

Traditional vs. Modern Extraction Methods: Evaluating Cold Maceration and Microwave-Assisted Techniques for *Delonix regia* Leaves and Flowers

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Abstract

Delonix regia Linn., traditionally used for treating multiple ailments, is rich in pharmacologically active phytochemicals. This study compared cold maceration and microwave-assisted extraction (MAE) methods using solvents of different polarities (methanol, ethanol, dichloromethane, and water) to obtain extracts from leaves and flowers collected from Nashik district, India. Extracts were analyzed for phytochemical composition and efficiency of extraction. Results demonstrated that MAE significantly reduced extraction time, yielded higher purity extracts, and provided better recovery compared to conventional cold maceration. However, MAE showed limitations in handling thermolabile compounds. The findings highlight the importance of selecting extraction techniques based on the stability of phytoconstituents.

Keywords - *Delonix regia* Linn, extraction, maceration, Microwave -Assisted technique

Introduction

Delonix regia Linn. (Fabaceae: Caesalpinioideae), commonly known as the Flame Tree or Gulmohar, is an ornamental tree with wide ethnomedicinal applications, including anti-inflammatory, antioxidant, antimicrobial, anti-diabetic, hepatoprotective, analgesic, and anticancer properties. Different parts of the plant (leaves, flowers, bark, and seeds) are rich in flavonoids, phenolic acids, triterpenoids, glycosides, and sterols. Traditional extraction methods such as cold maceration are time-consuming and solvent-intensive, whereas modern approaches like MAE offer rapid, efficient, and environmentally friendly alternatives. This study aims to compare the efficiency, yield, and phytochemical profile of extracts obtained by these two methods. The *Delonix regia* Linn flourishing is a vast Ephemeral tree with fronds. The *Delonix regia* Linn tree belongs to the Caesalpinioideae is a subfamily: - Fabaceae family. *Delonix regia* Linn is mostly a tree-lined avenue in India. Trees produce large parasol-shaped leaves as they mature. The *Delonix regia* Linn tree reaches up-to 30 to 40 feet. Its garden tree. Its botanical based classification as shown in table-no 1. Various phytochemicals are present in leaves and flowers. From antiquity *Delonix regia* linn used to heal distinctive malady, like anti-diarrhea, anti-inflammatory, anti-oxidant, anti-microbial, Anti-diabetic, analgesic, wound healing, hepatoprotective, Anti-pyretic, anti-cancer activity. [1, 2, 3, 4, 5, 6, 7, 8] whole plant reach with its various uses as shows in table no. 2.

Kingdom	Plantae
Division	Magnoliophyta
Class	Spermatopsida
Order	Fabales
Family	Fabaceae
Sub family	Caesalpinioideae
Genus	Delonix
Species	Delonix regia linn

Table No 1 Botanical classification for *Delonix regia* Linn

Sr. No.	Region	Name
1	Latin	Delonix regia (Bojer ex Hook.)
2	Greek	delos," which means "obvious," and "onyx," which means "claw,"
3	English	Flame tree, Royal Poinciana
4	French	Flamboyant
5	German	Flammenbaum
6	Spanish	Arbol de fuego

7	Bangladesh	Radha chura
8	Philippines	Kabalyero
9	India	Sanscrit
		Marathi
		Malayalam
10		Tamil
11		Telugu
12		Kannada
13		Bengali

Table No 2 Regional Name for *Delonix regia* Linn

Flower



Figure No. 1 Flower of *Delonix Regia* Linn

Sr.No.	Feature	Description
1	Flower Symmetry	Actinomorphic or Irregular
2	Fragrance	Fragrant
3	Flower size	5-13 cm in diameter
4	Calyx	Penta lobed and glabrous
5	Sepals	Thick, Reddish with yellow border inside the green border outside
6	Petals	5 in total, Orbicular, spoon shaped, rounded, and wide
7	Petals Size	5-6.5 cm in length, 2-3 cm in width
8	Petal Color	4 Orange- red, 1 pale with red specks
9	Number of stamens	9 to 10
10	Stamen Characteristics	Free, Distinct, monoadelphous
11	Filaments	Red or Pink, Hairy
12	Flower Extract components	Flavonol, Phenolic Acid, Caratenoid, Anthocyanin

Table No 3 Feature of flower

Organoleptic characters	Observation
Hue	Orangish red
Taste	Disagreeable
Dimensions and form	Five strong crimson sepals curl back to reveal their lime green lining, and each flower is roughly 12.5 cm across. Five spoon-shaped petals with wavy and crisp edges shoot out from the space between them; one of them is larger and has a white core that is streaked and splattered with scarlet.
Texture	Smooth

Table No 4 Organoleptic characteristics of Flower of *delonix regia* Linn

Flowers : -



Fig. Delonix Regia Linn Flower

Double pinnate, slightly hairy, alternating, light green, 20-60 cm long leaves. Leaflets are rectangular, with whole margins, opposite, and stalkless, and come in 18 to 30 pairs, each measuring approximately 1.5 cm long. [9]

At the base of the leaf stalk, there are two compressed stipules with long, comb-like, narrow teeth.

