial procedures among the several involved in creating natural colorants. Because natural pigments are good for the environment, they are interesting. The present studies have been conducted on chromatographic and spectroscopic analysis of *Mirabilis jalapa* flower pigment. Distilled water is used as a solvent to extract pigment, producing the maximum results. Analytical protocols like Thin Layer Chromatography (TLC), and UV-visible spectroscopy were carried out. The pigments from floral extracts showed widespread absorption peaks in the visible spectrum, according to UV-visible experiments. Spectral patterns were used to analyze the pigment's color stability. The pharmacological effects of *Mirabilis jalapa* flower pigments are enlisted and described.

INTRODUCTION

Mirabilis jalapa, also known as the "four o'clock flower," is rich in pigments like carotenoids and anthocyanins, attracting research due to its unique color variations. It is a Nyctaginaceae species with a unique flowering pattern, opening late afternoon and closing the next morning. It is an herbaceous perennial that grows 30 to 120 cm tall with smooth, ovate leaves and fleshy nodes on stems. Its trumpet-shaped flowers have a bright, petal-like calyx (Ali Esmail Al-Snafi et al., 2021) (Figure 1). It belongs to the Kingdom Plantae, within the clades Angiosperms and Eudicots (Naveed et al., 2010). It is classified under the order Caryophyllales, the family Nyctaginaceae, and the genus *Mirabilis*, with *Mirabilis jalapa* as the species. *Mirabilis jalapa*, also known as the "Marvel of Peru," is native to Peru, and is a decorative plant found in disturbed habitats, gardens, and waste areas. It thrives in warm temperatures, well-drained soils, and moderate rainfall, preferring sunny sites (Mabberley, 2017).

Flowers showcase nature's diverse color spectrum, while organic or inorganic pigments are essential for color display in the food, textile, cosmetic, and pharmaceutical sectors ((Saxena, 1985), (Escribano, J., Carvajal, M., & Morales, 2007). Natural-source pigments are gaining attention for potential medical benefits and eco-friendliness (Venil et al., 2013). Carotenoids, flavonoids, and anthocyanins are common natural pigments found in flowers, serving biological and aesthetic purposes. Anthocyanins, water-soluble pigments with red, blue, and purple hues, are affected by pH, temperature, light, oxygen, and sugars (Gould, 2004). Carotenoids are lipid-soluble pigments that give flowers distinctive red, orange, and yellow hues, attract pollinators, and protect against oxidative stress (Britton, 1995). The possible medical benefits of *Mirabilis jalapa* have been thoroughly investigated in recent years, with flower extracts receiving special attention. Bioactive chemicals from the flowers of *Mirabilis jalapa* are commonly extracted using water (Sinha, A., & Bhattacharyya, 2011), (Mishra, A., & Singh, 2015), (Kumar, A., & Gupta, 2013).

Aqueous extraction is more appropriate for conventional medical procedures because water is frequently utilized as a solvent. Research has indicated that aqueous extracts exhibit noteworthy biological activity as well, especially in applications related to antibacterial and anti-inflammatory properties. Studies looking into *Mirabilis jalapa's* therapeutic potential have used both extraction techniques, demonstrating the plant's utility in complementary and alternative medical practices (Khan, M. I., & Khan, 2016), (Choudhary, M. I., & Memon, 2010). In the present research work, aqueous methods are used for the extraction of flower pigments. Chromatographic & spectroscopic analysis of *Mirabilis jalapa* flower pigment was done (Patil et al., 2022).

Figure 1: Flower of *Mirabilis jalapa* L.

MATERIAL AND METHODS

Collection of the test flowers

Freshly bloomed *Mirabilis jalapa* flowers were collected from the botanical garden of Yashvantrao Chavan Institute of Science Satara and refrigerated for the entire night in clear domed trays. Following a thorough cleaning in distilled water, they were allowed to air dry in a laboratory at 25 ± 2 °C by soaking them in blotting paper towels under shade (Harborne, 1998).

Extraction of pigment

The aqueous extraction began by placing the flower petals in a mortar, to which 10–20 ml of distilled water was added. The petals were then ground thoroughly using the pestle, ensuring that the cells were broken down completely and a homogeneous mixture was formed. This step followed the protocol for aqueous extraction detailed by (Harborne, 1998). Once the petals were fully homogenized, the mixture was transferred to a clean container and incubated at room temperature for 30–60 minutes. During this incubation period, the mixture was stirred periodically to enhance the extraction of pigments. After incubation, the mixture was subjected to filtration using Whatman filter paper 110 to remove any solid residues. For further purification of the extract, centrifugation was performed at 10,000 rpm for 10 minutes, yielding a clear supernatant. (Harborne, 1998). The final extract was then carefully stored in a dark, airtight container to prevent any degradation due to light exposure. For short-term storage, the extract was kept at 4°C, while for long-term preservation, freezing was employed. This storage method was in line with the protocols recommended by (Sarker, S. D., Latif, Z., & Gray, 2006) for natural product isolation.

