IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

# Recent Advances In Extraction And Enrichment Methods Of Quercetagetin From Tagetes Erecta

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**Abstract**

Tagetes erecta is a crude drug known for its medicinal qualities. Enormous amounts of by products are synthesized in the medicinal usage of T. erecta flowers, including leaves that shall be used to develop novel eco-friendly phenolic extracts with extended value for the Pharma industry. To increase the phenol content in the leaf extracts, this study used a Box–Behnken design with Response Surface Methodology, considering three extraction methods (Soxhlet distillation, heat, and vacuum-assisted extraction), three cropping practices (without fertilizer, chemical fertilizer, and vermicompost), and three phenological stages (plants without buds, with buds, and in flower). Prodrugs from plants fertilized with vermicompost (Eisenia foetida, 10 t ha−1), collected during the blossoming stage and extracted via Soxhlet distillation, exhibited the highest phenol content (25.66 mg GAE/g). Further chemical characterization of the optimized extract (UV-Vis, UV-fluorescence, FTIR, GC-MS,HPLC)confirmedtheoccurrenceof polyphenols in the extract, including quercetin, chlorogenic, gallic, p-coumaric, 3-hydroxycinnamic, and caffeic acids. This underscores the significance of T. erecta leaf residues as a valuable source of bioactive molecules,associating the importance of integrating herbal practices and pharmaceutical extraction methods to enhance the phenolic content in leaf extracts from this species.

**Keywords:** Tagetes erecta, phenolic compounds; residues; optimization; FTIR,extraction, Quercetagetin.

## INTRODUCTION

Marigold flowers are prominent ornamental plants that draw a lot of attention because of their vibrant hues and advantageous health properties. These flowers, which belong to the Asteraceae family and are formally known as Tagetes erecta, are available in a variety of stunning hues, including orange, yellow, red, and brown, to name just a few. Marigold flowers are easy to utilize in gardens, landscapes, and adorned pots because of their variety of hues; therefore, it can be said that they play a significant part in increasing the beauty of the surrounding area. In addition to being lovely and appealing, marigolds are edible flowers that are particularly employed in cooking. . [1]

In addition to their culinary applications, marigold flowers are used to extract lutein and zeaxanthin for lutein supplements. Usually, people take these minerals to avoid age-related macular degeneration. On the culinary map, marigold flowers are categorized as both decorative plants and delicious garnishes, and they seem to have a rich flavor. Marigolds and other edible flowers are used to enhance the look and flavor of salads, desserts, and beverages. Additionally, these flowers contain high-quality components, particularly secondary metabolites that are important for human health.Depending on the flower's geographic origin and the growth

environment, including weather, light, temperature, soil, and fertilizers, marigold blooms may also include phytochemicals, which are linked to the plant's secondary metabolites. [1]

The total carotenoid content (TCC) group of secondary metabolites found in marigold flowers, including as lutein and zeaxanthin, are particularly significant.[2] Since these substances are essential for eye health, using marigolds can help prevent age-related eye conditions.[3] Marigolds also contain flavonoids, a family of antioxidants that are essential for protecting the human body against internal radicals. It has been demonstrated that flavonoids like quercetin have a strong antioxidant activity, which may be advantageous to people.[4] It is essential to identify and assess the components of marigold flowers by using laboratory analysis to determine their quality. The extraction of active chemicals is a common technique in the analysis.[5]

Visible near-infrared spectroscopy (Vis-NIRS) technology is a non-destructive technique that can be used to overcome these challenges and accurately assess the quality of marigold blooms. It is important to emphasize that, in addition to being quicker and easier than traditional extraction, spectrophotometry, and chromatography methods, Vis-NIRS is also more environmentally friendly because it eliminates the need for hazardous chemicals. This technology is a suitable option for routine examination since it offers significant advantages, such as shorter analysis times, lower expenses, and improved accessibility. Further more even if the method is cost-effective for routine analysis, the initial expense of equipment and calibration development might be high, which further restricts its wider accessibility. Vis-NIRS offers a novel way to evaluate the quality of marigold flowers, particularly edible blooms. Non-destructive sample analysis is made possible by this technology. [6–10]

