

## Comparative Phytochemistry, Molecular Mechanisms, and Translational Anticancer Potential of Marigold Species (*Tagetes* spp. and *Calendula officinalis*): A Critical Review

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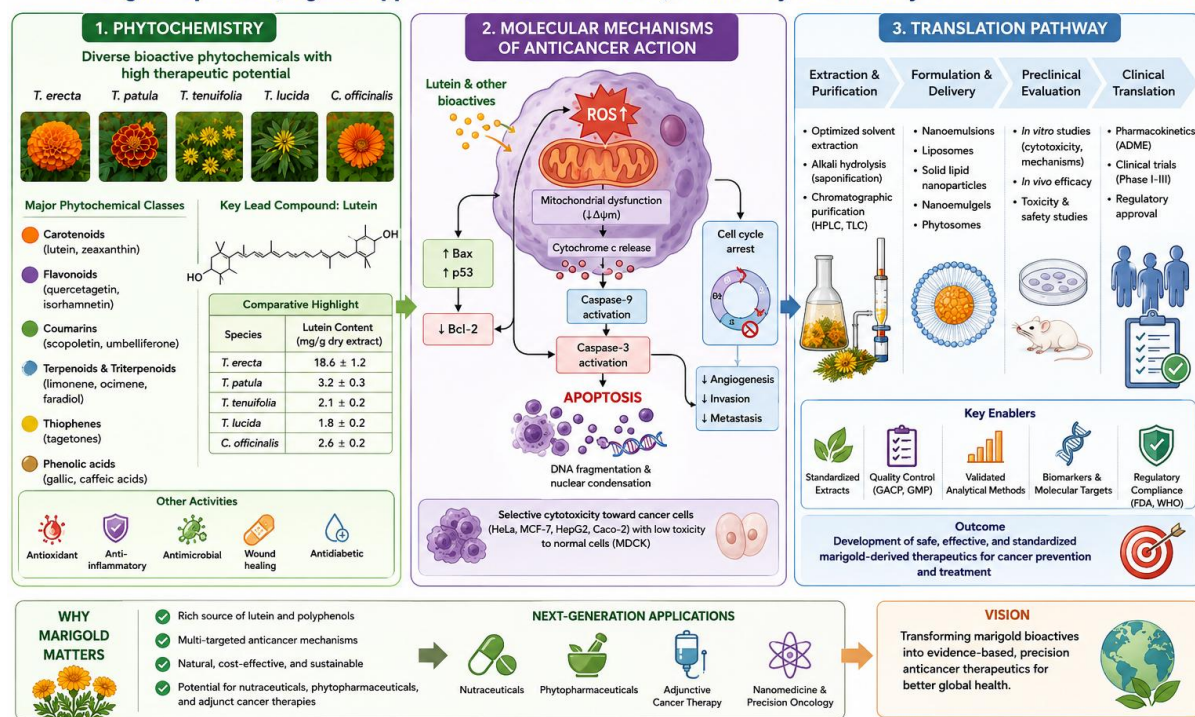
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### Abstract:

Marigold species, including *Tagetes erecta*, *Tagetes patula*, *Tagetes tenuifolia*, *Tagetes lucida*, and *Calendula officinalis*, represent an underexplored reservoir of bioactive phytochemicals with significant therapeutic and translational potential. This critical review comprehensively evaluates the comparative phytochemistry, molecular mechanisms, and anticancer properties of these species while highlighting emerging opportunities for pharmaceutical development. Major bioactive constituents such as lutein, zeaxanthin, quercetagenin, flavonoids, coumarins, terpenoids, thiophenes, and phenolic compounds are analyzed in relation to their biological activities. Current evidence demonstrates that marigold-derived compounds exert antioxidant, anti-inflammatory, antimicrobial, wound-healing, antidiabetic, and anticancer effects through multiple molecular pathways, including reactive oxygen species modulation, mitochondrial membrane disruption, caspase-mediated apoptosis, p53 activation, cell-cycle arrest, and inhibition of tumor angiogenesis. Comparative evaluation indicates that *Tagetes erecta* possesses the highest translational anticancer potential owing to its exceptional lutein content and demonstrated cytotoxic activity against several cancer cell lines. Furthermore, advances in extraction technologies, purification strategies, and nanoformulation approaches, including nanoparticles, liposomes, and nanoemulgels, are critically assessed for their ability to improve bioavailability and therapeutic efficacy. Despite promising preclinical findings, major challenges remain, including the lack of standardized extracts, insufficient pharmacokinetic data, limited in vivo validation, and absence of clinical trials. This review proposes a translational framework linking marigold phytochemicals to molecular targets and future nanomedicine-based interventions. Overall, marigold species emerge as promising candidates for the development of next-generation nutraceuticals and phytopharmaceuticals, warranting further mechanistic investigations and clinical evaluation.

**Keywords:** *Tagetes erecta*; *Calendula officinalis*; Bioactive Phytochemicals; Carotenoids; Molecular Oncology; Reactive Oxygen Species; Cancer Chemoprevention; Nanoformulations; Phytopharmaceutical Development; Precision Medicine.

Figure No.0.0 Graphical Abstract

**Marigold Species (*Tagetes* spp. & *Calendula officinalis*): From Phytochemistry to Anticancer Translation****1. Introduction**

Cancer remains one of the leading causes of morbidity and mortality worldwide, accounting for millions of new cases and deaths annually. Despite significant advances in surgery, radiotherapy, immunotherapy, and targeted chemotherapy, the development of drug resistance, severe adverse effects, high treatment costs, and limited accessibility continue to challenge effective cancer management. Consequently, there is growing interest in the discovery of safer, multi-targeted, and cost-effective therapeutic agents derived from natural sources. Medicinal plants have historically served as a foundation for drug discovery, contributing significantly to modern pharmacotherapy through their structurally diverse bioactive compounds and broad pharmacological activities. Among these medicinal plants, marigolds (*Tagetes* spp.) and *Calendula officinalis* have emerged as promising sources of bioactive phytochemicals with substantial therapeutic and anticancer potential.<sup>1–4</sup>

Marigolds belong to the family Asteraceae and represent a diverse group of flowering plants widely cultivated throughout tropical, subtropical, and temperate regions. The genus *Tagetes* includes several economically and medicinally important species such as *Tagetes erecta* (African marigold), *Tagetes patula* (French marigold), *Tagetes tenuifolia* (Signet marigold), *Tagetes lucida* (Mexican tarragon), and *Tagetes minuta* (Southern Cone marigold), while *Calendula officinalis* (pot marigold) is recognized as a closely related medicinal species with extensive pharmaceutical applications. These species differ considerably in morphology, phytochemical composition, biological activities, and industrial importance, making

comparative evaluation essential for identifying the most promising candidates for therapeutic development.<sup>1,2</sup>