Preliminary phytochemical screening

Using conventional techniques, qualitative phytochemical screening was carried out to determine whether *Mirabilis jalapa* flower extracts contained bioactive chemicals. To find alkaloids, flavonoids, terpenoids, and glycosides, the following tests were performed. Dragendorff's test was performed to check the alkaloids, and a Lead Acetate test was followed for flavonoid detection. To examine terpenoids, the Salkowski test was performed and Cardiac Glycosides The Keller-Killiani test was used to detect the glycosides (Sarker, S. D., Latif, Z., & Gray, 2006).

Thin layer chromatography (TLC)

This investigation uses a glass sheet coated with silica gel for adsorption. The stationary phase is the adsorbent layer, while the mobile phase is a solvent or mixture drawn up the plate. The plates are prepared with Silica Gel G and GF in an 8:2 ratio using ethyl acetate. A small sample is placed on a plate, dipped in the appropriate solvent, and deposited in bands. The solvent dissolves the sample mixture, causing different compounds to move at different speeds. UV light is used to view the TLC plate (Silva, F. A. M., & de Almeida, 2018).

UV-Visible Spectroscopy

A UV-visible spectrophotometer was used to perform the UV-Vis spectrum characterization of the extracted pigments. The process entailed: preparing a blank using the same extraction solvent. Filling a quartz cuvette with pigment extract. Finding the pigment's absorption maxima (λmax) requires scanning the sample between 400 and 700 nm (Pérez-Gálvez, A., & Carrión, 2003).

RESULTS AND DISCUSSION

Collection of the test flowers & extraction of pigment

Aqueous extraction (Harborne, 1998) was the extraction technique used. A deep-colored solution was produced by the ethanol-based extraction, indicating that anthocyanins and other polar pigments were effectively dissolved. Even though it was gentler, the aqueous extraction produced a noticeable shade shift that suggested the presence of water-soluble pigments such as betalains and flavonoids. Clear extracts were obtained by filtering through the Whatman No. 110 filter paper, which successfully eliminated solid residues. The solution was further purified by centrifuging it for ten minutes at 10,000 rpm, which ensured that the fine particles were removed. The final extracts were suitable for spectroscopic investigation since they were stable and devoid of suspended particles.

Preliminary phytochemical screening

The initial phytochemical screening of the *M. jalapa* extract revealed the presence of flavonoids, glycosides, terpenoids, and alkaloids, which are examples of bioactive components in different concentrations across aqueous extraction (Table 1). The aqueous extract included a modest amount of alkaloids and terpenoids while it contained a moderate amount of glycosides and flavonoids (Edeoga et al., 2005).

Table 1: Phytochemical screening results of aqueous extract of flowers of *M. jalapa* varieties

Sr. No.	Sample	Alkaloids	Terpenoids	Glycosides	Flavonoids

1	Control (Solvent)	-	-	-	-
2	Aqueous extract	+++	+++	++	++

Thin layer chromatography (TLC)

Different pigment bands were successfully extracted from *Mirabilis jalapa* preparations using TLC analysis. The presence of anthocyanins, flavonoids, and carotenoids was shown by the ethanol extract's numerous bands in the red, purple, and orange areas. Lighter bands in the yellow to light purple range were produced by the aqueous extract, indicating that flavonoid-based pigments predominated. Values of the Retention Factor (Rf): Other bands corresponded to different kinds of pigments, and the observed Rf values ranged from 0.25 to 0.85. Rf values for the most noticeable bands were roughly 0.32 for the red-violet band, 0.54 for the yellow band, 0.78 for the orange band, and 0.38 for the carotenoids (Figure 2).