Globally, the number of persons with metabolic syndrome conditions like obesity and diabetes mellitus is rising quickly. The International Diabetes Federation estimates that over 415 million individuals globally have diabetes in 2015, and by 2040, that number is predicted to rise to 642 million.[11] Metabolic syndrome, on the other hand, can seriously harm bodily systems like blood vessels and nerves.[12,13] Inhibiting the activity of the important digestive enzymes in the digestive system is one treatment strategy to delay the absorption of fats and carbs. The digestive system's primary enzymes, α-glucosidase, α-amylase, and pancreatic lipase, catalyze the hydrolysis of carbohydrates and lipids into molecules that are easily digested. [14]

The inflorescence of marigold (Tagetes erecta L.), a popular ornamental plant and traditional Chinese medicine, may be found all over the world.[15] A significant amount of marigold is grown in China in order to extract lutein. But once lutein is extracted using hexane, the leftovers are typically thrown away or used only as fertilizer. The residues contain significant amounts of beneficial compounds as flavonoids and polyphenols[16] Based on its molecular structure, quercetagetin, the main flavonoid component in the extract from marigold (Tagetes erecta L.) inflorescence remnants, has a distinctive flavonol molecule with an extra C6-OH group (Fig. 1). Its antioxidant activity has been the subject of numerous reports.[17, 18] According to several scientific studies, quercetin significantly inhibits lipase, α-glucosidase, and α-amylase.[19, 20

**1) basic structure and chemistry**

A naturally occurring flavonol, quercetagetin is a member of the polyphenolic class of flavonoids, which are found in many different types of plants. The chemical name for this compound is 3,5,6,7,3',4'-hexahydroxyflavone, and its molecular weight is 318.24 g/mol. Quercetagetin is structurally composed of the usual flavone backbone (C6–C3–C6 system), which is made up of two aromatic rings (A and B) connected by a heterocyclic pyrone ring (C).[21] Strong

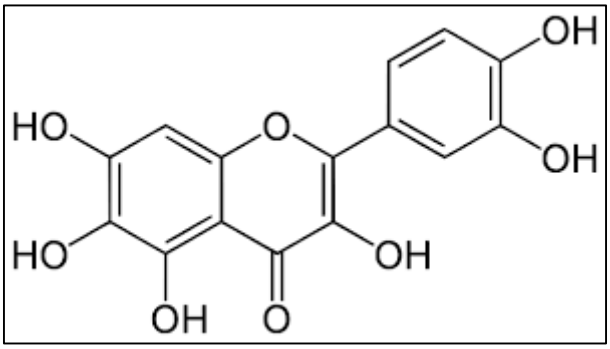



**fig.1 Chemical structures of quercetagetin**

antioxidant qualities are conferred by the presence of many hydroxyl groups, which enable hydrogen donation and stabilize free radicals.[22] The B-ring has hydroxyl groups at positions 3' and 4', the C-ring at position 3, and the A-ring has hydroxyl substituents at positions 5, 6, and 7.[23] Quercetagetin's greater polarity and improved metal chelating ability are attributed to its distinct hydroxylation pattern, which sets it apart from other flavonoids like quercetin and kaempferol.[24] The molecule is typically yellow in color and shows distinctive UV-visible absorption maxima about 255–265 nm and 370–380 nm, which correspond to π→π* transitions within its conjugated aromatic system.[25]

Because quercetagetin's biological activities, such as its antioxidant, anti-inflammatory, and antibacterial actions, are heavily dependent on the location and quantity of hydroxyl substituents, its structural characteristics are also crucial.[26] It is a molecule of increasing pharmacological interest, particularly in the realm of phytopharmaceutical and nutraceutical formulations, due to its strong reactivity toward metal ions and reactive oxygen species (ROS).[27]

| **Property** | **Description** |
|---|---|
| **Chemical name** | 3,5,6,7,8,3′,4′-Heptahydroxyflavone |
| **Molecular formula** | $C_{15}H_{10}O_8$ |
| **Molecular weight** | 318.24 g/mol |
| **Chemical class** | Flavonol (a subclass of flavonoids) |