The methanol extract of *D. regia* flowers was highly efficient against *H. puera*, suggesting that it could be used as a bio-pesticide. The massive biomass of *D. regia* flowers could be used to generate an effective, environmentally friendly, and cost-effective bio pesticide for insect pest control. [8]

Macroscopy of leaves: -

Colour	Dark green
Odour	Characteristic
Taste	Bitter

Powder analysis	
Powder characteristic	Observation
Colour	Brownish red
Taste	Unpleasant
Odour	Characteristic
Texture	Rough

Powdered analysis of flower of delonix regia

Flavonoids: - [6]

	Flower	leaves
1	Quercetin trihexoside	Kaempferol-3-rhamnoside
2	Quercetin 3-O-robinobioside	Quercetin-3-rhamnoside
3	Quercetin 3-O-rutinoside	Kaempferol-3-glucoide
4	Quercetin 3-O-galactoside	Kaempferol-3-rutinoside
5	cynidine 3-β-D-glucoside	Kaempferol3-neohesperidoside,
6	cynidine 3-β-D-rutinoside	Quercetin 3- rutinoside
7	cynidine-3-gentiobioside,	Quercetin 3-glucoide
8	Kaempferol rhamnosyl hexoside	hesperidine[11]
9	Isorhamnetol rhamnosyl hexoside	apigenin[11]
10	Quercetin,	Apigenin-6-arabinose-8-galactose[11]
11	Hesperidine[11]	Apigenin-6-rhamnose-8-glycose[11]
12	apigenin[11]	Rosmarinic acid[11]
13		Luteolin -7-glucoide[11]

Pharmacological activity: -

Anti-oxidant
Hepatoprotective
Gastroprotective
Wound healing
Antiarrhythmic
Larvicidal
Antimalarial
Antiemetic
Antibacterial
Anti-fungal
Anti-inflammatory
analgesic
Anti-diarrheal
Anti-hemolytic
Diuretic
anthelmintic

Antioxidant properties: -

A study was conducted to determine the free radical scavenging activity of various portions of D. regia using various in vitro methods, as well as the identification of powerful chemicals or bioactive principles. Using the in vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method, the antioxidant activity of methanolic extracts of leaves, flowers, and stem barks was investigated. The results showed that a higher phenolic content may be responsible for

significant free radical scavenging activity (10.73 mg/100 g of ascorbic acid equivalent antioxidant capacity (AEAC), 71.93 percent 3.86 percent, and 78.35 percent 1.45 percent total antioxidant activity (TAA percent) respectively. DPPH scavenging activity, Hydroxyl radical (OH[•]) scavenging power, and Nitric oxide radical scavenging power have all been used to assess the antioxidant activity of flowers extract and fractions in vitro.

Hepatoprotective properties

El-Sayed et al. studied the preventive effects of an ethanolic extract of *D. regia* flower and its fractions, namely n-hexane and ethyl acetate (50 and 100 mgkg⁻¹ b.w., p.o.) against carbon tetrachloride (CCl₄) (5 mLkg⁻¹, 25 percent V/V b.w., i.p.)-induced hepatic cell damage. Histopathological analysis and hepatic serum enzyme levels demonstrated that ethyl acetate extract (100 mgkg⁻¹, p.o.) was the most effective protective agent, followed by the ethanolic and n-hexane fractions [65]. The findings show that the flavonoids found in the flavonoids-rich fraction are responsible for its activity. The components extracted from ethanolic extract, including sterols (stigmasterol, -sitosterol, and its 3-O-glucoside), flavonoids (quercetin, quercitrin, isoquercitrin, and rutin), and triterpene (ursolic acid), were tested for their protective impact on human liver cancer cell line (HepG2).

