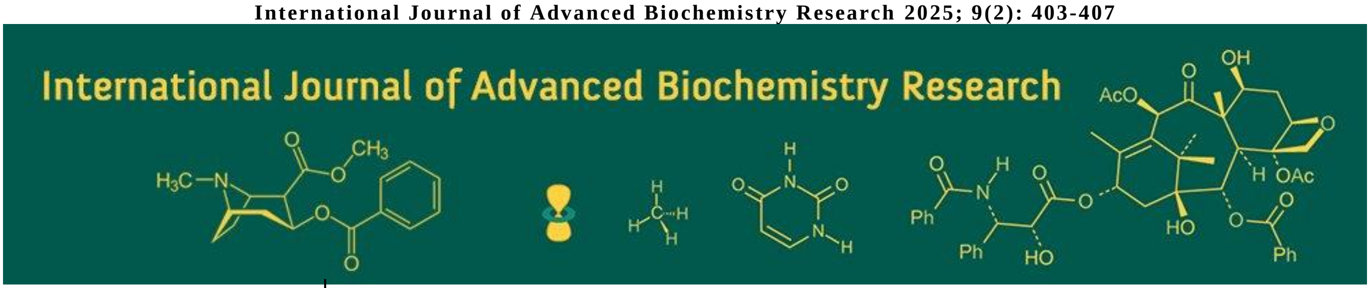

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A comprehensive review on extraction techniques for natural dyes from flowers

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Abstract
Natural dyes and their modified products play a crucial role in promoting sustainable development. By adopting natural dyes derived from flowers, we not only reduce our environmental footprint but also support local communities engaged in sustainable practices. They have several advantages including their high safety, biodegradability, soft tones and functional properties like antimicrobial, UV protective etc. Pigments present in flowers like anthocyanins, carotenoids, betalains etc are responsible for production of colours. Extraction of natural dyes are categorized into conventional and modern methods. Conventional methods include solvent extraction, aqueous extraction, alkaline and acid extraction. The non-conventional or modern extraction methods include ultrasound-assisted, microwave-assisted, enzyme-assisted and supercritical fluid extraction. The adoption of such techniques not only supports environmental sustainability but also offers economic benefits by lowering operational costs and increasing the overall yield of the desired dye.

Keywords: Natural dyes, solvent extraction, aqueous extraction, ultrasound-assisted extraction, microwave assisted extraction, enzyme-assisted extraction

Introduction

In a world increasingly driven by sustainability and a desire to reconnect with nature, the ancient art of deriving natural dyes from flowers has experienced a remarkable resurgence. The naturally occurring bioactive compounds present in flowers possessing anti-inflammatory, and antioxidant properties have the potential to be used as natural dyes. Natural dyes from flowers have been utilized across the globe for countless generations, providing humanity with a kaleidoscope of rich, organic hues that evoke the very essence of the natural world. These dyes, extracted from petals, leaves, and other botanical sources, carry with them a profound connection to both cultural heritage and ecological responsibility. As we seek alternatives to synthetic dyes, which often come at the cost of the environment, the exploration of natural dyes from flowers emerges as a compelling and sustainable solution.

Extraction is one of the critical phases in the processing of natural dyes. Extraction methodologies are primarily categorized into conventional and non-conventional techniques. Conventional approaches encompass solvent extraction, aqueous extraction, as well as alkaline and acid extraction. The traditional chemical separation method, commonly used in modern practices, is widely employed for extracting and separating natural compounds. This procedure is straightforward, requiring no additional apparatus.

The potential risks posed by chemical solvents, such as residual traces in final products and decreased extraction efficiency, have driven the pursuit of eco-friendly extraction methods that focus on minimizing or completely eliminating the use of organic solvents. Commercially, there has been a growing interest in more sustainable extraction methods, fuelled by rising consumer demand for eco-friendly alternatives devoid of toxic chemicals and reduced health related risks (Tiwari, 2015) [35].

Microwave-assisted extraction, along with other cutting-edge extraction technologies like ultrasound-assisted, supercritical fluid, high-pressure liquid, pulsed electric field, and enzyme-assisted extraction, are undeniably yield efficient as well as time and energy saving. Yet, among other things, the difficulties such as implementation in a large scale, high expenses, maintenance, and use of specific enzymes make these approaches difficult to scale up (Netravati *et al*., 2022) [23].

Natural dyes from flowers

The beautiful array of flowers found in nature can be utilized to extract excellent natural dyes for colouring textile fibres and fabrics. Flower-derived dyes are eco-friendly, cost-effective, and help maintain ecological balance. Nearly all rainbow-hued flowers produce dye when boiled in a dye bath. While these coloured substances typically have little affinity to the material they are applied to, only certain flowering plants provide dyes that act as permanent natural colours. Below are some examples of flowers that yield dyes.