**2) Extraction methods**

1]Soxhlet extraction

2]ultrasound-assisted extraction

3]microwave-assisted extraction

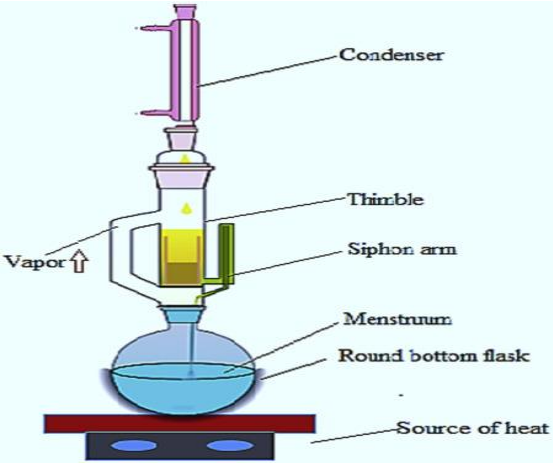
4]supercritical water extraction

5]enzyme-assisted extraction

**1] soxhlet extraction**

With a few minor adjustments, the Soxhlet distillation process was carried out as described.[28] A Grade 645 cellulose extraction thimble (Fisher brand TM, Toronto, ON, Canada) containing twenty grams of FD leaf powder was inserted into the Soxhlet extractor's primary chamber, which was situated above the collecting flask and under a reflux condenser. Water was used as the extraction solvent in the round-bottom flask. A heating mantle was used to heat the solvent. The resultant vapors rose and came into contact with the condenser when the water reached its boiling point. These vapors were then condensed and dripped into the thimble that held the plant material of T. erecta.

After the thimble was sufficiently filled with solvent, the solvent was returned to the collecting flask by siphoning it off using a siphon tube. Plant material/solvent (distilled water) ratios of 0.001, 0.005, and 0.01 g/mL were used, and the reflux was sustained for six hours.[29] Since soxhlet distillation is a popular technique for obtaining polyphenolic-rich extracts from plants and foods, it was used for this investigation. Plant components are extracted repeatedly using fresh solvent parts in this straightforward and cost-effective method, which modifies the mass transfer equilibrium. Additionally, there is no need for a post-leaching filtration step because the temperature in the extraction area is kept relatively high.[30]

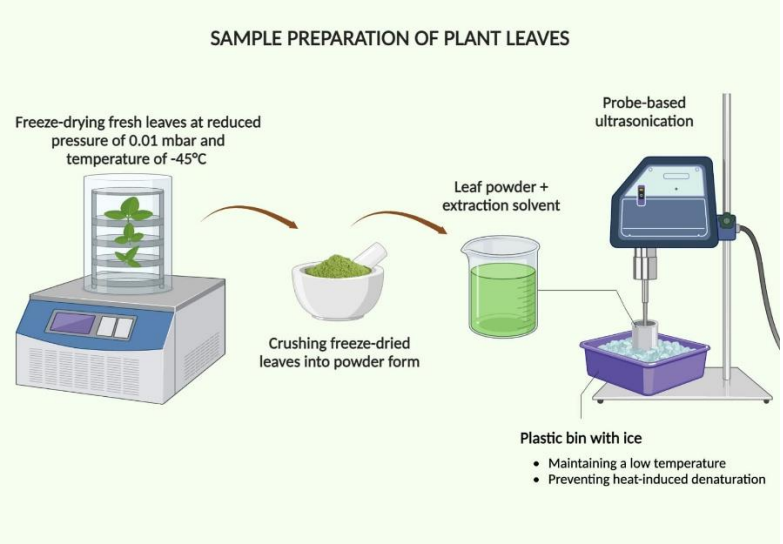



**2] ultrasound-assisted extraction**

A sophisticated and effective method that is frequently used to recover bioactive phytochemicals like flavonoids from plant matrices, such as Tagetes erecta (marigold), is ultrasound-assisted extraction (UAE). This technique creates acoustic cavitation bubbles in the solvent solution by using high-frequency ultrasonic vibrations (usually 20–40 kHz). By creating microturbulence and rupturing plant cell walls, these bubbles' implosion improves solvent penetration and the mass transfer of intracellular substances like quercetagetin into the extraction media.[31, 32]

When compared to traditional maceration or Soxhlet procedures, UAE offers a number of advantages in the extraction of quercetagetin from Tagetes erecta. These include shorter extraction times, less solvent usage, and increased thermolabile chemical yields as a result of gentler temperature conditions.[33] Hydroethanolic or methanolic solvents (50–80% ethanol) are typically employed under ideal conditions, which include an extraction period of 20–40 minutes, a temperature of 40–60 °C, and an ultrasonic frequency of about 35 kHz.[34] Quercetagetin yield is greatly influenced by optimizing factors such solvent concentration, solid-liquid ratio, and sonication power.[35]