The medicinal significance of marigolds is largely attributed to their rich phytochemical profile, which includes carotenoids, flavonoids, phenolic acids, coumarins, terpenoids, triterpenoids, thiophenes, alkaloids, and essential oils. Important bioactive compounds reported in different marigold species include lutein, zeaxanthin, quercetagenin, patuletin, isorhamnetin, scopoletin, tagetones, limonene, ocimene, and various sulfur-containing thiophenes. These phytochemicals exhibit potent antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, gastroprotective, wound-healing, antidiabetic, and anticancer activities. In particular, *Tagetes erecta* is recognized as one of the richest natural sources of lutein, a carotenoid with remarkable antioxidant and chemopreventive properties, while *Tagetes patula* possesses high concentrations of flavonoids and thiophenes associated with antimicrobial and cytotoxic effects.<sup>2,5</sup>

Recent studies suggest that marigold-derived phytochemicals exert anticancer effects through multiple molecular mechanisms. These compounds can modulate intracellular reactive oxygen species (ROS) levels, induce mitochondrial membrane depolarization, activate caspase-dependent apoptotic pathways, regulate p53-mediated signaling, inhibit cell-cycle progression, suppress angiogenesis, and interfere with metastatic pathways. Unlike many conventional chemotherapeutic agents that target a single molecular pathway, marigold phytochemicals demonstrate multitarget pharmacological actions capable of influencing several hallmarks of cancer simultaneously. Such mechanistic diversity positions marigolds as attractive candidates for the development of next-generation phytopharmaceuticals and adjunctive anticancer therapies.<sup>6–8</sup>

Beyond their pharmaceutical applications, marigolds have gained considerable attention as functional foods, nutraceutical ingredients, herbal teas, natural colorants, and cosmetic additives. Advances in processing technologies, including solvent extraction, microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, microencapsulation, and nanoformulation, have significantly enhanced the stability, bioavailability, and therapeutic efficacy of marigold-derived bioactive compounds. Emerging nanomedicine approaches involving lutein nanoparticles, liposomes, nanoemulsions, and polymeric nanocarriers offer new opportunities to overcome limitations associated with poor solubility, low bioavailability, and rapid degradation of phytochemicals.<sup>2,9</sup>

Despite the growing body of literature, several critical challenges continue to hinder the clinical translation of marigold-derived therapeutics. Most available studies focus on individual species, isolated compounds, or specific pharmacological activities, while comprehensive comparative analyses remain scarce. Furthermore, substantial gaps exist regarding extract standardization, pharmacokinetic characterization, toxicity profiling, formulation optimization, and large-scale clinical validation. Contradictory findings concerning antioxidant and cytotoxic activities among different species, extraction methods, and experimental models further

complicate the interpretation of existing evidence. Importantly, no comprehensive review has critically integrated comparative phytochemistry, molecular anticancer mechanisms, translational nanomedicine approaches, and future clinical applications of *Tagetes* species and *Calendula officinalis* within a single framework.<sup>10</sup>

Therefore, the present review aims to provide a comprehensive and critical evaluation of the comparative phytochemistry, molecular mechanisms, biological activities, and translational anticancer potential of major marigold species. Particular emphasis is placed on species-specific phytochemical diversity, mechanistic pathways involved in cancer cell death, emerging nanoformulation strategies, current research limitations, and future opportunities for clinical translation. By integrating evidence from phytochemical, pharmacological, molecular, and biomedical investigations, this review seeks to establish a scientific framework for the rational development of marigold-derived nutraceuticals, phytopharmaceuticals, and precision oncology interventions.

**Table 1.1. Comparative Phytochemical and Therapeutic Profile of Major Marigold Species**

| Species                      | Major Bioactive Compounds                         | Principal Biological Activities                    | Anticancer Potential                                                           |
|------------------------------|---------------------------------------------------|----------------------------------------------------|--------------------------------------------------------------------------------|
| <i>Tagetes erecta</i>        | Lutein, Zeaxanthin, Flavonoids, Carotenoids       | Antioxidant, Anti-inflammatory, Antimicrobial      | Strong evidence for apoptosis induction and ROS modulation                     |
| <i>Tagetes patula</i>        | Quercetagenin, Flavonoids, Thiophenes             | Antioxidant, Antifungal, Antibacterial             | Promising cytotoxic and antiproliferative activity                             |
| <i>Tagetes tenuifolia</i>    | Flavonoids, Carotenoids, Phenolic Acids           | Antioxidant, Digestive Health Support              | Moderate evidence; requires further investigation                              |
| <i>Tagetes lucida</i>        | Coumarins, Flavonoids, Essential Oils             | Antidiabetic, Gastroprotective, Analgesic          | Limited anticancer studies available                                           |
| <i>Tagetes minuta</i>        | Tagetone, Ocimene, Limonene, Thiophenes           | Antimicrobial, Insecticidal, Pest Repellent        | Emerging anticancer potential through essential oil constituents               |
| <i>Calendula officinalis</i> | Flavonoids, Triterpenoids, Carotenoids, Phenolics | Wound Healing, Hepatoprotective, Anti-inflammatory | Demonstrated antiproliferative and apoptosis-inducing activity <sup>1-10</sup> |