Table 1: List of some common dye yielding flowers

Flowers	Colour of dye	Reference
Celosia cristata	Brownish colour	Shanker and Vankar (2005) [30]
Gulmohar	Red	Vankar and Shanker (2009) [36]
Rose	Red	Ekrami *et al.* (2011) [9]
Tecoma stans	Yellow	Chandra *et al.* (2012) [6]
Rhododendron arboreum	Violet	Kim *et al.* (2013) [15]
Gerbera jamesonii	Red	Shivaji *et al.* (2014) [31]
Pot marigold	Yellow, Orange	Okoduwa *et al.* (2015) [24]
Golden trumpet	Yellow	Okoduwa *et al.* (2015) [24]
Nerium (Yellow)	Yellow	Selvam *et al.* (2015) [29]
Flame of Forest	Orange red	Daberao *et al.* (2016) [7]
Hibiscus	Red	Hayat and Jacob (2016) [11]
Ixora coccinea	Red and Purple	Ghurde *et al.* (2016) [10]
Marigold	Yellow, Orange	Vastrad *et al.* (2017) [36]
Delphinium zalil	Yellow	Kiumarsi *et al.* (2017) [16]
Butterfly Pea	Dark blue	Ludin *et al.* (2018) [18]
Nerium (Pink)	Pink	Thamaraiselvi and Deepthi (2018) [34]
Antirrhium majus	Orange	Patel (2019) [25]
Caesalpinia pulcherima	Red	Lakshmana *et al.* (2019) [17]
Gomphrena globosa	Purple	Roriz *et al.* (2020) [28]
Nerium oleander	Dark blue	Anantha and Giri (2020) [2]
Gladiolus	Red	Ijaz (2020) [12]
Tropaeolum majus	Yellow	Singh *et al.* (2021) [32]
Mussaenda erythrophylla	Red	Manicketh *et al.* (2022) [19]
Nyctanthes	Yellow	Adeel *et al.* (2022) [1]
Bougainvillea	Pink	Rasool *et al.* (2023) [27]
Lantana camara	Yellow	Datta *et al.* (2023) [8]

Extraction methods of natural dyes from flowers

Aqueous extraction: This is a widely utilized method due to its simplicity and effectiveness in isolating plant constituents, particularly those amenable to steam distillation without molecular degradation. This method is practical for extracting dyes from flowers. This involves making coloured parts into small fragments, making them into fine powder and soaking them to disrupt the structure of cells. These compounds, either hydrophobic or sparingly soluble, possess a distinct vapor pressure at 100 °C. During boiling, these substances volatilize with the steam and, after condensation, the crude extract is separated via oil-water separator for removing the aqueous phase, thus isolating the desired plant extracts. However, this boiling process can diminish the yield of thermolabile dyes, warranting the recommendation of lower extraction temperatures. Despite its efficacy in producing dyes for textile applications, the method is constrained by prolonged processing durations, substantial water consumption, and elevated temperatures (Mia *et al.*, 2021) [21].

Jothi (2008) [14] conducted a study on extracting natural dyes from African marigold (Tagetes erecta) flowers for application on pure silk and cotton fabrics. The process involved crushing the flowers and dissolving them in distilled water, followed by boiling for 2 hours in a beaker placed over a water bath to expedite extraction. After the 2-hour period, all the colour was extracted from the flowers. The resulting solution was then filtered for immediate use.

Marigold dye is rich in flavonoids and carotenoids. Jha *et al.* (2015) [13] discovered that a dark yellow natural dye can be effectively obtained from *Tagetes erecta* flowers. They conducted the extraction at various temperatures to determine optimal conditions, finding that the strength of the dye extract increased with rise in temperature. The aqueous extraction method achieved the highest dye yield at 95 °C.

An experiment was carried out to extract anthocyanin pigment from Butterfly pea flowers for food applications. Among five different methods—acidified aqueous, solvent, acidified solvent, microwave-assisted extraction, and aqueous extraction using distilled water—the aqueous extraction method proved to be the most efficient. Aqueous extraction method yielded the highest total monomeric anthocyanin content of 7925.29±36.07 mg/l (Netravati *et al.*, 2022) [23].

Solvent extraction: This is a simple method with minimal equipment investment, making it easy to learn and apply in various practical scenarios. This technique allows for simpler purification, solvent removal, and reuse in comparison to the aqueous approach. Solvent extraction using alcohol typically shows higher efficiency as compared with aqueous extraction. Because of the properties of colorants, water-soluble pigments are generally extracted using water or ethanol, which are hydrophilic organic solvents, while fat-soluble pigments are extracted with solvents such as hexane. For efficient extraction, solvent should have a high solubility for both target solute and any other substances present (Mia *et al*., 2021) [21]. Key factors that influence extraction include the degree of grinding, temperature, time for extraction, type of equipment used, and choice of solvent. Processing at low temperatures helps reduce degradation, but this approach has some downsides, including greenhouse gas emissions, toxic residue and difficulties in processing waxy compounds and chlorophyll.