According to studies, UAE increases cell wall disintegration and facilitates solvent access to intracellular pigments, which increases the extraction efficiency of flavonols and quercetagetin derivatives from Tagetes erecta flower extracts.[36, 37] Additionally, the technique is compatible with later enrichment or purification procedures such column chromatography or liquid-liquid partitioning.
In accordance with contemporary green chemistry concepts, UAE is generally regarded as a sustainable, quick, and repeatable method appropriate for both analytical and industrial extraction of quercetagetin from Tagetes erecta.[38]



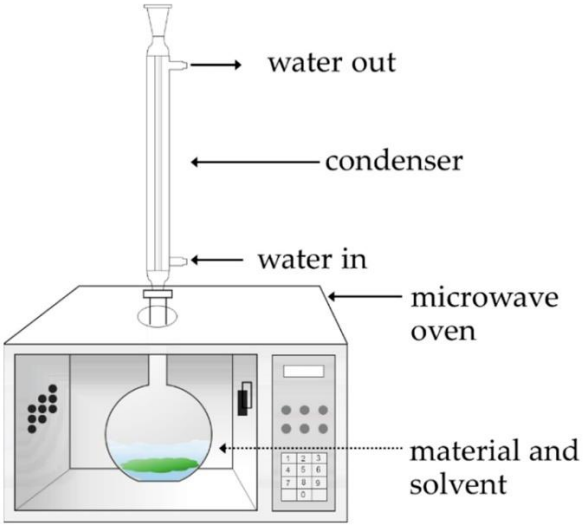

**3]microwave-assisted extraction**

A cutting-edge green extraction technique called Microwave-Assisted Extraction (MAE) uses microwave energy to quickly heat the solvent and plant matrix, increasing the extraction efficiency of bioactive substances like flavonoids and phenolics from plant materials. The method relies on electromagnetic waves interacting with polar molecules in the sample to cause localized heating and cell rupture, which makes it easier for intracellular substances like quercetagetin to be released.[39]

Compared to traditional extraction methods like maceration or Soxhlet extraction, MAE provides notable benefits for Tagetes erecta (marigold), which contains quercetagetin as a key flavonol. Reduced solvent consumption, shorter extraction times, increased yields, and improved thermolabile chemical preservation are the primary advantages.[40] Rapid solvent penetration into plant tissues is made possible by microwave energy, which also improves mass transfer and efficiently breaks down cell walls to increase quercetagetin recovery.[41]

To achieve optimal yield, MAE parameters such microwave power, extraction duration, solvent type, and solid-to-solvent ratio must be optimized. According to studies, the polarity and dielectric constant of aqueous ethanol or methanol solutions make them appropriate solvents for the extraction of flavonoids such quercetagetin.[42] For example, it has been demonstrated that ethanol–water (70:30 v/v) may efficiently extract flavonoids from Tagetes erecta when microwaved at 400–600 W for 5–10 minutes.[43]

Additionally, MAE is regarded as an energy-efficient and ecologically friendly method. By using less organic solvents and cutting down on process waste, it is consistent with the ideas of green chemistry and sustainable extraction techniques.[44] For analytical or medicinal uses, the resultant quercetagetin-rich extract can be further refined or enhanced utilizing chromatographic methods. Microwave-assisted extraction is a potential method for large-scale processing in the phytochemical and pharmaceutical sectors because it is a quick, effective, and environmentally friendly way to separate quercetagetin from Tagetes erecta.[45]