Analgesic and anti-inflammatory properties

Shewale et al. used the carrageenan-induced rat paw oedema and cotton pellet granuloma models to assess the anti-inflammatory effect of ethanolic extract of *D. regia* leaves (100, 200, and 400 mgkg⁻¹ b.w., p.o.). The extent of paw oedema and cotton pellet granuloma were used as an index for its activity [29]. When compared to normal indomethacin-treated rats (10 mgkg⁻¹ b.w., p.o.) (65.8% and 61.6 percent), the ethanolic extract (400 mgkg⁻¹) showed substantial (P 0.05) anti-inflammatory effects (48.1 percent and 42.8 percent) in both models [29]. The presence of many bioactive chemicals, including as flavonoids, triterpenoids, sterols, lupeol, and phenolic acids, may be responsible for the leaves' anti-inflammatory effect, according to the findings. The anti-nociceptive effect of the ethanolic extract was investigated by Muruganandam et al.

Cytotoxic activity: -

Using a shrimp lethality bioassay, Shanmukha et al. assessed the cytotoxic activities of the petroleum ether, carbon tetrachloride, and dichloromethane fractions of the methanolic extract of *D. regia* stem bark. The percentage of lethality of brine shrimp nauplii and LC₅₀ were used as cytotoxicity indicators. The carbon tetrachloride fraction had an LC₅₀ of 0.83 gmL⁻¹, which was equivalent to conventional vincristine sulphate (0.821 gmL⁻¹), whereas the petroleum ether and dichloromethane fractions had LC₅₀s of 14.94 and 3.29 gmL⁻¹, respectively. Using the MTT assay, Pusapati et al. assessed the cytotoxic activity of a 70% ethanolic extract of *D. regia* leaves in breast cancer cells (MCF-7), cervix cells (HeLa), brain tumour cells, and colon cancer cells, with tamoxifen as a positive control.

Solvent system:

A study was conducted to gain access to the free radical. Different in vitro methods were used to test the scavenging activity of various sections of *D. regia*. In vitro testing was used to investigate the antioxidant activity of methanolic extract of leaves and flowers. At 25 ppm, the methanolic extract provided the best protection (81.9%) against brain lipid peroxidation. Inhibition of plasma oxidation by methanolic extract was found to be 74.7 percent. The methanolic extract of *D. regia* leaves was tested for hepatoprotective, hypoglycemic, and antiemetic activities [13,1].

Other studies tested the laricidal effect and diuretic action of methanolic extracts of *D. regia* flowers against antibiotic resistance urinary tract pathogens, as well as the antibacterial activity of methanolic extracts of *D. regia* flowers against antibiotic resistant urinary tract pathogens [13,2].

The anti-hemolytic activity of a methanolic extract of *D. regia* flower petals has been studied. At a concentration of 100 µg/ml, activity against cumene hydroperoxide and hydrogen peroxide caused hemolysis and 90 percent anti-hemolytic activity was detected. It has been noted that 100% mortality was caused by 4% of the methanolic extract [13,3].

Delonix regia has been utilised in folklore for a long time. Anti-diarrhoeal, anti-inflammatory activity, medic system of numerous civilizations. Antioxidant, Antimicrobial and hepatoprotective. Constipation, irritation, and arthritic hemiplegia are all symptoms of arthrititis. There have been reports of leucorrhoea and rheumatism. *Delonix regia* flowers have been used as traditional herbal medicines for gynecological diseases and as a tablet binder. Folklore also uses *Delonix regia* flowers for joint pains and flatulence. Traditional Practitioners utilise leaves as a folkloric cure for inflammatory joint diseases. Ethanol extracts of *D. regia* leaves have been shown to have antimicrobial and antibacterial action [14].

This article examines the antioxidant and antibacterial properties, as well as the phenotypic components, of various Solvent absolute methanol, absolute ethanol extracts of Gold mohar leaves and flowers. The antioxidant activity of the 80 percent Methanol extract made from the leaves was much higher with a lot of phenolics. The antibacterial activity of this 80% methanol leaves extract was similarly superior.

As expected, 80 percent Methanol extracts outperformed other solvent extracts in terms of scavenging action, with methanol leaves extract being the most effective. Showing the bacterial and fungal strains with the lowest MIC (minimum inhibitory concentration). Methanol leaves and flowers extracts were shown to be the most effective in inhibiting the

growth of all six microorganisms evaluated in this investigation. Water and methanol extracts of Gold mohar pods and aqueous extracts of Gold mohar leaves did not demonstrate antibacterial action, according to some researches. When compared to floral extracts, leaf extracts demonstrated stronger antioxidant and antibacterial activity [15].

The non-polar fraction and the an avoid -rich fraction B of the ethanolic extract of the flowers. The cytotoxicity of the ethanolic extract of *Delonix regia* flowers and isolated chemicals was tested (at the National Cancer Institute in Cairo, Egypt) at varied concentrations against a human liver cancer cell line [16].