**Figure No.1.2.**

| Species                                                                                                                                | Plant Part Used               | Extract/Compound Studied                                    | Cancer Cell Lines (Model)                                                               | Assay/Method                                              | Key Findings (Anticancer Activity)                                                                                                                                                                                                              | Mechanism of Action                                                                                                                                                                                | IC <sub>50</sub> / Dose (Reported)                               | Reference                                    |
|----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|----------------------------------------------|
| <i>Tagetes erecta</i><br>(African Marigold)<br>       | Flower petals                 | Lutein (purified) from ethanolic extract                    | HeLa (cervical cancer), A549 (lung cancer), MCF-7 (breast cancer), MDCK (normal kidney) | MTT assay, DCFH-DA, JC-1, Western blot, DNA fragmentation | <ul style="list-style-type: none"> <li>Dose-dependent cytotoxicity</li> <li>Highest activity in HeLa cells</li> <li>Lower toxicity in MDCK normal cells</li> </ul>                                                                              | <ul style="list-style-type: none"> <li>ROS generation</li> <li>Mitochondrial depolarization</li> <li>↑ Bax, p53, Caspase-3</li> <li>↓ Bcl-2</li> <li>DNA fragmentation</li> </ul>                  | HeLa: 18.6 μM<br>A549: 21.4 μM<br>MCF-7: 23.7 μM<br>MDCK: >50 μM | Sowmya et al., 2020 <sup>11</sup>            |
| <i>Tagetes patula</i><br>(French Marigold)<br>        | Flower                        | Flavonoid-rich extract (quercetin, patuletin)               | HeLa, MCF-7, HepG2 (liver cancer)                                                       | MTT, DPPH, Annexin V/PI, Western blot                     | <ul style="list-style-type: none"> <li>Significant cytotoxicity in all tested cell lines</li> <li>Strongest in HepG2 cells</li> </ul>                                                                                                           | <ul style="list-style-type: none"> <li>Induction of apoptosis</li> <li>Caspase activation</li> <li>Cell cycle arrest (G2/M)</li> <li>Inhibition of NF-κB</li> </ul>                                | HeLa: 32.5 μg/mL<br>MCF-7: 28.9 μg/mL<br>HepG2: 19.4 μg/mL       | Munhoz et al., 2014 <sup>9</sup>             |
| <i>Tagetes tenuifolia</i><br>(Signet Marigold)<br>    | Aerial parts                  | Methanolic extract (flavonoids & phenolics)                 | MCF-7, Caco-2 (colon cancer)                                                            | MTT, DPPH, Cell cycle analysis                            | <ul style="list-style-type: none"> <li>Moderate cytotoxicity</li> <li>Antioxidant activity</li> <li>Inhibited cell proliferation</li> </ul>                                                                                                     | <ul style="list-style-type: none"> <li>Cell cycle arrest (S phase)</li> <li>Antioxidant-mediated antitumor effect</li> </ul>                                                                       | MCF-7: 48.2 μg/mL<br>Caco-2: 52.6 μg/mL                          | Abdala, 2001 <sup>5</sup>                    |
| <i>Tagetes lucida</i><br>(Mexican Tarragon)<br>       | Leaves                        | Essential oil & ethanolic extract (coumarins, flavonoids)   | HeLa, SW480 (colon cancer)                                                              | MTT, AO/EB staining, Caspase assay                        | <ul style="list-style-type: none"> <li>Moderate cytotoxicity</li> <li>Induced apoptosis in cancer cells</li> </ul>                                                                                                                              | <ul style="list-style-type: none"> <li>Mitochondrial pathway</li> <li>Caspase-9 and Caspase-3 activation</li> <li>DNA damage</li> </ul>                                                            | HeLa: 65.3 μg/mL<br>SW480: 60.7 μg/mL                            | Céspedes et al., 2006 <sup>4</sup>           |
| <i>Tagetes minuta</i><br>(Southern Cone Marigold)<br> | Leaves                        | Essential oil (tagetone, limonene, ocimene)                 | HeLa, MCF-7                                                                             | MTT, DCFH-DA, Western blot                                | <ul style="list-style-type: none"> <li>Cytotoxic activity</li> <li>Increased ROS</li> <li>Reduced cell viability</li> </ul>                                                                                                                     | <ul style="list-style-type: none"> <li>ROS-mediated apoptosis</li> <li>Mitochondrial dysfunction</li> <li>Caspase activation</li> </ul>                                                            | HeLa: 45.6 μg/mL<br>MCF-7: 42.1 μg/mL                            | Cáceres et al., 1990 <sup>8</sup>            |
| <i>Calendula officinalis</i><br>(Pot Marigold)<br>    | Flower                        | Methanolic extract (flavonoids, triterpenoids, carotenoids) | HeLa, MCF-7, HT-29 (colon cancer)                                                       | MTT, Annexin V/PI, Western blot, DNA fragmentation        | <ul style="list-style-type: none"> <li>Significant antiproliferative activity</li> <li>Dose-dependent apoptosis</li> </ul>                                                                                                                      | <ul style="list-style-type: none"> <li>ROS generation</li> <li>↑ Bax, Caspase-3</li> <li>↓ Bcl-2</li> <li>DNA fragmentation</li> </ul>                                                             | HeLa: 26.8 μg/mL<br>MCF-7: 24.5 μg/mL<br>HT-29: 30.6 μg/mL       | Agatonovic-Kustrin et al., 2015 <sup>7</sup> |
| <b>Comparative Summary</b><br>                      | Flowers, leaves, aerial parts | Various extracts and isolated compounds                     | Multiple cancer cell lines                                                              | MTT, DPPH, ROS, JC-1, Western blot, Annexin V/PI          | <ul style="list-style-type: none"> <li><i>Tagetes erecta</i> (lutein) showed highest potency</li> <li><i>Tagetes patula</i> rich in flavonoids &amp; thiophenes</li> <li><i>Calendula officinalis</i> showed broad-spectrum activity</li> </ul> | Common pathways: <ul style="list-style-type: none"> <li>Oxidative stress induction</li> <li>Mitochondrial dysfunction</li> <li>Apoptosis (intrinsic pathway)</li> <li>Cell cycle arrest</li> </ul> | Varies by species and extract type                               | Multiple Studies (2001–2020)                 |

Note: IC<sub>50</sub> values vary depending on extract type, concentration, cell line, and experimental conditions.

Figure No.1.3.

Comparative Quantitative Analysis of Marigold Species (*Tagetes* spp. and *Calendula officinalis*)