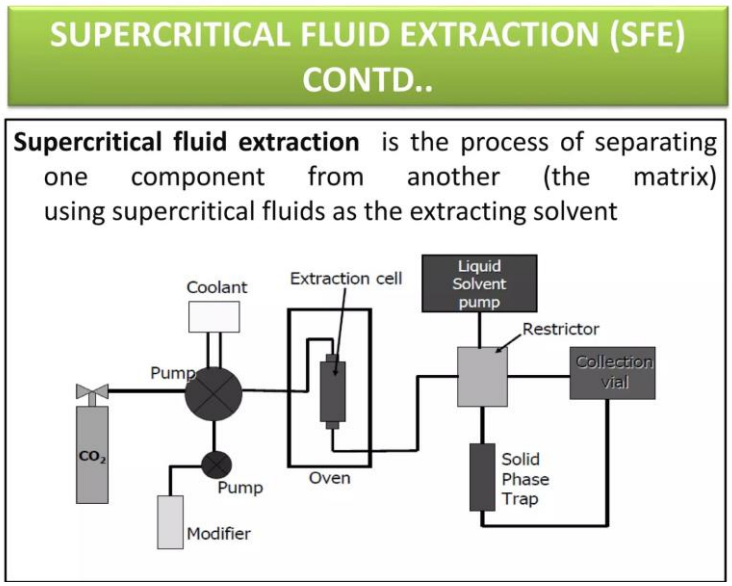



**4] supercritical water extraction**

Supercritical Water Extraction (SWE) is a cutting-edge green extraction technique that uses water at pressures more than 22.1 MPa and temperatures over its critical point (374°C) to extract bioactive chemicals from plant matrices.[46] Water's dielectric constant drastically drops under supercritical conditions, resembling an organic solvent that may dissolve nonpolar and moderately polar substances like flavonoids.[47] This characteristic reduces the need for dangerous organic solvents by facilitating the effective extraction of thermally stable phenolic compounds, such as quercetagetin, from natural sources.[48]

Target chemicals can be extracted selectively based on polarity in SWE because the density and diffusivity of water can be precisely adjusted by varying temperature and pressure.[49] Compared to traditional solvent extraction methods, the method has a number of benefits, such as shorter extraction times, higher extraction efficiency, and better environmental sustainability.[50] Furthermore, because SWE produces low oxidation and degradation under carefully regulated supercritical conditions, it frequently produces extracts with high purity and bioactivity.[51]

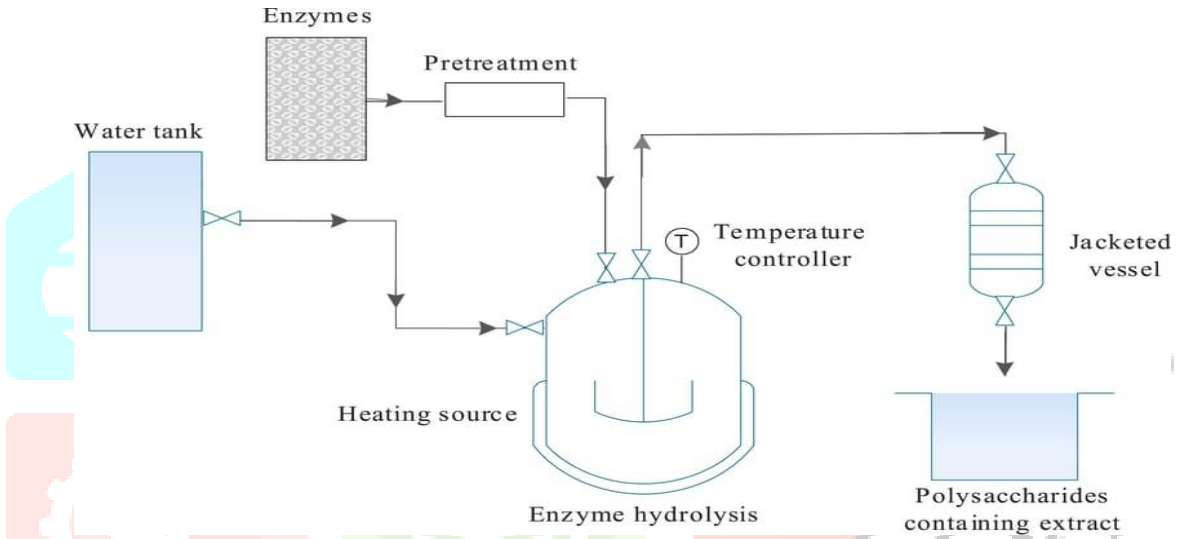
SWE is a promising method for extracting polyphenolic chemicals from a variety of plant sources, including flavonols and flavones like quercetagetin, according to recent investigations.[52] As a result, its possible use in the extraction of quercetagetin from Tagetes erecta may offer a more effective and sustainable substitute for conventional solvent-based extraction techniques.[53]