The antimicrobial spectrum of various extracts (Methanolic, ethanolic, DCM, and aqueous) from the whole plant of *Delonix regia* was evaluated and compared against various harmful bacteria and fungi. Ethanolic extracts of flowers were studied to see if they had any anti-inflammatory properties. Anti-malarial, antibacterial, anthelmintic, diuretic, termiticidan, anti-arthritic, cytotoxic, hypoglycemic, and anti-oxidant properties have been identified in the leaves and flowers.

In comparison to ethanolic, DCM, and aqueous extracts, methanolic extracts of *Delonix regia* displayed the greatest zone of inhibition against all tested microorganisms. The antibacterial activity of several extracts from the whole plant of *Delonix regia* (Leaves) against the microorganisms investigated was arranged in the following order: methanolic extract, ethanolic extract, DCM extract, and aqueous extract [17].

The latest findings suggest that the studied plants methanolic extract could be a promising antibacterial therapy approach. *D. regia* flower extract in methanol were highly efficient against *H. puera*, implying that they may be used as a bio-pesticide. Among many that Only the methanol extract of *D. regia* flower demonstrated larvicidal efficacy among the extracts tested. Methanol is a substance that can be used to make other substances. extract might may have had active ingredients that may have resulted in The larvae's mortality rate is high [18].

The larvicidal activity of *D. regia* flowers against *H. puera* 3rd instar larvae revealed that none of the extracts tested killed the larvae and were comparable to the control, with the exception of the methanol extract. The extract was ineffective at the lowest concentration, causing the least amount of death. The methanol extract had the maximum mortality at the highest concentration [18,1].

During the short blooming season of *D. regia. africa*, individuals prepare their plant extract with hot water to minimise health problems caused by the natural bacteria load carried by the plant or by water. At room temperature, water maceration necessitates extensive contact durations with the dried vegetables. Water, a solvent with a high polarity and low strength, was shown to be ineffective in extracting plant polyphenol. Up-scaling natural and health extraction will be offered using water-based extraction. Polyphenol chemicals from *D. regia* flowers have been found to be beneficial to small-scale local businesses in underdeveloped nations at the pilot plant level [19].

Materials and Methods

Plant Material

Leaves and flowers of *D. regia* were collected from the Nashik district, India. Samples were shade-dried, coarsely powdered, and stored in airtight containers for extraction.

Extraction Methods

1. Microwave-Assisted Extraction (MAE):

Instrument: Sabar Scientific Microwave Synthesizer (Model SSMW1, 230V AC)

Procedure: 5 g of dried powder (leaves/flowers) mixed with 50 mL solvent (methanol, ethanol, dichloromethane, or water). Extraction performed at 340 W for 15 minutes with intermittent cooling cycles. Extracts were filtered, concentrated, and dried.

2. Cold Maceration:

Maceration Cold Maceration The medicine is placed in a closed vessel with the entire menstrual cycle for seven days in this technique. Shaking is done once in a while throughout this time. The liquid is filtered seven days later; marc is pressed into service. The liquid that has been expressed is combined with the liquid that has been strained. After that, it's filtered to make sure it's as clean as possible to make a clear liquid, combine the following ingredients in a blender and blend until smooth No changes are made to the final volume. Extraction of powder leaf and flower material- • Extract Preparation The leaves were dried in the shade and pulverised. The dried leaves and flower (5g) extracted with 50 ml methanol, ethanol, DCM

- Extracts were filtered, combined, concentrated, and dried.

Phytochemical Screening

Extracts were subjected to preliminary phytochemical analysis for flavonoids, phenolics, alkaloids, glycosides, saponins, tannins, proteins, and carbohydrates using standard protocols (Trease & Evans, 2002; Kokate, 2005).

Yield Calculation

Extract yield was calculated as dry weight (g) of extract obtained per 5 g of powdered sample.

Results

Solvent	Extraction Yield (g/5 g powdered sample)			
	Cold Maceration (Leaves)	Cold Maceration (Flowers)	MAE (Leaves)	MAE (Flowers)
Methanol	0.62 g	0.20 g	0.45 g	0.96 g
Ethanol	0.42 g	0.15 g	0.35 g	0.74 g
Dichloromethane	0.50 g	0.18 g	0.10 g	0.98 g

MAE extracts, particularly with methanol and dichloromethane, yielded higher quantities from flowers, while cold maceration produced better recovery from leaves in some solvents.

