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Floral pigments and its cytotoxic activity: An update

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In India, approximately 80,000 tonnes of flower waste are produced every year. The increase in floral waste has driven attention to utilise it in various fields. Flowers, with their intricate properties, are applicable in the dye, paper, incense, perfumery, and pharmaceutical industries. It can also be used for vermicomposting. The pigments extracted from temple flowers are known to show antibacterial and antifungal activity and are finding their application as anti-cancerous agents. This paper comprises an overview of the major floral pigments such as carotenoid, flavonoids and xanthophyll, which are found in temple flowers and their extraction procedures using conventional methods such as agitation, centrifugation, Soxhlet extraction and non-conventional methods such as enzymatic extraction, microwave-assisted extraction, pressurised liquid extraction and ultrasound-assisted extraction. This is followed by a brief discussion of the cytotoxic effect *in vitro* using cell lines such as MCF-7, HeLa, and DU145.

Keywords: Anti-cancer agents, Cell lines, Cytotoxicity, Floral pigments

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Introduction

India is a land of devotees, deities, temples, and celebrations where flowers are offered, used in decoration, and have importance in social disciplines. Flowers are used in celebrations of humans' birth to death. Everyday use of flowers has left them untreated due to religious beliefs and led to waste mismanagement, resulting in solid waste[1]. In India, approximately 80,000 tonnes of flower waste is produced every year[2]. This is an issue of concern, due to which the application of flower waste in various fields is the need of the hour.

The employment of solid waste fermentation in the treatment of flower waste has aided in the production of certain valuable products like biofuels, bioethanol, bio-surfactants, compost, dyes, incense sticks, food, organic acids, pigments, polyhydroxybutyrate-co-hydroxyvalerate and sugar syrup. The accumulating floral waste has also led to the formation of industries of handmade paper[3].

Flower waste also serves a purpose in the dye industry, where it is shown to be a better alternative to synthetic dyes. The major advantage of floral dyes is that they are eco-friendly, easily available, inexpensive, and non-allergic to the skin. Few dyes have been shown to have medicinal properties like antioxidant and radical scavenging activity.

There are reports where floral wastes are applicable in the medicinal fields. Waste flowers of *Madhuca* are found to be used in the treatment of bronchitis, and they also possess antibacterial, antifungal and antivirus activity. *Lilium* is used to cure jaundice[3]. *Hibiscus rosa-sinensis* flower has antioxidant, antimicrobial, cytotoxic and antigenotoxic effects[4]. Pigments like carotenoid, betalain and anthocyanins in flowers exhibit antioxidant properties, which have applications in the treatment of cancer.

Advancements in cancer treatment have led to the development of various chemotherapeutic drugs currently in clinical use. However, these drugs often come with side effects, including nephrotoxicity and neurotoxicity[5]. The drawbacks in chemically synthesised drugs lead to the search for new alternatives from biological sources and are now a vast field that needs attention.

Various studies have reported that flowers of different varieties serve as a significant source of medicinal pigments. Traditionally, plants and their extracts have medicinal value that is applicable in every field of medicine. Starting from the wound healing process by the action of curcumin present in turmeric (*Curcuma longa* L.)[6] to the anticancer potential of phytochemicals luteolin, chlorogenic acid,

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rutin, quercetin, and apigenin of the *Chrysanthemum* cultivars[7]. These pigments have proven to be effective in the treatment of various lifestyle diseases.

Several developments have been made in the domain of flower pigment extraction processes over the course of time. Scientists have come up with conventional and non-conventional methods of extraction, of which soxhlet and solvent methods have been deemed the most popular[8]. Characterisation is necessary to identify the pigment of action from the flower extract. The method observed frequently was High-Performance Liquid Chromatography (HPLC), along with various methods of mass spectroscopy[9].

The action of these phytochemicals has been well studied, and it has been observed that they are potential sources of bioactive compounds that can be used in the treatment of cancer. The cytotoxic effect of these phytochemicals has been studied against cell lines, and the outcomes have been assayed *via* cytotoxic studies like 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay, Water Soluble tetrazolium (WTS) assay, etc. At the molecular level, the interaction of the phytochemicals and the cellular components was determined[7,10].

This review focuses on the medicinal properties of various pigments present in flowers, which are usually found as temple waste. In addition, we discuss methods of extraction of pigments, their cytotoxic effects on cell lines and the mechanism of cell death.

Temple flowers and their pigments

The major flowers studied in this review include Marigold, Hibiscus, Rose and Chrysanthemum (Table 1, Fig. 1).