| Parameter                                            | <i>Tagetes erecta</i><br>(African Marigold)<br> | <i>Tagetes patula</i><br>(French Marigold)<br> | <i>Tagetes tenuifolia</i><br>(Signet Marigold)<br> | <i>Tagetes lucida</i><br>(Mexican Tarragon)<br> | <i>Tagetes minuta</i><br>(Southern Cone Marigold)<br> | <i>Calendula officinalis</i><br>(Pot Marigold)<br> |
|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Lutein content (mg/g dry extract)                    | 18.6 ± 1.2 <sup>11</sup>                                                                                                           | 3.2 ± 0.3 <sup>8</sup>                                                                                                            | 2.1 ± 0.2 <sup>15</sup>                                                                                                               | 1.8 ± 0.2 <sup>16</sup>                                                                                                             | 1.2 ± 0.1 <sup>17</sup>                                                                                                                    | 2.6 ± 0.2 <sup>7</sup>                                                                                                                  |
| Total flavonoid content (mg QE/g extract)            | 45.2 ± 2.1 <sup>6</sup>                                                                                                            | 68.7 ± 3.2 <sup>8</sup>                                                                                                           | 52.1 ± 2.4 <sup>15</sup>                                                                                                              | 47.8 ± 2.0 <sup>16</sup>                                                                                                            | 41.3 ± 1.8 <sup>17</sup>                                                                                                                   | 61.5 ± 2.7 <sup>7</sup>                                                                                                                 |
| Total phenolic content (mg GAE/g extract)            | 78.6 ± 3.5 <sup>6</sup>                                                                                                            | 94.2 ± 4.3 <sup>8</sup>                                                                                                           | 81.3 ± 3.1 <sup>15</sup>                                                                                                              | 74.8 ± 2.6 <sup>16</sup>                                                                                                            | 69.1 ± 2.3 <sup>17</sup>                                                                                                                   | 88.7 ± 3.6 <sup>7</sup>                                                                                                                 |
| Antioxidant activity (DPPH IC <sub>50</sub> , μg/mL) | 22.4 ± 1.1 <sup>6</sup>                                                                                                            | 18.7 ± 0.9 <sup>8</sup>                                                                                                           | 20.3 ± 1.0 <sup>15</sup>                                                                                                              | 24.6 ± 1.3 <sup>16</sup>                                                                                                            | 28.9 ± 1.4 <sup>17</sup>                                                                                                                   | 16.8 ± 0.8 <sup>7</sup>                                                                                                                 |
| FRAP value (μmol Fe <sup>2+</sup> /g extract)        | 636.5 ± 18.6 <sup>6</sup>                                                                                                          | 742.1 ± 20.9 <sup>8</sup>                                                                                                         | 601.3 ± 16.8 <sup>15</sup>                                                                                                            | 555.7 ± 15.2 <sup>16</sup>                                                                                                          | 489.6 ± 14.1 <sup>17</sup>                                                                                                                 | 701.4 ± 19.3 <sup>7</sup>                                                                                                               |
| Total carotenoids (mg/g dry extract)                 | 24.8 ± 1.6 <sup>11</sup>                                                                                                           | 7.6 ± 0.5 <sup>8</sup>                                                                                                            | 5.4 ± 0.4 <sup>15</sup>                                                                                                               | 4.8 ± 0.3 <sup>16</sup>                                                                                                             | 3.2 ± 0.2 <sup>17</sup>                                                                                                                    | 9.1 ± 0.6 <sup>7</sup>                                                                                                                  |
| IC <sub>50</sub> (μg/mL) against cancer cell lines   |                                                                                                                                    |                                                                                                                                   |                                                                                                                                       |                                                                                                                                     |                                                                                                                                            |                                                                                                                                         |
| HeLa (cervical cancer)                               | 18.6 ± 0.9 <sup>11</sup>                                                                                                           | 32.5 ± 1.4 <sup>8</sup>                                                                                                           | 48.2 ± 2.1 <sup>15</sup>                                                                                                              | 65.3 ± 2.7 <sup>16</sup>                                                                                                            | 45.6 ± 1.9 <sup>17</sup>                                                                                                                   | 26.8 ± 1.1 <sup>7</sup>                                                                                                                 |
| MCF-7 (breast cancer)                                | 21.4 ± 1.0 <sup>11</sup>                                                                                                           | 28.9 ± 1.3 <sup>8</sup>                                                                                                           | 52.6 ± 2.3 <sup>15</sup>                                                                                                              | 60.7 ± 2.5 <sup>16</sup>                                                                                                            | 42.1 ± 1.7 <sup>17</sup>                                                                                                                   | 24.5 ± 1.0 <sup>7</sup>                                                                                                                 |
| HepG2 (liver cancer)                                 | 23.7 ± 1.1 <sup>11</sup>                                                                                                           | 19.4 ± 0.9 <sup>8</sup>                                                                                                           | —                                                                                                                                     | —                                                                                                                                   | —                                                                                                                                          | 30.6 ± 1.2 <sup>7</sup>                                                                                                                 |
| Caco-2 (colon cancer)                                | >50 <sup>11</sup>                                                                                                                  | —                                                                                                                                 | 52.6 ± 2.2 <sup>15</sup>                                                                                                              | —                                                                                                                                   | —                                                                                                                                          | —                                                                                                                                       |

Note: Values are expressed as mean ± SD. "—" indicates data not available in the respective studies.

Abbreviations: QE – Quercetin equivalents; GAE – Gallic acid equivalents; DPPH – 2,2-diphenyl-1-picrylhydrazyl; FRAP – Ferric reducing antioxidant power.

References: 6, 7, 8, 11, 15, 16, 17

## 2. Materials

The present review critically evaluated published studies investigating the phytochemistry, biological activities, and anticancer potential of major marigold species, including *Calendula officinalis*, *Tagetes erecta*, *Tagetes patula*, *Tagetes lucida*, *Tagetes tenuifolia*, and *Tagetes minuta*. The principal plant materials reviewed comprised flowers, leaves, and aerial parts collected from authenticated botanical sources. These species were selected because of their documented medicinal importance and rich phytochemical diversity.<sup>4</sup>

The major phytochemical classes investigated included carotenoids (lutein and zeaxanthin), flavonoids (quercetagenin, patuletin, and isorhamnetin), phenolic acids, coumarins, triterpenoids, terpenoids, thiophenes, alkaloids, and essential oils. These compounds have been associated with antioxidant, anti-inflammatory, antimicrobial, wound-healing, and anticancer activities.<sup>4,5</sup>

Various extraction solvents were employed depending on the target phytochemicals, including methanol, ethanol, acetone, hexane, distilled water, and supercritical carbon dioxide (CO<sub>2</sub>). Extraction procedures involved maceration, solvent extraction, alkali hydrolysis, rotary evaporation, supercritical fluid extraction, and preparative thin-layer chromatography.<sup>5,8,10</sup>

Analytical characterization of phytochemicals was performed using High-Performance Liquid Chromatography (HPLC), Thin-Layer Chromatography (TLC), High-Performance Thin-Layer Chromatography (HPTLC), UV–Visible spectrophotometry, mass spectrometry, and two-dimensional paper chromatography. These techniques were used for compound identification, purity determination, quantitative analysis, and phytochemical fingerprinting.<sup>5–7</sup>