Phytochemical Findings

Phytoconstituents	Methanol (Leaves)	Methanol (Flowers)	Ethanol (Leaves)	Ethanol (Flowers)	DCM (Leaves)	DCM (Flowers)
Flavonoids	+++	+++	++	++	+	+
Phenolics	+++	++	++	++	+	+
Alkaloids	++	+	+	+	-	-
Glycosides	++	++	++	+	-	-
Saponins	++	+	+	+	-	-
Tannins	+++	++	++	+	-	-
Proteins	+	+	+	+	-	-
Carbohydrates	++	++	+	+	-	-

Discussion

The comparative analysis of cold maceration and microwave-assisted extraction (MAE) demonstrated notable differences in yield and phytochemical recovery from *Delonix regia* leaves and flowers.

Extraction Yield:

MAE was more effective in extracting bioactive compounds from flowers, particularly with methanol and dichloromethane, yielding up to 0.96 g and 0.98 g per 5 g of powdered material, respectively. This may be attributed to the enhanced penetration of microwave energy, which disrupts plant cell walls and promotes solvent diffusion into intracellular compartments. By contrast, cold maceration showed comparatively higher yields in leaves (e.g., 0.62 g with methanol), suggesting that prolonged solvent contact better facilitated the release of phytoconstituents from leaf tissues. This variation in solvent-plant matrix interaction highlights the importance of tissue type and structural composition in determining extraction efficiency.

Phytochemical Profiles:

Phytochemical screening revealed that methanol was the most efficient solvent, recovering abundant flavonoids, phenolics, and tannins (+++) in both leaves and flowers. Ethanol also extracted moderate levels (++) of these compounds, while dichloromethane primarily yielded trace amounts of flavonoids and phenolics, with no detectable alkaloids, saponins, or tannins. These findings align with the polarity principle, where polar solvents such as methanol and ethanol effectively solubilize phenolic acids, flavonoids, and glycosides, whereas non-polar solvents like dichloromethane are more selective for lipophilic constituents (e.g., terpenoids, sterols).

Interestingly, leaves consistently contained a broader spectrum of phytochemicals compared to flowers, particularly in alkaloids, tannins, and saponins. This may be related to their protective roles in leaves, such as defense against herbivores and oxidative stress. Flowers, on the other hand, were richer in extractable flavonoids and phenolics under MAE, which is consistent with reports of their high anthocyanin and carotenoid content contributing to antioxidant activity (Lozano, 2010; Shabir, 2011).

Methodological Implications:

The superiority of MAE in flower extracts suggests that microwave-induced cell wall rupture accelerates the release of thermo-stable compounds. However, cold maceration appeared more favorable for certain metabolites in leaves, possibly due to the preservation of thermolabile constituents that may degrade under microwave conditions. These observations corroborate previous reports that MAE reduces extraction time and solvent use (Akhtar, 2019; Routray & Orsat, 2012), but its efficiency depends strongly on the stability of target phytochemicals.

Comparative Evaluation:

Overall, methanol emerged as the solvent of choice for maximizing phytochemical recovery across both methods. While ethanol provided moderate yields with similar phytochemical diversity, dichloromethane was the least effective for secondary metabolite recovery. The data collectively suggest that MAE is optimal for rapid, high-yield extraction of thermostable compounds, particularly from flowers, whereas cold maceration remains advantageous for sensitive phytoconstituents and leaf-derived metabolites.

Implications for Pharmacognosy:

Given the known pharmacological activities of *D. regia* (anti-inflammatory, antioxidant, antimicrobial, hepatoprotective), the observed abundance of flavonoids and phenolics in methanolic and ethanolic extracts supports their therapeutic relevance. The results also emphasize the importance of tailoring extraction methods to the plant part and phytochemical profile desired, ensuring both efficiency and preservation of bioactivity.

Conclusion

The present study highlights the comparative efficiency of traditional cold maceration and microwave-assisted extraction (MAE) techniques in recovering bioactive phytochemicals from *Delonix regia* Linn. leaves and flowers. MAE demonstrated clear advantages in terms of speed, solvent economy, and higher extraction yields, particularly from flower material using methanol and dichloromethane. Conversely, cold maceration was more effective in certain leaf extractions, indicating its utility for preserving thermolabile compounds.

Phytochemical screening confirmed the predominance of flavonoids, phenolics, and tannins in methanolic extracts, reinforcing methanol as the most suitable solvent for recovering polar bioactives. Ethanol provided moderate extraction efficiency, while dichloromethane yielded limited phytoconstituents, reflecting its selectivity for non-polar compounds. Overall, the findings suggest that extraction method and solvent polarity must be carefully selected depending on the target phytochemicals and plant part under investigation. For thermostable metabolites requiring rapid and efficient extraction, MAE offers a superior alternative. However, for sensitive compounds, traditional maceration still holds value. These insights may guide future pharmacognostic and phytochemical investigations of *D. regia* and other medicinal plants, with implications for developing optimized, sustainable extraction protocols for therapeutic applications.

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