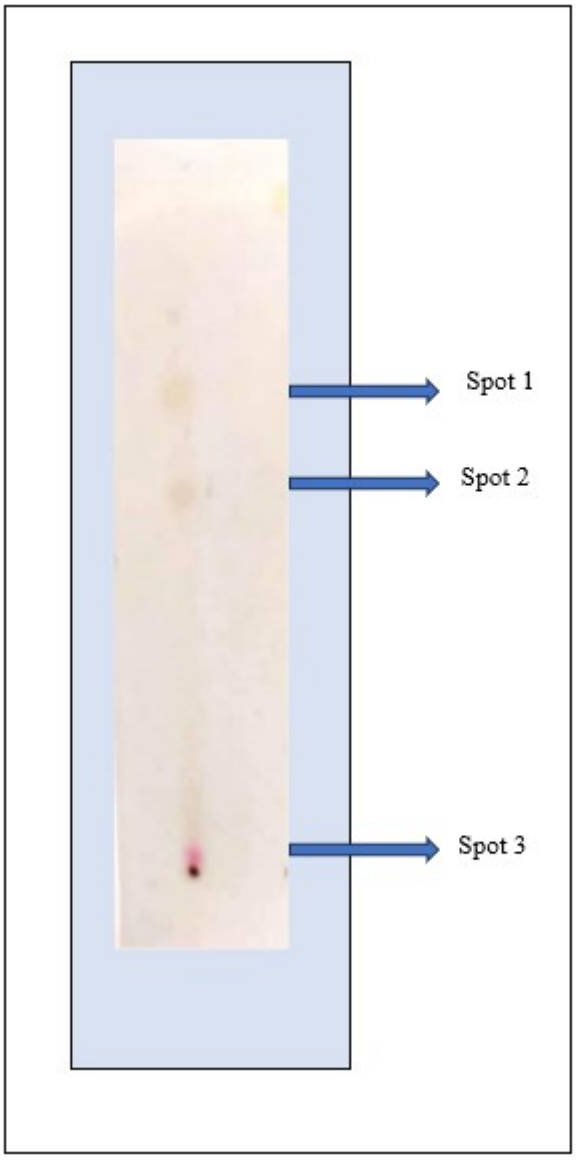

Figure 2: TLC result of *Mirabilis jalapa* L.

UV-Visible Spectroscopy

The UV-Vis absorption spectra of pigments from *Mirabilis jalapa* reveal three peaks at 479 nm, 534 nm, and 664 nm. These peaks correspond to specific pigment components in the flowers, such as betalains, betacyanins, and chlorophylls. These absorption profiles reveal a combination of betalains and other pigments, providing insight into the rich coloration of *Mirabilis jalapa* flowers (Table 2 & Figure 3) (Patil et al., 2022).

Table 2: Absorption spectra of pigments from *Mirabilis jalapa*

Peak	Wavelength	Absorbance
1	479	2.432

2	534	2.919
3	664	0.543

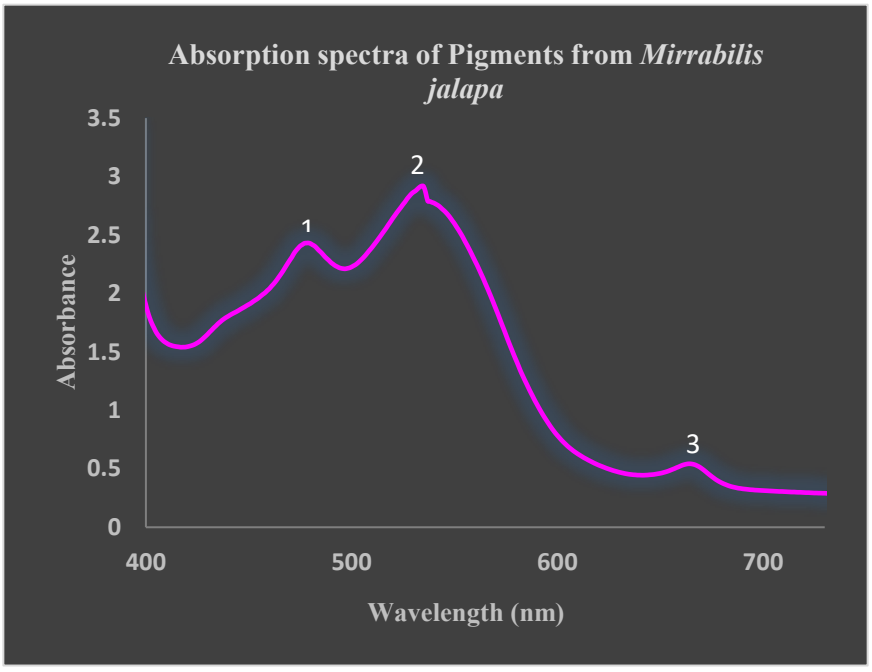

Figure 3: Absorption spectra of pigments from *Mirabilis jalapa*

Storage and stability of extracted pigment

For short-term use, the extracted pigments were kept at 4°C; for long-term preservation, freezing was used. Since there were no discernible color changes or precipitation over time, the storage conditions were successful in preventing pigment deterioration. This supports earlier research by (Sarker, S. D., Latif, Z., & Gray, 2006), which showed that appropriate storage conditions are essential to preserving pigment stability.