**5]enzyme-assisted extraction**

Using particular hydrolytic enzymes like cellulase, pectinase, and hemicellulase to break down plant cell walls, enzyme-assisted extraction (EAE) is an efficient and environmentally friendly method that increases the release of intracellular bioactive substances like flavonoids, such as quercetagetin.[54] Polysaccharides like cellulose, hemicellulose, and pectin found in the stiff cell wall matrix of Tagetes erecta petals can prevent solvent penetration during traditional extraction techniques.[55] By hydrolyzing glycosidic bonds, enzymes increase the permeability of plant tissues and make it easier for quercetagetin to be released into the extraction medium.[56]

In order to improve yield, the EAE process typically entails controlling variables including temperature, pH, incubation duration, and enzyme concentration. According to studies, enzymatic pretreatment greatly improves flavonoid recovery when compared to solvent extraction alone.[57] For instance, cellulase-assisted extraction has demonstrated increased flavonol extraction efficiency from marigold flowers at mild temperatures (40–50°C) and slightly acidic pH (4.5–5.0) without degrading thermolabile chemicals.[58] Furthermore, because EAE preserves the structural integrity and bioactivity of the extracted chemicals while consuming less energy and solvent, it is seen as a green substitute for traditional techniques.[59]




**4] Enrichment / purification techniques**

1) liquid liquid extraction

2) Solid Phase Extraction (SPE) using C18 Cartridges

3) Sephadex LH-20

4) counter-current chromatography

1) liquid-liquid extraction

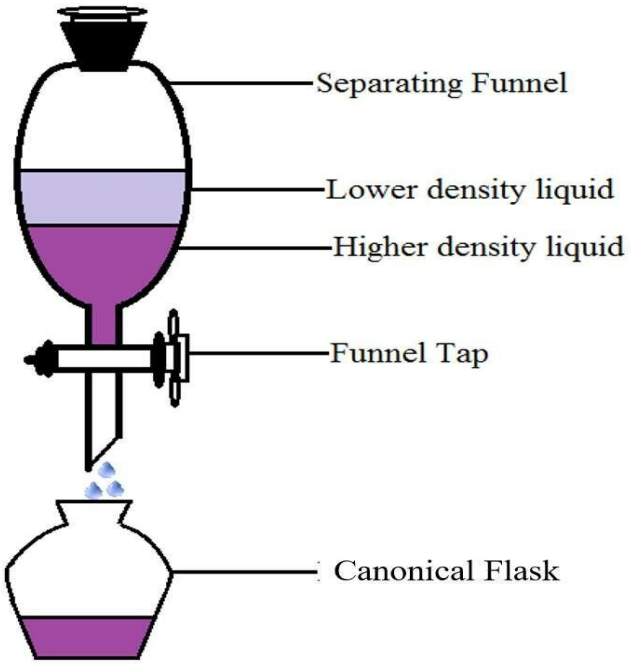
One of the most popular methods for enriching and purifying flavonoids like quercetagetin from plant matrices is liquid–liquid extraction (LLE), sometimes referred to as solvent partitioning. A target compound is distributed between two immiscible liquid phases, usually an organic solvent phase and an aqueous phase, according to the varied solubility of the compounds in each phase.[60]

LLE is typically carried out following first extraction using solvents like ethanol or methanol in the enrichment of quercetagetin from Tagetes erecta. To separate quercetagetin from non-polar contaminants and other phenolic compounds, the crude extract is first concentrated and then partitioned using immiscible solvents such as ethyl acetate, n-butanol, or chloroform.[61]

Because of its somewhat polar hydroxyl substituents, quercetagetin's partition coefficient is greatly influenced by the polarity of the solvent.[62]

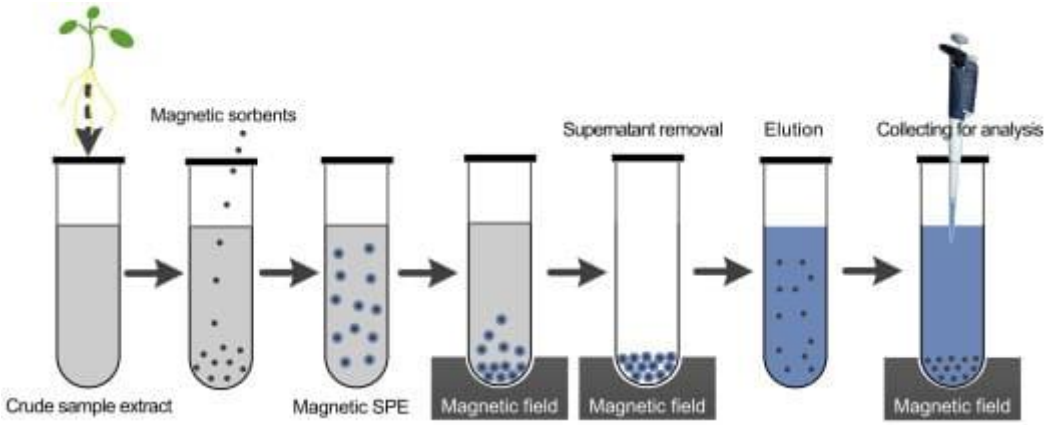
Quercetagetin is typically found in high concentrations in the ethyl acetate fraction, which can then be further purified using chromatographic techniques like preparative HPLC or column chromatography.[63] By lowering the extract's complexity, LLE not only improves downstream purification efficiency but also enriches quercetagetin.[64]