Pigments are substances that are coloured and absorb light selectively. Among the major classes of pigments, carotenoids, flavonoids, and xanthophylls have shown medicinal properties that aid in our study. Tables 2–4 depict the class of pigments with sub-classes and their property.

Extraction of flower pigments

Over the years, there have been advancements in the extraction techniques of various bioactive compounds and pigments from various parts of the plant, like the stem, leaves, flowers, etc. Factors like temperature and pH are significant in the extraction of floral pigments as they are susceptible to colour change at times, leading to decolourisation. Carotenoids are pigments which are highly sensitive to heat and light as they are feasible to undergo thermal degradation and photodegradation. The

Table 1 — Flowers commonly found in temple waste with the scientific name and the various pigments present in them

Common Name	Scientific Name	Pigments Present	Pigment of Interest	References
Marigold	*Tagetes erecta*	α-Carotene Antheraxanthin β-Carotene Lutein Phytofluene Quercetagetin	α-Carotene	11
Hibiscus	*Hibiscus rosa-sinensis*	Anthocyanins Kaempferol Myricetin Proanthocyanidin Quercetrin, Rutin	Quercetin	12, 13
Rose	*Rosa*	Anthocyanin Lycopene Rubixathin Taraxaxanthin Lutein Zeaxanthin β - carotene Isoquercitrin Quercitrin Tiliroside	Lycopene Lutein Zeaxanthin	14, 15
Chrysanthemum	*Chrysanthemum morifolium*	Anthocyanidine Violanthin Lutein α-carotene β-carotene	β-Carotene	9

Table 2 — Properties of floral carotenoids

Pigment	Properties	References
α-Carotene	1. Present in food and shows provitamin-A activity. 2. Antioxidant and anti-carcinogenic and enhances the immune system function.	16
β-Carotene	1. Pro-vitamin A which is optimal and naturally occurring. 2. Lipid radical scavenger and a singlet oxygen quencher.	17
Lycopene	1. Prevents cardiovascular diseases and cancer. 2. Decreases the level of cholesterol by inhibiting the enzyme essential for cholesterol synthesis.	18
Phytofluene	1. Therapeutic in treating neoplasm.	19
Zeaxanthin	1. Combat light-induced damage mediated by reactive oxygen species. 2. Zeaxanthin-dependent quenching is seen in higher plants.	20

Table 3 — Properties of floral flavonoids

Pigment	Properties	References
Anthocyanidine	1. Antioxidant and anti-obesity effect.	21
Anthocyanin	1. Antimicrobial, neuro-protective and anti-cancer effects. 2. Improve visual health.	21
Isoquercitrin	1. Reactive Oxygen Species ROS scavengers 2. Exhibit dose-dependent antioxidant activities.	22
Kaempferol	1. Potent anti-inflammatory effect. 2. Antioxidant, antimicrobial, anticancer, cardioprotective, neuroprotective, antidiabetic effect	23
Myricetin	1. Antioxidative properties, anticarcinogen and antimutagen. 2. Therapeutic potential in cardiovascular diseases and diabetes mellitus.	24
Proanthocyanidin	1. Antioxidant, anticancer, anti-diabetic, neuroprotective, and antimicrobial. 2. Present in plants as a defence against biotic and abiotic stressors.	25
Quercetagetin	1. Exhibits *in vitro* antioxidant, anti-diabetic and antilipemic activities. 2. Used in treatment of tumours, cancer, and cardiovascular diseases.	26
Quercitrin	1. Exhibits strong antioxidant and anti-inflammatory properties and applied in cardiovascular disease, osteoporosis & pulmonary disease. 2. Scavenges ROS to protect mesenchymal stem cell from ROS-induced oxidative damage.	27
Rutin	1. Exhibits nutraceutical effect and used in the treatment of cancer, diabetes, hypertension, and hypercholesterolemia. 2. Used as an antimicrobial, antifungal, and anti-allergic agent.	28
Tiliroside	1. Antioxidant, anti-obesity, antidiabetic, and other effects. 2. Used in the treatment of various ailments and as a food supplement.	29

Table 4 — Xanthophyll present in flowers and their properties

Pigment	Properties	References
Antheraxanthin	1. Exhibits High antioxidant activities. 2. Shows potent lipid peroxidation inhibitory activities.	30
Lutein	1. Filter of high-energy blue light 2. Antioxidant that quenches and scavenges photo-induced ROS.	31
Rubixathin	1. Exhibit antioxidant activity. 2. Formerly used as a food colourant.	32
Violanthin	1. Present in KabasuraKudineer (poly-herbal formulation), which may inhibit covid -19. 2. Antioxidant, antimicrobial and anti- hypocholesterolaemic, antifungal and antibacterial, antidiabetic.	33,34

choice of the extraction method should be such that the extract contains a maximum quantity of bioactive compounds or pigments of interest with a minimum amount of solvent.