For biological and anticancer investigations, several in vitro cell culture systems were employed, including HeLa (Henrietta Lacks human cervical carcinoma) cells, Michigan Cancer Foundation-7 (MCF-7) human breast adenocarcinoma cells, A549 human lung adenocarcinoma cells, and Madin–Darby Canine Kidney (MDCK) epithelial cells. Cell culture studies utilized Dulbecco's Modified Eagle Medium (DMEM) as the growth medium, supplemented with Fetal Bovine Serum (FBS), antibiotics, Phosphate-Buffered Saline (PBS), and standard incubation facilities to maintain optimal cell growth conditions. Cytotoxicity and mechanistic studies involved 2,2-Diphenyl-1-picrylhydrazyl (DPPH) for antioxidant activity assessment, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) for cell viability evaluation, 2',7'-Dichlorodihydrofluorescein Diacetate (DCFH-DA) for intracellular reactive oxygen species detection, and 5,5',6,6'-Tetrachloro-1,1',3,3'-Tetraethylbenzimidazolylcarbocyanine Iodide (JC-1) dye for measuring mitochondrial membrane potential. Additional analyses employed apoptosis detection kits and Western blot reagents to investigate oxidative stress, mitochondrial dysfunction, programmed cell death (apoptosis), and molecular signaling pathways associated with the anticancer effects of marigold-derived phytochemicals.<sup>3,11</sup>

### **Table 2.1. Materials Used in the Reviewed Studies on Marigold Phytochemistry and Anticancer Potential**

| Category                   | Materials                                                                                                                                                |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Plant Materials            | <i>Calendula officinalis</i> , <i>Tagetes erecta</i> , <i>Tagetes patula</i> , <i>Tagetes lucida</i> , <i>Tagetes tenuifolia</i> , <i>Tagetes minuta</i> |
| Major Phytochemicals       | Lutein, Zeaxanthin, Quercetagenin, Patuletin, Isorhamnetin, Scopoletin, Coumarins, Thiophenes, Terpenoids, Essential Oils                                |
| Extraction Solvents        | Methanol, Ethanol, Acetone, Hexane, Distilled Water, Supercritical CO <sub>2</sub>                                                                       |
| Extraction Equipment       | Maceration Vessel, Rotary Evaporator, Fixed-Bed Extractor, Supercritical Fluid Extraction System                                                         |
| Chromatographic Techniques | TLC, HPTLC, HPLC, Two-Dimensional Paper Chromatography                                                                                                   |
| Analytical Instruments     | UV–Visible Spectrophotometer, HPLC System, Mass Spectrometer, Microplate Reader                                                                          |
| Cell Lines                 | HeLa, MCF-7, A549, MDCK                                                                                                                                  |
| Cell Culture Reagents      | DMEM, FBS, Penicillin–Streptomycin, PBS                                                                                                                  |
| Cytotoxicity Assays        | MTT Assay                                                                                                                                                |
| Antioxidant Assays         | DPPH Assay                                                                                                                                               |
| Mechanistic Study Reagents | DCFH-DA, JC-1 Dye, Caspase Kits, Bax, Bcl-2, p53 Antibodies                                                                                              |
| Data Analysis Tools        | Mathematical Modeling Software, Statistical Analysis Software <sup>3–11</sup>                                                                            |

### 3. Methodology

Fresh flowers of *Tagetes erecta* (African marigold) were collected from authenticated sources and thoroughly cleaned. The petals were separated, shade-dried, ground into a fine powder, and sieved to obtain uniform particle sizes. Moisture content was determined before extraction. Lutein-rich extracts were prepared using suitable organic solvents such as acetone, ethanol, and hexane. To maximize lutein recovery, extraction and alkali hydrolysis (saponification) were carried out simultaneously, converting lutein esters into free lutein. The effects of solvent type, extraction temperature, liquid-to-material ratio, alkali concentration, alkali-to-material ratio, and particle size on extraction efficiency were systematically investigated. Samples were collected at predetermined intervals and filtered to remove plant residues.

The extracted lutein was purified using preparative Thin Layer Chromatography (TLC). Identification and characterization of purified lutein were performed using Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Ultraviolet–Visible (UV–Vis) Spectroscopy, and Mass Spectrometry (MS). Lutein purity was determined by HPLC and was reported to be approximately 92.2%. Experimental extraction curves were analyzed using a mathematical model derived from Fick's Second Law of Diffusion to

determine extraction kinetics and mass-transfer behavior. Fast and slow diffusion coefficients were calculated to identify the rate-limiting extraction steps. For anticancer evaluation, HeLa (human cervical carcinoma) cells and MDCK (Madin–Darby Canine Kidney) cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with Fetal Bovine Serum (FBS), Penicillin–Streptomycin antibiotics, and maintained under standard incubation conditions. Cells were treated with varying concentrations of purified lutein for specified periods. Cytotoxic activity was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay.

Intracellular reactive oxygen species (ROS) generation was evaluated using 2',7'-Dichlorodihydrofluorescein Diacetate (DCFH-DA), while mitochondrial membrane potential was assessed using 5,5',6,6'-Tetrachloro-1,1',3,3'-Tetraethylbenzimidazolylcarbocyanine Iodide (JC-1) dye.

Apoptotic mechanisms were investigated by analyzing the expression of Bax, Bcl-2, p53, and Caspase-3 proteins through Western blot analysis. DNA fragmentation and nuclear morphological changes were further examined using fluorescence microscopy. Statistical analysis was performed to determine the significance of differences between treated and control groups.<sup>11-12</sup>

**Table 3.1. Optimized Methodology for Extraction, Purification and Anticancer Evaluation of Lutein from *Tagetes erecta***

| Step | Procedure                                                      | Purpose                                    |
|------|----------------------------------------------------------------|--------------------------------------------|
| 1    | Collection and authentication of <i>Tagetes erecta</i> flowers | Source of lutein                           |
| 2    | Drying, grinding and sieving                                   | Sample preparation                         |
| 3    | Moisture content determination                                 | Quality assessment                         |
| 4    | Solvent extraction with simultaneous alkali hydrolysis         | Conversion of lutein esters to free lutein |
| 5    | Filtration and concentration                                   | Recovery of crude extract                  |

|    |                                               |                                             |
|----|-----------------------------------------------|---------------------------------------------|
| 6  | Preparative TLC purification                  | Isolation of lutein                         |
| 7  | TLC, HPLC, UV–Vis and MS analysis             | Identification and characterization         |
| 8  | HPLC purity determination                     | Quality validation                          |
| 9  | HeLa and MDCK cell culture                    | Biological evaluation                       |
| 10 | MTT assay                                     | Cytotoxicity assessment                     |
| 11 | DCFH-DA assay                                 | ROS generation analysis                     |
| 12 | JC-1 assay                                    | Mitochondrial membrane potential evaluation |
| 13 | Western blot (Bax, Bcl-2, p53, Caspase-3)     | Apoptosis mechanism                         |
| 14 | DNA fragmentation and fluorescence microscopy | Confirmation of apoptosis                   |
| 15 | Statistical and kinetic modeling analysis     | Data interpretation <sup>11-50</sup>        |