Temperature, the number of extraction cycles, the solvent-to-sample ratio, and the pH of the aqueous phase all affect how effective LLE is. To increase recovery yield and purity, these parameters must be optimized.[65] Numerous investigations have shown that the enrichment and recovery of flavonoids from marigold extracts are improved when LLE is combined with solid-phase extraction or ultrasound-assisted extraction.[66]




2) Solid Phase Extraction (SPE) using C18 Cartridges

Quercetagetin and other flavonoids may be effectively and consistently extracted from complicated plant preparations using Solid Phase Extraction (SPE). Reversed-phase C18 cartridges, which selectively retain phenolic chemicals based on hydrophobic interactions, are commonly used in this process. After conditioning the cartridge with methanol and water, the aqueous or hydroalcoholic extract is loaded. Quercetagetin is then eluted with a greater concentration of methanol or acetonitrile after polar impurities are eliminated using water or diluted methanol.[67] By minimizing interference from sugars, proteins, and colors, this technique improves the purity of flavonoids. Additionally, SPE is compatible with additional analytical methods like HPLC and LC-MS and enables the concentration of target chemicals with little solvent consumption.[68]



3) Sephadex LH-20

One of the most popular gel filtration and adsorption resins for the enrichment and purification of polyphenolic chemicals from plant matrices, such as flavonoids like quercetagetin, is Sephadex LH-20. It is composed of hydroxypropylated dextran gel, which offers adsorption and size exclusion capabilities appropriate for the separation of phenolic compounds according to polarity and molecular weight differences.[69] The crude ethanolic or methanolic extract is usually dissolved in a small amount of solvent and loaded onto a Sephadex LH-20 column that has been previously equilibrated with an appropriate solvent system, such as methanol, ethanol, or methanol–water mixtures, in order to enrich quercetagetin from Tagetes erecta extract.[70]

To enable the selective elution of flavonoids, the column is then eluted with increasing polarity solvents, most frequently methanol or aqueous methanol. Quercetagetin can be eluted in later fractions with higher methanol concentrations because of its numerous hydroxyl groups and moderate polarity, which allow it to attach to the LH-20 matrix effectively.[71]

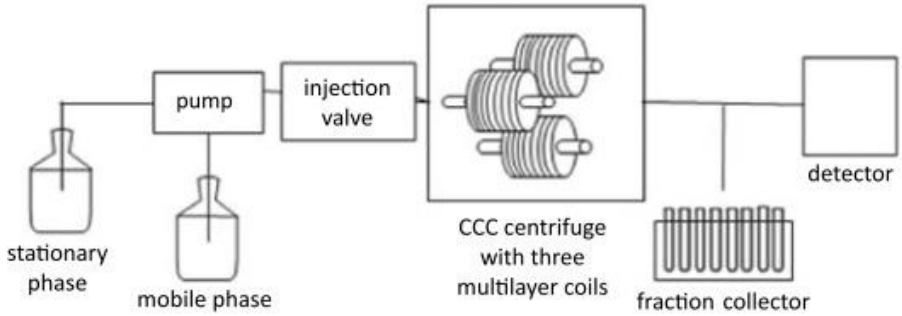
By efficiently eliminating sugars, chlorophylls, and other low-polarity substances, this method greatly raises the concentration and purity of quercetagetin in the enriched fraction. Because it can be recycled after regeneration and preserves the structural integrity of the flavonoid, Sephadex LH-20 is especially beneficial.[72] For flavonols like quercetagetin and quercetin, studies have shown that Sephadex LH-20 chromatography offers a high recovery yield and purity (>90%), making it a perfect enrichment procedure before additional analysis utilizing HPLC or LC-MS techniques.[73]