Pharmacological effects of *Mirabilis jalapa* flower pigment

The study explores the pharmacological effects of the *Mirabilis jalapa* flower, specifically its antimicrobial and antiparasitic properties (Plant & Linn, 2004). The leaf extract showed moderate activity against *Staphylococcus aureus*, while two antimicrobial peptides, Mj-AMP1 and Mj-AMP2, showed broad-spectrum antifungal activity against 13 plant pathogenic fungi. The extract from the white-flowered variety showed the highest antibacterial activity. The leaf extract showed the most potent antiparasitic and antioxidant properties, with the water extract being the most potent (Hajji et al., 2010). The study also examined the effects of the hydroethanolic extract and its terpenoid and flavonoid fractions on skin wound healing in rats (Ali Esmail Al-Snafi et al., 2021). The cytotoxic properties of *Mirabilis jalapa* leaves and bark were tested using brine shrimp lethality bioassay techniques. The bark extract showed significant cytotoxic activity, while the protein showed anticancer effects against T47D and SiHa cell lines (Augustine et al., 2013). The extract showed significant anti-inflammatory activity in carrageenan-induced rat paw edema and cotton pellet-induced granuloma models. The extract also suppressed paw edema in formaldehyde and Complete Freund's adjuvant-induced arthritis in rats (Walker et al., 2008). The extract of *Mirabilis jalapa* root showed hypoglycemic and hypolipidemic effects in diabetic mice and rats. They also showed antihistaminic and immunomodulatory effects. The extract of *Mirabilis jalapa* flowers inhibited gut smooth muscle contractility and stimulated rabbit aortic muscle contraction (Aoki et al., 2008) (Figure 4).

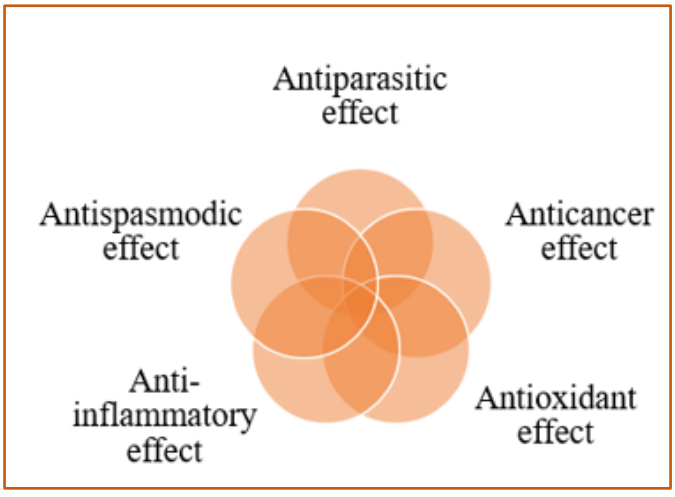

Figure 4: Pharmacological effects of *M. jalapa* flower pigment (Source : (Ali Esmail Al-Snafi et al., 2021)**)**

DISCUSSION

The study utilized chromatographic and spectroscopic analysis of *Mirabilis jalapa* flower pigments to identify and characterize bioactive compounds, including flavonoids, alkaloids, terpenoids, and glycosides. The study reveals that the aqueous extract of *M. jalapa* flower contains key secondary metabolites such as alkaloids, flavonoids, glycosides, and terpenoids. This suggests that water is a more effective solvent for extracting bioactive compounds due to its ability to dissolve both polar and non-polar constituents. The chromatographic analysis revealed the presence of flavonoids, anthocyanins, and other polyphenolic compounds. UV-visible spectroscopic analysis confirmed the presence of these pigments, indicating their stability and absorbance in specific UV-Vis regions (Kumar et al., 2010). Plant-based phytochemicals are used as a model to create medications that are less harmful and more successful at preventing the growth of germs (Ahmad & Beg, 2001). According to the (Chakraborty, 2009) findings, we examined the presence of terpenoids and flavonoids in water extract in the current study. *Mirabilis jalapa* is a medicinal plant widely used to cure numerous ailments globally. The study reveals *M. jalapa* flower's rich phytochemical profile, potential applications in natural dye production, medicinal formulations, and antioxidant research, with high flavonoids and anthocyanins, and solvent-dependent extraction efficiency.

CONCLUSION

The study of *Mirabilis jalapa* flower pigments using chromatographic and UV-Vis spectroscopy has revealed the presence of various pigment classes, including betalains, which are characteristic of the species. The results highlight the complex nature of the plant's pigmentation and the effectiveness of combining TLC and UV-Vis spectroscopy for qualitative analysis. This research could lead to further investigation into individual pigment compound's isolation and potential applications in the pharmaceutical, food, and cosmetic industries. Further research work is required to determine whether the pigments found in *Mirabilis jalapa* flowers may be used as a natural food coloring or for commercial purposes.

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