Among the extraction techniques, it is broadly classified into conventional and non-conventional methods (Fig. 2).

The non-conventional or green extraction methods (Fig. 2) are another category of extraction processes where the process is green as they avoid using harmful organic solvents or use organic solvents in less quantity, giving an extract free of solvent. This method is beneficial in the extraction of thermolabile compounds. It has been shown to produce

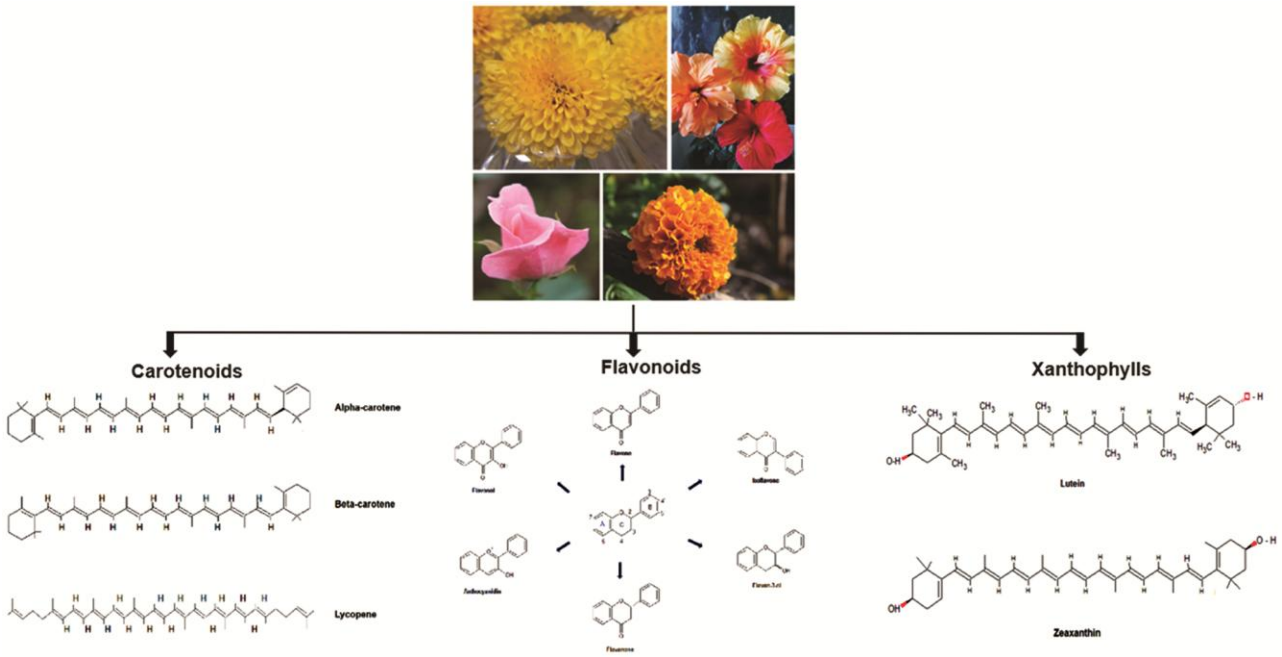

Fig. 1 — Temple flowers and their major pigments.

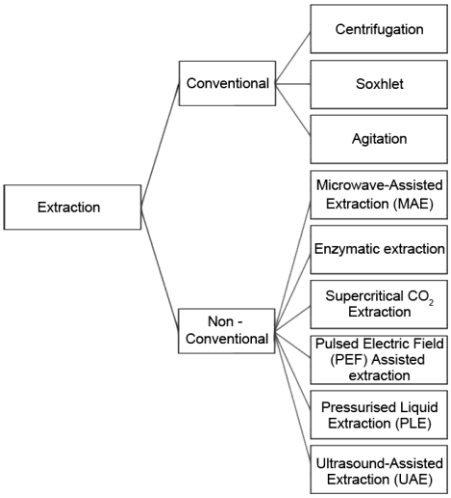

Fig. 2 — Schematic representation of extraction methods.

greater carotenoid content when compared to the conventional forms with high purity and yield[8]. Table 5 depicts the methods of extraction of carotenoids with yield and the material used.