Solvent extraction can be efficiently executed using a Soxhlet apparatus. The process entails securely enclosing finely ground plant material inside a porous bag made of durable filter paper or clean linen. The solvent is placed in bottom flask, and thimble containing plant material is inserted into the extraction chamber. As the solvent is heated, it evaporates and travels through the condenser, where it condenses and makes contact with the plant material, extracting the desired compounds. When the solvent in the extraction chamber reaches top of siphon, it flows back into the flask, taking the extracted compounds along with it. The cycle continues until extraction is complete (Bhalekar *et al*., 2022) [4].

Different solvents were tested for extracting natural dyes from leaves of *Zingiber officinale* and flowers of *Clitoria ternatea*, which are intended to be used as photosensitizers in TiO_2-based dye-sensitized solar cells (Ludin *et al*., 2018) [18]. The solvents included ethanol, methanol, water, acetonitrile, acetone, chloroform, ethyl acetate, n-butyl, ethyl ether, and n-hexane. Among the solvents, ethanol extracted from *Clitoria* flowers yielded maximum absorbance value of 1.702 au. The study also explored the impact of solvent, temperature, and pH on the yield of natural extracts. Ethanol was selected as solvent with an optimum temperature of 80 °C for extraction from *Z. officinale* leaf and 70 °C for *C. ternatea* flower. It was determined that solvents with higher polarity, such as ethanol, are more effective at extracting polar molecules like anthocyanins.

Alkaline and Acid extraction: Such techniques leverage weak acids and alkalis to treat natural dyes containing glycosides. These additives expedite the extraction process by hydrolysing glucosides, thereby enhancing colour production. Alkaline extraction is particularly effective for dyes with phenolic groups. Following the extraction, acid precipitation can be used to isolate the dye (Mia *et al*., 2021) [21]. However, a significant drawback of this method is its potential to reduce colour yield, as some natural dyes are sensitive to pH changes.

Colorants from Bougainvillea were extracted using aqueous and acidic media and used for environment friendly dyeing of silk and cotton (Rasool *et al*., 2023) [27]. It was observed that the highest amount of colorant was extracted in aqueous medium, as opposed to acidic medium. Aqueous dye extract applied for 45 minutes resulted in strong colour on silk.

In an experiment conducted by Sreevidhya (2010) [33], the solvent extraction was identified as an optimal technique for standardizing extraction, preservation, and utilization of natural colour from marigold (*Tagetes erecta*) flowers. The process resulted in the production of an orange-red, water-insoluble, sticky fluid with a distinct marigold fragrance, achieving a lutein recovery rate of 22.86%.

Ultrasound-assisted extraction (UAE): This method uses ultrasound waves to create pressure fluctuations in a medium, causing bubbles to form and collapse, a process called "acoustic cavitation." This process enhances the extraction of target compounds by improving solvent penetration and increasing the surface area between the solid and liquid phases, leading to more efficient extraction. The extraction continues until equilibrium is reached. Various UAE systems, such as ultrasonic baths, horns, and continuous horn-based units, operate at different frequencies. Direct contact between the sample and transducers improves extraction efficiency and reduces energy loss. For large-scale extractions, multiple transducers in a flow-through system can be used for continuous extraction (Tiwari, 2015) [35].

Ultrasound-assisted extraction was effectively applied to optimize extraction of natural pigments from *Bougainvillea glabra* flowers (Maran *et al*., 2015) [20]. Using the Box-Behnken response surface design, temperature of extraction 55 °C, 88W ultrasonic power, extraction duration 37 minutes and solid-to-liquid ratio of 1:17 g/ml were found to be optimum.

Muruganandham *et al*. (2023) [22] investigated yellow dye extraction from *Cassia alata* flower petals and optimized the process using various techniques, including an ultrasonic bath (40 KHz, 500 W) and an ultrasonic probe (390 W to 650 W). UV–Visible spectrophotometric analysis revealed that the highest absorption of yellow dye in methanolic extraction (287 and 479 nm) occurred at 50 °C for 45 minutes using the ultrasonic water bath extraction, which outperformed the ultrasonic probe and direct heating methods. The same method also yielded the best dyeing results for cotton, silk, and leather fabrics, which showed excellent washing and rubbing fastness, retaining strong color even after two washes with boiling soap solution.