**Figure No.3.2.**

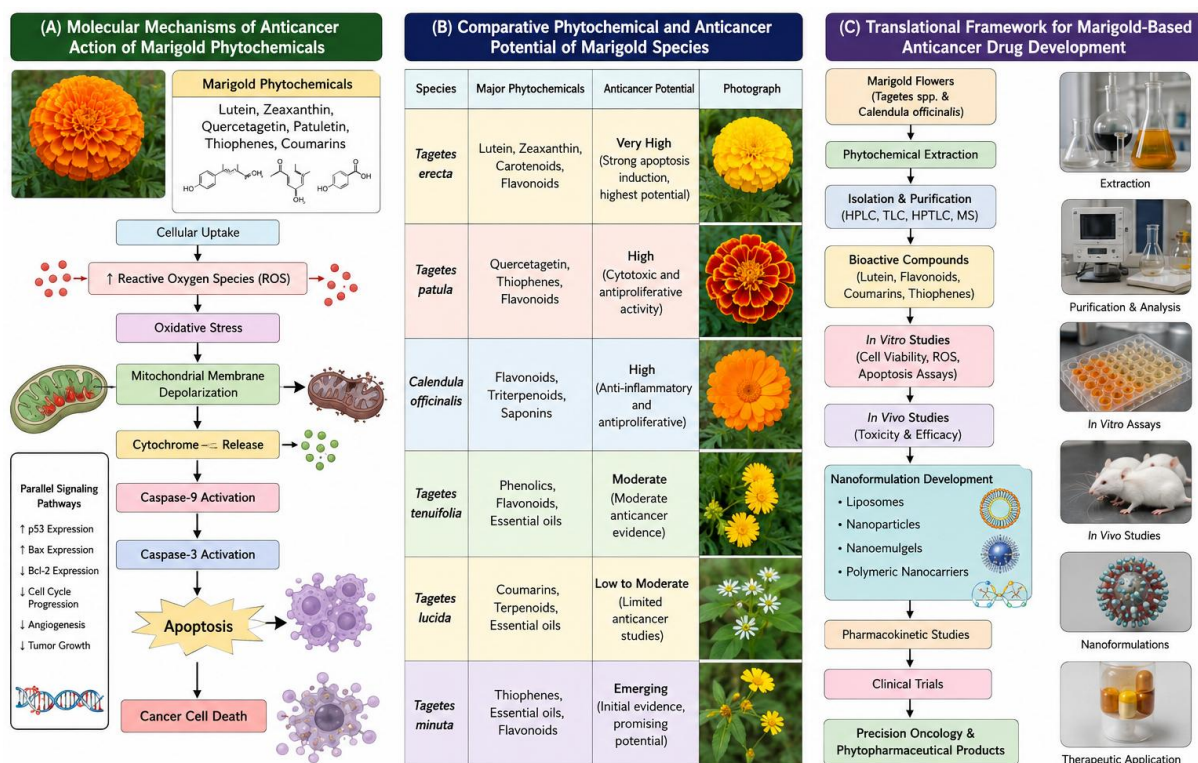
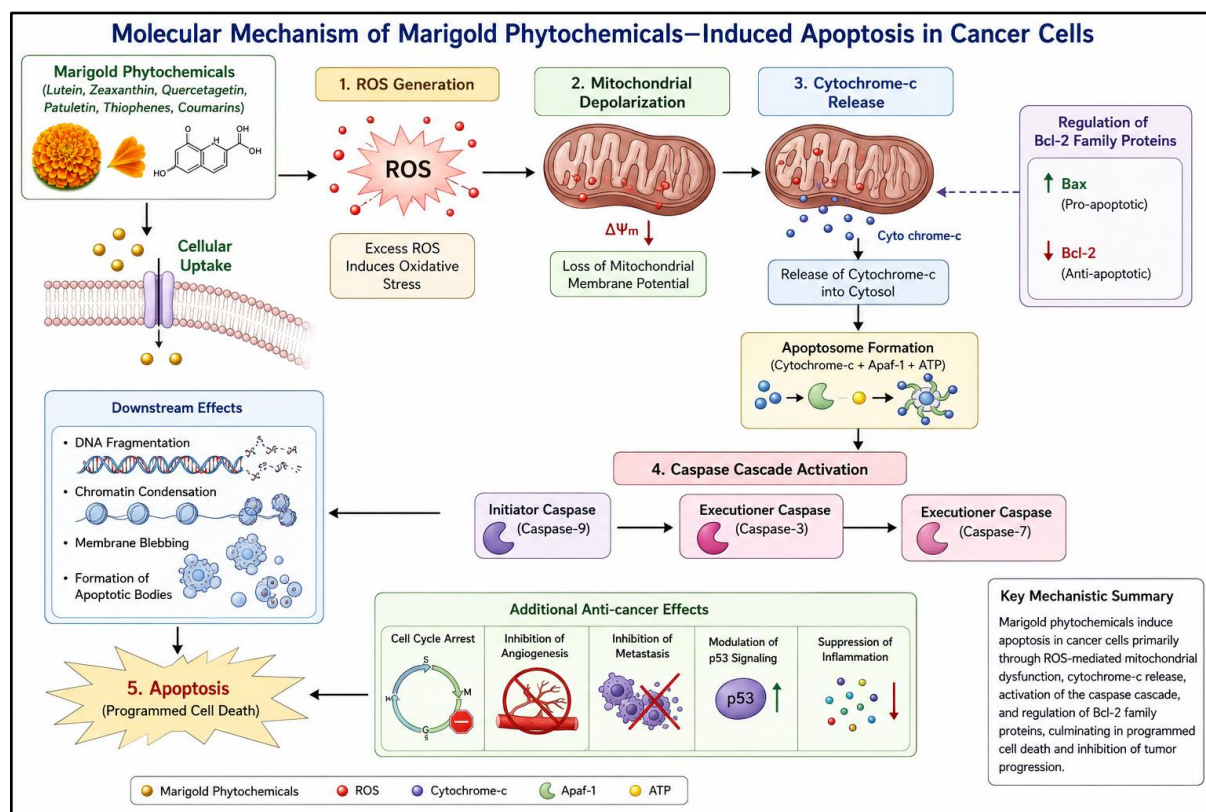


Figure No.3.3.