The study by Zhang *et al.* reported that the usage of super-critical fluid in the extraction of carotenoids has given the maximum yield of 6.872 mg/g[43]. The sample used was *Tagetes erecta* L., and the optimal conditions used for the experiment were 55°C, 50 MPa and 180 min. The extraction solvent used was Acetone. In the literature, the advantages of the application of Supercritical Carbon dioxide in the extraction process are low toxicity, low cost, and ease of separation of the extracted product. Henceforth, modifications in the traditional methods have led to greater extraction yields.

Deepika *et al.* used *T. erecta* L. flowers and segregated them based on their blooming[42]. The samples were dried, ground, and powdered and were subjected to solvent extraction; here, the solvent used was acetone and hexane in a ratio of 1:1. The extraction conditions were 24 hours at room temperature (20-24 h). The yield of the carotenoid extract from open flowers was 4.135 mg/g.

The extraction method mentioned above by Deepika *et al.* is feasible in simple lab conditions[42]. In a comparison of the yield of carotenoids from flowers, *T. erecta* L. flowers gave the maximum yield of 6.872 mg/g *via the* supercritical CO2 Extraction SFE method of extraction. Next in line is the *Chrysanthemum morifolium* flower, where the carotenoid extracted from this flower was 0.506 mg/g carotenoid of fresh weight. The solvents used in this extraction process were methanol, water, and formic acid (70:28:2, v/v/v), where the powdered samples were incubated for 15 minutes. Later, it was subjected to ultrasonication for 40 minutes. The effect of ultrasonication has enabled the cells to open and result in higher carotenoid content.

Lutein is found 60-70% in marigold flowers and has applications in the food and pharmaceutical

Table 5 — Methods of extraction of carotenoids

Entries	Flower used	Method of extraction	Solvents used in extraction	Yield	Reference
1	*Hibiscus rosa – sinensis*	Solvent extraction	Ethanol, Ethyl acetate and Chloroform	0.00091 mg/g	35
2	*Chrysanthemum morifolium*	Solvent extraction and HPLC-MS	N-hexane: Acetone: Absolute Ethanol 2:1:1, v: v: v	0.0118– 0.16 mg/g (α-carotene) 0.005–0.27 mg/g (β-carotene)	36
3	*Chrysanthemum morifolium*	Solvent extraction and HPLC	N-hexane: Acetone: Absolute ethanol 2: 1: 1, v: v: v	0.009–0.015 mg/g	37
4	*Chrysanthemum morifolium*	Solvent extraction and HPLC	N-hexane: Acetone: Absolute ethanol 2: 1: 1, v: v: v	0.133–0.192 mg/g	38
5	*Hibiscus rosa – sinensis*	Solvent extraction	Ethanol	0.162 mg/g	39
6	*Calendula officinalis*	alkaline hydrolysis	Ethanol (0.2 % t-BHT)/Acetone — 6:4 (v/v)	0.22–0.904 mg/g of β carotene	40
7	*Dendranthema grandiflorum*	Solvent extraction	Ethanol + 0.1% Ascorbic acid	0.45–18 mg/g	9
8	*Tagetes patula*	Alkaline hydrolysis	Ethanol (0.2 % t-BHT)/Acetone — 6:4 (v/v)	0.465 mg/g of β carotene	40
9	*Chrysanthemum morifolium*	Ultra-sonication	Methanol, Water, and Formic acid	0.506 mg/g	41
10	*Tagetes erecta*	Solvent extraction	Acetone + Hexane	4.165 mg/g of carotenoid	42
11	*Tagetes erecta*	supercritical fluid extraction (SFE)	Supercritical Carbon dioxide	6.872 mg/g of carotenoid	5
13	*Hibiscus rosa – sinensis*	Solvent extraction	Acetone: Hexane (1:1 v/v)	6.2 µg/mL	43

industries; its extraction is of importance. Lutein is found naturally in acylated form, where it is chemically bound to various kinds of fatty acids. In *T. erecta* L. flowers, the main lutein present is lutein di-palmitate (50%); due to the high lipophilicity of the long-chain carbon skeleton of lutein fatty acid esters, it becomes difficult to extract them. Henceforth, the extraction of lutein fatty acid esters should be followed by de-esterification reactions such that the lutein is free of esters[45].

Table 6 shows the methods of extraction of lutein with yield and the material used. Here, the various techniques used in the extraction of lutein give an overview of the techniques in use in the current scenario and will give output to interpret the better technique to be used.

Among the papers discussed above, the technique by Boonnoun *et al.* provides a better quantity of lutein than other techniques[45]. This technique was a one-step process of simultaneous DME extraction and de-esterification of marigold flowers. For the extraction process, the DME to dried marigold flowers ratio was 33:0.5 (g/g), and the Ethanol to dried marigold flowers ratio was 10:0.5 (mL/g); for the de-esterification process 5% w/v Potassium hydroxide Ethanol (KOH-EtOH) concentration was used at a temperature of 35°C and simultaneous extraction and de-esterification time was 1 hour. This reaction condition yielded 20.71 mg free lutein /g dried marigold flowers.