Microwave assisted extraction (MAE): Microwave-assisted extraction (MAE) uses electromagnetic energy to generate heat, which helps release solutes from the sample into the solvent. There are two types of MAE systems: closed and open. In a closed MAE system, extraction occurs in a sealed vessel under high pressure and temperature, allowing for fast and efficient extraction but with limited sample throughput and potential loss of volatile components. In an open MAE system, extraction happens at atmospheric pressure, providing higher sample throughput and safer operation, especially for heat-sensitive compounds. It allows solvent addition during the process and is commonly used in analytical applications. Open systems are commonly used for extracting active compounds and in analytical chemistry (Chan *et al*., 2011) [5].

Netravati *et al*. (2022) [23] explored an efficient, cost-effective, and eco-friendly method for extracting anthocyanin pigments from Butterfly pea flowers for food applications. Among various methods tested, microwave-assisted extraction (MAE) provided the highest recovery (73.17±1.76%) and exhibited superior antioxidant activity, measured by DPPH, FRAP, and ABTS assays. The MAE method also resulted in high levels of total phenolics and flavonoids, producing a brilliant-blue colour with notable colour values. The increased phenolic and flavonoid content in MAE with water was due to the microwave's ability to break cell membranes, improving compound extraction efficiency.

Supercritical fluid extraction (SFE): Supercritical fluid extraction (SFE) is a promising alternative to traditional solvent extraction due to the unique properties of supercritical fluids, which have characteristics of both liquids and gases, such as high density, viscosity, and solubility with lower surface tension (Zougagh *et al*., 2004) [38]. These properties enhance fluid penetration into the extraction matrix as pressure increases. This combines extraction, separation, and solvent removal in one unit, improving process flow and efficiency. The method offers advantages like faster extraction, high selectivity, and the ability to operate at room or low temperatures. It also reduces solvent pollution and preserves natural flavours and nutrients, especially in heat-sensitive or chemically unstable products (Mia *et al*., 2021) [21].

Enzyme assisted extraction: Enzyme-assisted extraction is a promising alternative to traditional solvent-based methods for extracting bioactive compounds from plants (Puri *et al*., 2012) [26]. Enzymes serve as effective catalysts, aiding in the extraction or synthesis of complex bioactive substances from natural sources. A key advantage of this method is its ability to enhance solvent pre-treatment, either by reducing solvent usage or increasing the yield of extractable compounds. It is particularly efficient and environmentally friendly compared to conventional extraction techniques.

Enzymes like cellulases, pectinases, and hemicellulases are essential for breaking down plant cell walls, improving the extraction of bioactive compounds. These enzymes hydrolyse cell wall components, enhancing the permeability of the walls and leading to higher yields of bioactive substances. Enzymes can be sourced from fungi, bacteria, plant/fruit extracts, or animal organs. To optimize enzyme-assisted extraction, it's crucial to understand the enzymes' catalytic properties, modes of action, ideal conditions, and the best enzyme or enzyme combinations for the specific plant material (Puri *et al*., 2012) [26].

Barzana *et al*. (2002) [3] discovered that the use of Viscozyme, a commercial enzyme cocktail mainly composed of polysaccharases, along with hexane, increased the carotenoid recovery yield from marigold flowers by over 45%. The yield was further enhanced to nearly 100% with the use of proteolytic enzymes (HT or Neutrase) or pectinolytic enzymes (Pectinex). This study led to the development of a novel solvent extraction process for marigold colorants from fresh flowers. The high added value of product, improved reaction yields, and the lack of negative environmental impacts make this method an appealing alternative to traditional methods.

Conclusion

The progression of extraction methods for natural dyes from flowers reveals a remarkable journey from conventional techniques to sophisticated, modern methodologies. Conventional approaches encompass solvent extraction, aqueous extraction, as well as alkaline and acid extraction. All these techniques are straightforward, requiring no additional apparatus. However, traditional solid-liquid extraction methods, such as Soxhlet extraction and maceration, are often time-consuming and require solvent in large amounts. Microwave-assisted extraction, along with other cutting-edge modern extraction techniques have significantly enhanced the yield, purity, and sustainability of natural dyes. These advanced techniques have streamlined the extraction process while also minimizing environmental impact, addressing the increasing demand for eco-friendly and sustainable practices. Despite these potential advantages, high costs associated with large-scale implementation, ongoing maintenance requirements, and the specific nature of enzyme activity make these approaches difficult to escalate. In an era where sustainability and eco-consciousness are of paramount importance, the utilization of natural dyes from flowers is a testament to our commitment to preserving the planet's beauty and diversity. It serves as a reminder that our creativity need not come at the cost of nature, but rather in harmony with it.

Conflict of interest

All authors declared that there is no conflict of interest.

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