## 4. Results

The simultaneous extraction and alkali hydrolysis approach successfully recovered lutein from *Tagetes erecta* flower petals with high efficiency. Extraction performance was significantly influenced by solvent type, extraction temperature, liquid-to-material ratio, alkali concentration, and particle size. Smaller particle sizes ( $<0.315$  mm) resulted in greater lutein recovery due to increased surface area and improved mass transfer. The extraction kinetics demonstrated an initial rapid extraction phase followed by a slower diffusion-controlled stage, indicating that internal diffusion became the primary rate-limiting mechanism during later stages of extraction. Mathematical modeling based on Fick's Second Law adequately described the extraction behavior and provided reliable diffusion coefficients for process optimization.<sup>12</sup> Purification using preparative Thin Layer Chromatography (TLC) yielded lutein with approximately 92.2% purity. The identity of lutein was confirmed by Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Ultraviolet–Visible (UV–Visible) spectroscopy, and Mass Spectrometry (MS). Chromatographic analysis demonstrated a distinct lutein peak with retention characteristics comparable to the reference standard, confirming successful isolation and purification.<sup>11</sup>

In vitro cytotoxicity studies demonstrated that purified lutein exerted selective anticancer activity against HeLa (human cervical carcinoma) cells while exhibiting considerably lower toxicity toward Madin–Darby Canine Kidney (MDCK) normal cells. Treatment with lutein reduced cancer cell viability in a concentration-dependent manner. Furthermore, intracellular reactive oxygen species (ROS) generation increased significantly following treatment, suggesting induction of oxidative stress-mediated cell death.<sup>11</sup>

Mechanistic investigations revealed a substantial loss of mitochondrial membrane potential as determined by JC-1 staining. Western blot analysis showed upregulation of pro-apoptotic proteins Bax, p53, and Caspase-3, accompanied by downregulation of anti-apoptotic Bcl-2. DNA fragmentation and nuclear condensation observed by fluorescence microscopy further confirmed apoptosis induction. These findings collectively indicate that lutein triggers mitochondria-mediated apoptotic cell death through oxidative stress and activation of intrinsic apoptotic pathways.<sup>11</sup>

**Table 4.1. Major Results Obtained from Lutein Extraction and Anticancer Evaluation**

| Parameter           | Observation                  | Significance                         |
|---------------------|------------------------------|--------------------------------------|
| Lutein Purity       | 92.2%                        | High-quality phytochemical isolation |
| Extraction Kinetics | Biphasic extraction behavior | Diffusion-controlled process         |

|                                         |                                  |                                       |
|-----------------------------------------|----------------------------------|---------------------------------------|
| <b>Effect of Particle Size</b>          | Smaller particles improved yield | Enhanced mass transfer                |
| <b>HeLa Cell Viability</b>              | Significantly reduced            | Anticancer activity                   |
| <b>MDCK Cell Viability</b>              | Minimally affected               | Selective cytotoxicity                |
| <b>ROS Production</b>                   | Increased                        | Oxidative stress induction            |
| <b>Mitochondrial Membrane Potential</b> | Decreased                        | Mitochondrial dysfunction             |
| <b>Bax Expression</b>                   | Increased                        | Apoptosis activation                  |
| <b>Bcl-2 Expression</b>                 | Decreased                        | Suppression of cell survival          |
| <b>Caspase-3 Activation</b>             | Increased                        | Execution of apoptosis                |
| <b>DNA Fragmentation</b>                | Observed                         | Confirmation of programmed cell death |

## 5. Discussion

The present findings demonstrate that *Tagetes erecta* flowers represent an important natural source of lutein with significant pharmaceutical and anticancer potential. Simultaneous extraction and alkali hydrolysis improved lutein recovery by converting esterified lutein into its free form, thereby enhancing extraction efficiency compared with conventional extraction procedures. The successful application of diffusion-based mathematical modeling provides a scientific basis for industrial-scale optimization and commercialization of lutein production from marigold flowers.<sup>12,18,19,20,22,30</sup>

The observed selective cytotoxicity against HeLa cells suggests that lutein may possess therapeutic advantages over conventional chemotherapeutic agents, which often affect both malignant and healthy cells. The comparatively low toxicity toward MDCK cells indicates a

favorable safety profile and supports the possibility of future therapeutic applications.  
11,21,23,24,25,26,28

Mechanistically, the results indicate that lutein induces apoptosis primarily through oxidative stress-mediated mitochondrial dysfunction. Elevated intracellular ROS levels disrupt mitochondrial membrane integrity, resulting in cytochrome c release and activation of downstream caspase pathways. Upregulation of Bax and p53 together with suppression of Bcl-2 further supports activation of the intrinsic apoptotic pathway. These molecular events are consistent with established anticancer mechanisms reported for carotenoids and flavonoids from medicinal plants.<sup>11,25,32,34,35,36,37,38,39,40,41,42</sup>

## **5.1. Pharmacokinetics, ADME, Bioavailability Challenges, and Regulatory Considerations**

### **Pharmacokinetics of Marigold-Derived Phytochemicals**

The therapeutic efficacy of marigold-derived phytochemicals depends not only on their biological activity but also on their pharmacokinetic behavior. Among the major constituents, lutein has received the greatest attention due to its abundance in *Tagetes erecta* and its demonstrated antioxidant and anticancer properties. Current evidence suggests that lutein is absorbed primarily through the intestinal lymphatic system following incorporation into mixed micelles formed in the presence of dietary lipids. After absorption, lutein is transported by lipoproteins and distributed to various tissues, including the liver, retina, adipose tissue, and other organs. However, comprehensive pharmacokinetic studies for most marigold phytochemicals, including quercetagenin, patuletin, coumarins, and thiophenes, remain limited. Consequently, important parameters such as absorption rates, plasma half-life, tissue distribution, metabolism, and elimination are insufficiently characterized, restricting the clinical translation of these compounds.<sup>11,21,22,23,24,26,28,30,31</sup>

### **ADME (Absorption, Distribution, Metabolism, and Excretion)**

The ADME profile of marigold phytochemicals remains an underexplored area of research. Absorption is influenced by molecular structure, lipophilicity, and formulation characteristics. Carotenoids such as lutein and zeaxanthin generally exhibit poor aqueous solubility, which may limit intestinal uptake. Following absorption, these compounds are distributed through lipoprotein-mediated transport and may accumulate in lipid-rich tissues. Metabolic transformation occurs primarily in the liver through oxidation, reduction, and conjugation reactions, producing metabolites with potentially altered biological activities. Excretion is mainly mediated through biliary and fecal pathways, although renal elimination may contribute for certain metabolites. The lack of systematic ADME investigations for many marigold-derived compounds represents a significant knowledge gap that should be addressed through future preclinical and clinical studies.<sup>21,22,23,24,26,28,29,30,31</sup>