Cytotoxicity studies using cell lines

Cell lines have been a boon in revolutionising scientific research and have found their application in antibody production, generation of artificial tissues (e.g., artificial skin), the study of gene function, and synthesis of biological compounds, e.g., therapeutic proteins testing drug metabolism and cytotoxicity, vaccine production[46].

Cytotoxicity can be described as the toxicity that is caused due to the activity of chemotherapeutic agents on living cells. Cytotoxicity assays provide information on the minimum dosage required to cause cell death. The methods of determination of cytotoxicity are by uptake of colours of the stains, tritium-labelled thymidine uptake assay, the

Table 6 — Methods of Extraction of Lutein

Entries	Flower used	Method of extraction	Solvents used in extraction and conditions	Yield	Reference
1	*Chrysanthemum morifolium*	Solvent extraction and HPLC-MS	N-hexane: Acetone: Absolute Ethanol 2:1:1, v: v: v	0.0015-0.245 mg/g	36
2	*Calendula officinalis*	Alkaline hydrolysis	Ethanol (0.2 % t-BHT)/Acetone — 6:4 (v/v)	0.046 mg/g	40
3	*Tagetes patula*	Alkaline hydrolysis	Ethanol (0.2 % t-BHT)/Acetone — 6:4 (v/v)	0.85-1.362 mg/g	40
4	*Tagetes erecta* L.	Enzyme-assisted aqueous two-phase extraction (EA-ATPE)	30% (w/w) ethanol/19% (w/w), Ammonium Sulphate, 4.2 U g^{-1} pectinase	5.59±0.13 mg/g	45
5	*Tagetes erecta* L.	Microwave and Enzyme co-Assisted Aqueous Two-Phase Extraction (MEAATPE)	28% (w/w) ethanol/20% (w/w), Ammonium Sulphate, Pectinase enzyme (0.45 Ug^{-1}), enzymatic hydrolysis for 150 min at 45 °C, microwave power of 270 W and microwave duration of 120 s.	7.32 mg/g	45
6	*Tagetes erecta*	Agitation	Dimethyl Ether DME (33:0.5 (w/w) One-step process	20.71 mg free lutein /g dried marigold	44

MTT method, water Soluble tetrazolium WST assay, and dehydrogenase-based assay[47].

Studies utilising *Chrysanthemum* extract

Hodaei *et al.* evaluated the cytotoxic effect of methanolic flower extracts of various *Chrysanthemum* cultivars in the human breast cancer cell lines (MCF-7) and human lymphocytes[7]. Using the MTT assay, they determined that a concentration of 312 µg mL^{-1} of the flower extracts of the cultivar "Dorna2" and "Farhood" could inhibit the viability of the cancer cell lines by 50%. The result indicated that the varied mixture of phytochemicals present in the *Chrysanthemum* extract had potential anticancer activity. The high risk of breast cancer is due to the high levels of estrogen bound to the estrogen receptors present in breast cancer cells. Flavonoids act as selective estrogen receptor modulators that change the activities of estrogen receptors, preventing the growth of breast cancer cells. The study showed that phytochemicals like luteolin, chlorogenic acid, rutin, quercetin, and apigenin from the flower extract of *Chrysanthemum* are the targets that belong to the estrogen receptors such as ESR1, ESR2 and PGR and are vital therapeutic targets of breast cancer (Fig. 3). The study also showed that the *Chrysanthemum* phytochemicals like rutin not only regulates breast cancer pathways but are also involved in the

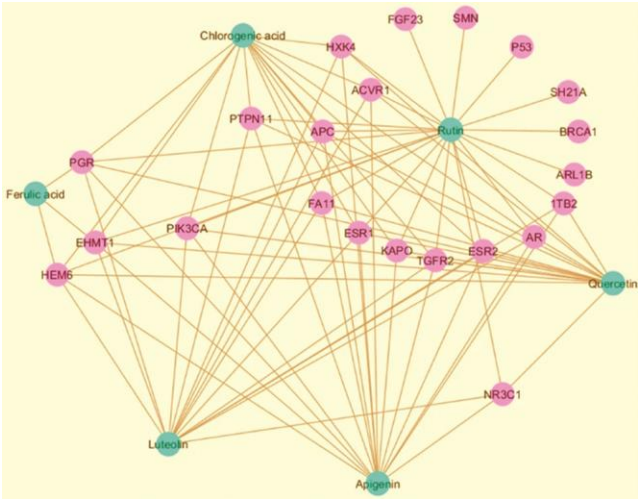

Fig. 3 — Compound-Target (C-T) network of *Chrysanthemum* bioactive compounds. Nodes represent bioactive compounds and targets. (Reproduced from Hodaei *et al.* 2021).

prevention of breast cancer from developing into gastric and other types of cancers.