4) counter-current chromatography

The liquid-liquid separation method known as Counter Current Chromatography (CCC) eliminates issues such irreversible adsorption and sample loss that are frequently seen in solid-phase chromatography by operating without the use of a solid stationary phase.[74] Under the influence of a centrifugal field, it depends on the differential partitioning of analytes between two immiscible liquid phases, one stationary and one mobile. Because of this special mechanism, CCC is especially well-suited for the enrichment and purification of natural substances like quercetagetin and other flavonoids.[75]

In order to enrich quercetagetin from Tagetes erecta, a biphasic solvent system such as n-hexane–ethyl acetate–methanol–water in optimum proportions is used to treat the crude extract obtained following solvent extraction (often methanol or ethanol) to CCC.[76] The partition coefficient (K value) of quercetagetin, which should ideally fall between 0.5 and 2 for efficient separation, is determined by the choice of solvent solution.[77] The compound of interest can frequently partition between the two phases during CCC operation because the mobile phase (organic phase) passes through while the stationary phase (often the more viscous aqueous phase) is kept in the coiled column under centrifugal force.[78]

Because there is less sample degradation and adsorption loss than with traditional column chromatography, this leads to good recovery and purity of quercetagetin.[79] The effectiveness of CCC in enriching flavonoids from plant matrices has been effectively shown in a number of investigations. For example, high-speed CCC with tailored solvent systems has successfully separated quercetagetin and its analogs, achieving purity above 95% with good recovery yield.[80] Because of its excellent selectivity, repeatability, and use of environmentally benign solvents, CCC is regarded as a potent enrichment method for separating bioactive flavonoids such as quercetagetin from Tagetes erecta.[81,82]



**Conclusions:**

T. erecta flowers have been widely studied as a source of bioactive molecules, primarily phenols and carotenoids. However, the species' leaves have received little attention and are currently considered a low-value waste. This research integrates, for the second time, agronomic aspects like fertilization type and the timing of the harvest during various phenological stages with chemical extraction techniques to maximize the phenol content of the leaves of T. erecta. Soxhlet extraction (0.01 g FD leaf powder/mL) in plants harvested at the blooming stage leads to high yields of phenols. Major identified polyphenols in the optimized extract included 2,4-di-tert butylphenol, 2,6-di-tert-butyl-4-methylphenol, 3-hydroxycinnamic acid, p-coumaric acid, gallic acid, 2-allyl-4-methylphenol, quercetin, chlorogenic acid, and caffeic acid, according to GC-MSandHPLCanalyses. This study demonstrated that fertilization with E. foetida (10 t ha−1) represents a better option than chemical fertilization[. The irrational use of agrochemicals represents an economic burden for farmers, contributing to soil degradation and greenhouse gas emissions. Transitioning to fertilization with Eisenia foetida is a more cost-effective option, thus ensuring the viability of the crops for farmers. The ideal time to harvest this species occurs 90 days after sowing, coinciding with the bloom of T. erecta. During this timeframe, farmers can reap dual benefits by selling bouquets while utilizing the leaves to produce extracts with potentially high phenolic content[83]. The optimization conducted in this study enabled the identification of optimal con ditions at the laboratory scale, which could be a foundation for scaling up to a pilot level by considering additional factors like energy use, temperature fluctuations, equipment performance, etc. Further research is required to evaluate the antioxidant properties of the optimized extract produced at a pilot scale, compared to those derived from T. erecta f lowers and food antioxidant additives, to determine its potential commercial value for the food industry. Further studies may be done by semisynthesizing the prodrug and purifying it . Pharmacological activity and toxicological studies could be done in future.

**Funding:** This research received no external funding.

**Data Availability Statement:** The original contributions presented in the study are included in the article, further inquiries can be directed at the corresponding authors.

**Acknowledgment:**

Author is thankful to the The Principal Dr. RR Pinjari and guide Dr. Rashid A. for guiding us for this review. Author is greatfull to the Sinhgad Institute of Pharmaceutical sciences, Lonavala, Pune, management for providing resources and facilities to accomplish this review.

**Conflicts of Interest:** The authors declare no conflicts of interest

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