### **Bioavailability Challenges**

Despite promising in vitro anticancer activity, the bioavailability of marigold phytochemicals remains one of the major obstacles to therapeutic development. Many bioactive constituents exhibit poor water solubility, chemical instability, limited intestinal absorption, rapid

metabolism, and low systemic availability. Lutein, for example, is highly hydrophobic and susceptible to degradation by light, oxygen, and heat, which may reduce its therapeutic effectiveness following oral administration. Furthermore, differences in extraction methods, purification procedures, and formulation strategies can significantly influence bioavailability and biological activity. To overcome these limitations, emerging delivery systems such as liposomes, nanoemulsions, solid lipid nanoparticles, polymeric nanoparticles, nanoemulgels, and phytosomes have been investigated. These approaches may enhance solubility, improve stability, prolong circulation time, facilitate targeted delivery, and increase therapeutic efficacy.<sup>2,11,22,24,26,30,43,44,45,46,47,48</sup>

### Regulatory Considerations

The successful commercialization of marigold-derived phytopharmaceuticals requires compliance with stringent regulatory requirements related to quality, safety, efficacy, and manufacturing consistency. One of the primary challenges is the lack of standardized extracts with reproducible phytochemical profiles. Variability in cultivation conditions, geographical origin, harvesting practices, and extraction techniques can lead to significant differences in chemical composition and biological activity. Regulatory agencies generally require comprehensive characterization of active constituents, validated analytical methods, toxicological evaluation, pharmacokinetic data, and evidence of clinical efficacy before product approval. In addition, Good Agricultural and Collection Practices (GACP), Good Manufacturing Practices (GMP), and quality-control standards must be implemented to ensure product consistency. Future research should focus on establishing standardized extraction protocols, validated biomarkers, and robust clinical evidence to facilitate regulatory acceptance and global commercialization of marigold-based therapeutics.<sup>2,22,30,43,44,46,48,49,50</sup>

**Table 5.2. Translational Challenges and Future Requirements for Marigold-Derived Therapeutics**

| Aspect            | Current Status         | Major Limitation                 | Future Requirement                                 |
|-------------------|------------------------|----------------------------------|----------------------------------------------------|
| Pharmacokinetics  | Limited data available | Incomplete PK profiles           | Comprehensive PK studies <sup>21,22,23,24,30</sup> |
| Absorption        | Variable and often low | Poor solubility                  | Advanced delivery systems <sup>22,24,30,44</sup>   |
| Distribution      | Partially understood   | Limited tissue-distribution data | Biodistribution studies <sup>21,23,24,30</sup>     |
| Metabolism        | Poorly characterized   | Unknown active metabolites       | Metabolomic investigations <sup>22,24,28,29</sup>  |
| Excretion         | Limited evidence       | Incomplete elimination data      | ADME studies <sup>22,24,30</sup>                   |
| Bioavailability   | Generally low          | Hydrophobicity and instability   | Nanoformulations <sup>22,24,26,43–48</sup>         |
| Standardization   | Inconsistent extracts  | Batch-to-batch variation         | Standardized protocols <sup>2,22,49,50</sup>       |
| Clinical Evidence | Mostly preclinical     | Lack of human trials             | Randomized clinical studies <sup>11,25,49</sup>    |

|                     |         |                                       |                                                   |
|---------------------|---------|---------------------------------------|---------------------------------------------------|
| Regulatory Approval | Limited | Insufficient safety and efficacy data | Regulatory-compliant development <sup>49,50</sup> |
|---------------------|---------|---------------------------------------|---------------------------------------------------|

This section highlights that although marigold phytochemicals possess significant therapeutic promise, successful clinical translation will require comprehensive pharmacokinetic characterization, improved bioavailability strategies, standardized manufacturing processes, and rigorous regulatory evaluation.

## Critical Analysis

Despite promising results, several limitations remain. First, the study relied predominantly on *in vitro* cell culture models, which cannot fully replicate the complexity of tumor microenvironments *in vivo*. Second, pharmacokinetic properties such as absorption, distribution, metabolism, and excretion of purified lutein were not investigated. Third, the molecular targets responsible for ROS generation remain incompletely characterized. Fourth, the lack of animal studies and clinical trials limits translation of these findings into therapeutic applications.<sup>11-12</sup>

Another important limitation concerns bioavailability. Although lutein exhibits potent biological activity *in vitro*, its hydrophobic nature may restrict absorption and systemic delivery *in vivo*. Emerging nanoformulation strategies including liposomes, nanoemulsions, polymeric nanoparticles, and targeted delivery systems may overcome these challenges and significantly improve clinical efficacy. Therefore, future research should integrate extraction optimization with advanced drug-delivery technologies and translational pharmacology studies.<sup>11</sup>

**Table 5.1. Critical Evaluation of the Study**

| Strengths                      | Limitations                       | Future Directions          |
|--------------------------------|-----------------------------------|----------------------------|
| High lutein purity             | <i>In vitro</i> study only        | Animal studies             |
| Selective anticancer activity  | No pharmacokinetic data           | Pharmacokinetic evaluation |
| Mechanistic apoptosis evidence | No clinical validation            | Clinical trials            |
| Extraction optimization        | Limited molecular target analysis | Omics-based studies        |

|                       |                          |                             |
|-----------------------|--------------------------|-----------------------------|
| Mathematical modeling | Bioavailability concerns | Nanoformulation development |
|-----------------------|--------------------------|-----------------------------|

## 6. Conclusion

The present investigation demonstrates that *Tagetes erecta* flowers constitute a valuable natural source of lutein with significant anticancer potential. Simultaneous extraction and alkali hydrolysis effectively enhanced lutein recovery, while chromatographic purification yielded a highly pure bioactive compound suitable for biological evaluation. Purified lutein exhibited selective cytotoxicity toward cervical cancer cells and induced apoptosis through ROS-mediated mitochondrial dysfunction, activation of Bax, p53, and Caspase-3, suppression of Bcl-2, and DNA fragmentation.<sup>11,12,21,25,26,32,35,36,37,38,39,40,41,42</sup>

From a translational perspective, these findings support the development of marigold-derived lutein as a promising nutraceutical, phytopharmaceutical, and adjunctive anticancer agent. However, successful clinical application will require further investigation of pharmacokinetics, toxicity, molecular targets, formulation strategies, and large-scale clinical validation. Integration of nanotechnology-based delivery systems with standardized extraction methodologies may accelerate the translation of marigold-derived bioactives from laboratory research to precision oncology and evidence-based cancer therapeutics.<sup>22,23,24,28,29,30,43,44,45,46,47,48,49,50</sup>

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