In their study, Ma *et al.* used *C. morifolium* extract-mediated silver nanoparticles to improve the cytotoxicity effect of A549 lung cancer cells[48]. On performing the cytotoxic assay, they confirmed that the viability of the A549 cells decreased as the concentration of the silver nanoparticles increased. Their result suggested that the silver nanoparticles transferred onto the surface of the cancer cells and

thus extended their proliferation ability, thereby undergoing a decrease in the number of cells and approaching the decline phase, leading to apoptosis and, finally, cell death. The 53% cell viability was observed at 200 µg/mL.

Kim *et al.* studied the anticancer effect of *Chrysanthemum indicum.* L. on the STAT3 signalling pathway in human prostate (DU145) cancer cells, human multiple myeloma cell lines (U266) and human breast carcinoma (MDA-MB-231)[10]. Using the MTT assay, they checked the cytotoxic effect of ethanol extracts and the four isolated fractions (hexane, Dichloromethane (CH_2Cl_2), Ethyl Acetate, EtOAc and Butanol (BuOH)) from *C. indicum*. The most potent cytotoxic effect was seen against DU 145 and U266 cancer cells but not in the case of the MDA-MB-231 cancer cells. The mortality rate went up to 50% when the concentration of MCI (methylene chloride fraction of *C. indicum*) was approximately 40 µg/mL in the case of DU145 and U266 cells. The MCI suppresses the activation of STAT3 and, in turn, blocks upstream JAK1 and JAK2 but not the Src (Sarcoma) pathway (Fig. 4). Since the STAT3-regulated gene product expression is downregulated, it corresponds to the accumulation of the cell cycle at sub-G1 phase, the induction of caspase-3 activation and finally leading to apoptosis. MCI also contains a lot of bioactive compounds such as sudachitin, acacetin, chrysoeriol, and hesperetin, of which the first three brought about cytotoxicity, suppressed the STAT3 activation and induced apoptosis.

Hairul *et al.* performed the aqueous extraction to obtain the flower extract of *C. indicum*[49]. The cell lines studied were human acute promyelocytic leukaemia HL- 60 cells (ATCC CCL- 240). The cells were treated with the flower extracts for a period ranging from 24-72 hours, and the concentration of the extract ranged from 12.5-400 µg/mL. The IC_{50} (half-maximal inhibitory concentration) results were 320, 205 and 168 µg /mL for 24, 48 and 72 hours respectively. Here, the flower extract showed low toxicity as the IC_{50} value was more than 100 µg/mL even after 72 hours, but important morphological alternations were observed like cell blebbing, cell shrinking and apoptotic body formation in HL-60 cells due to the high toxicity of the extracts at IC_{50} concentration. The authors reported that the extract was efficient in causing morphological changes but failed to show anti-cancer properties due to the low cytotoxicity.

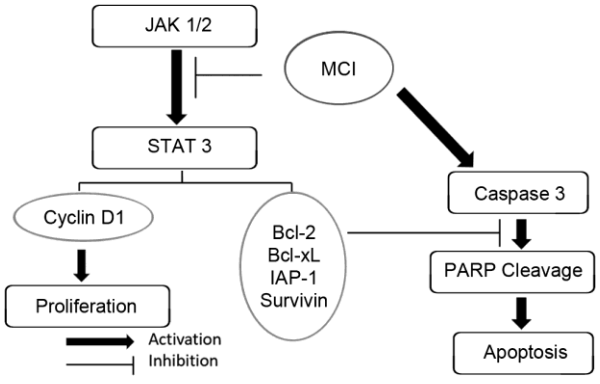

Fig. 4 — Schematic diagram indicating the effect of MCI on STAT3 signalling pathway and apoptosis. (Reproduced from Kim *et al.* 2012).

Studies utilising Chamomile extracts

Sak *et al.* have used Melanoma SK-MEL-2 and epidermoid carcinoma KB cells as model systems to perform the cytotoxic assay of the methanol flower extracts of pot marigold and German chamomile (*Matricaria recutita* L.)[50]. The sulforhodamine B cytotoxic assay was performed and resulted in 37.4% growth inhibition on SK-MEL-2 and 20.8% growth inhibition on KB cells at a concentration of 100 µg/mL of the methanol extract of marigold flowers. The results for methanol extracts of German chamomile (*M. recutita* L., Asteraceae) were IC_{50} 40.68±2.92 µg/mL against SK-MEL cells and IC_{50} 71.42±2.34 µg/mL against KB cells. They conclude that the phenolic compounds present in German chamomile (*M. recutita* L., Asteraceae), such as apigenin, luteolin, quercetin and patuletin had a better cytotoxic potential against SK-MEL cells than against the KB cells, thus, reporting that the methanolic extracts of chamomile (*M. recutita* L.) had potential anti-melanoma activity.

Studies utilising *Calendula* extracts

Gómez de Cedrón *et al.* performed the extraction of pigments from *C. officinalis*, commonly known as Marigold, *via* the non-conventional method of supercritical fluid extraction (SFE) with the aid of supercritical CO_2[(Ref. 51)]. The cell lines focused here was the pancreatic cancer cells MiaPaCa-2 and Panc-1. The marigold SFE was investigated for its impact on the mitochondrial oxidative respiration of pancreatic cancer cells, and the results showed 39.8 (±4.6) µg/mL for MiaPaca-2 cells, indicating 50% inhibition of cell proliferation after 48 hours of treatment. The Lethal Concentration 50 (LC50), which represents the concentration of extract needed

for cell death, was found to be 78.5 (±1.4) µg/mL after 48 hours of treatment. The study showed that the marigold SFE pre-treated cells produced reduced maximal extracellular acidification rate (ECAR) levels when compared to the control cells, indicating that the treatment compromised aerobic glycolysis. Marigold SFE pre-treated cells also showed reduced ATP content in a dose-dependent manner. *BMP8B* functions as a tumour suppressor in pancreatic cancer, wherein it inhibits invasion and tumour growth of pancreatic cancer xenografts. Upregulation of *BMP8B* was found in marigold SFE pre-treated cells, indicating that the extracts of the marigold SFE had the ability to inhibit cell invasion by means of Matrigel-coated chambers, in addition to EMT and stemness markers in a dose-dependent manner.

Abutaha *et al.* have utilised the Soxhlet method for the extraction process from the plant material *C. arvensis* L.[52]. Cell lines used in the study were MCF-7 and MDA-MB-231, on which cytotoxicity of the plant extract was measured using the MTT assay. Among these, the extract of *Calendula arvensis* flower ethyl acetate extract (CAF EtOAC) had a greater impact on the cell lines in the study, and the activity was in a concentration-dependent manner. The results of the assay for the estrogen-positive MCF-7 cells were found to be 70 µg/ mL, is IC_{50} (half-maximal inhibitory concentration) and for triple-negative MDA-MB-231 cells the IC_{50} value was 78 µg/mL. A Lactate Dehydrogenase Assay (LDH) assay was performed to confirm the cytotoxic effect of the extract, wherein the levels of LDH increased in the media of the treated MDA-MB-231 and MCF-7 cells. LDH increase is associated with apoptosis. To confirm apoptosis, Hoechst 33258 staining was performed. Subsequently, evidence supporting apoptosis, such as DNA fragmentation, cytoplasmic condensation, detachment, and cell shrinkage, was observed. Apoptosis effects were observed in both cell lines in a concentration-dependent manner upon treatment with the extract (CAF EtOAC). The *Bcl-2* family genes, *Bcl-2* anti-apoptotic and *Bax* pro-apoptotic genes, have the ability to control the activation of apoptosis by their expression. The ratio of *Bcl-2* and *Bax* genes determines cell survival and death. The treatment of the extract CAF EtOAC against cell lines MCF-7 and MDA-MB-231 has resulted in notable upregulation of *Bax* and downregulation of *Bcl-2* after 24 hours. By performing Gas Chromatography-Mass Spectroscopy (GC-MS), they concluded the presence of linolenic and palmitic acid in the CAF EtOAC, which has potential cytotoxic effects and antimetastatic properties.

Abudunia *et al.* obtained methanol and hexane flower extracts of *C. arvensis* using Soxhlet extraction, while aqueous extracts were obtained through maceration in cold water[53]. For the cytotoxic study of the extracts, cancer cells from myeloid cell lines were used. MTT cytotoxic assay was performed, and the IC_{50} value was found to be 31 µg/mL for both methanolic and aqueous extracts. This suggests that both extracts are efficient cytotoxic bioagents. When compared to other extracts, the methanolic extract exhibited an 89% inhibition of myeloid cells at a concentration of 100 mg/mL at 24 hours (P <0.05), proving to be a significant antimyloid cancer agent.

Studies utilising Rose extracts

Turan *et al.* studied the effect of *Rosa canina* extract on human colon adenocarcinoma (WiDr, ATCC-CCL-218) cancer and human colon normal epithelial cell lines (CCD 841 CoN, ATCCCRL-1790)[54]. The MTT assay was performed and the IC_{50} was determined at a concentration of 270 µg/mL. Mitochondrial membrane potential (MMP) analysis was also carried out, and it was seen that all the concentrations of *R. canina* reduced the MMP in WiDr cells. The highest MMP reduction was noted as 62.9% at a concentration of 540 µg/mL. This study assessed the capability of different *R. canina* extract concentrations in order to reduce the growth of WiDr cells *via* an increase in apoptosis levels using the Annexin V or propidium iodide double-staining assay. The obtained results conclude that *R. canina* extract induces cell apoptosis in a concentration-dependent manner. Mitochondrial depolarisation is also recognised as a crucial step of apoptosis induction. Alterations in MMP following *R. canina* extract treatment were examined, revealing a concentration-dependent induction of MMP loss by the *R. canina* extract. Therefore, it was noted that the cell death type induced by the *R. canina* extract is mitochondria-dependent apoptosis. Additionally, it is suggested that the down-regulation of the telomerase activity in the polyphenol-rich *R. canina* extract contributes to its apoptotic and antiproliferative activity in WiDr cells.

Shokrzadeh *et al.* investigated the cytotoxic effect of *Rosa damascene*-sourced essential oils in the A549 human cancer cell line and in the normal cell line (NIH3T3)[55]. On performing the MTT assay to check for cytotoxicity, the IC_{50} values for A549 were seen at

a concentration OD 39 µg/mL, and in the case of NIH3T3, IC_{50} was observed at 8 µg/mL. Based on the MTT assay, the sensitivity of cancer cells against rose oil was significantly higher than that of normal cells. This could be a result of malfunctioning cancerous cells, impairment disorders in the immune cell process, or increased permeability and absorption due to the high proliferation rate. The main compound present in roses is Geraniol, which is a monoterpene alcohol and increases the sensitivity of the cell to toxic compounds by decreasing the amounts of thymidilatesyntase (TS) and thymidine kinase (TK) enzymes seen in the case of colon cancer cells. It is found that essential oils bring about changes in their internal and external mitochondrial membrane fluidity, which thereby increases their permeability, thus inducing cell death by apoptosis as well as necrosis.

Zamiri-Akhlaghi *et al*. studied the cytotoxic effects of *R. damascena* extract in human cervix carcinoma cell line[56]. Cell viability was determined using the MTT assay. This study determined the IC_{50} based on a time interval of 24 hours. At 24 hours, IC_{50} was noted at a concentration of 2135 µg/mL; at 48 hours, the IC_{50} concentration was at 1540 µg/mL; and at 74 hours, the IC_{50} concentration was at 305.1 µg/mL. This data obtained showed that the *R. damascena* extract had cytotoxic activity against HeLa cell line in a dose-dependent manner, with promising morphological changes like reduction in cell and cell rounding. The authors concluded that the ingredients of *R. damascena* extract possess antitumor and anticancerogenic activities. *R. damascena* could also be used as a chemotherapeutic agent in cancer treatment.

Conclusion

This review concludes that flowers, mainly varieties of *Tagetes erecta* L. are good sources of phytochemicals. The major flavonoids present in this flower are α-Carotene, β-Carotene and Lutein. These flavonoids have multiple therapeutic values such as antimicrobial, anticancer, etc. This paper discusses a comparison made between the conventional and non-conventional methods of extraction of phytochemicals from flower sources, with the aid of solvents like methanol and ethanol. Green methods of extraction are better options than conventional methods in terms of the amount of solvent used for extraction. Green methods of extraction are safer for the environment, analysts, and users. A comparison was also made on the cytotoxicity of flower extracts on various cell lines. Among the various papers discussed, MTT assay is a common method used to check cell viability. Various cell lines are being used as models to study the cytotoxicity of the different floral extracts, which also implies the impact of the cytotoxic effect of the floral pigments over a wide range of cancer cell lines. As major studies are done *in-vitro*, the effect of these extracts *in-vivo* must be studied. This also paves the way for the usage of floral extract in the treatment of various cancer conditions. Advances are yet to be made in the detection and interaction of phytochemicals from floral extracts. In retrospect, more varieties of floral waste can be studied for therapeutic values obtained from their phytochemicals and other sources.

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Conflict of Interest

The authors declare no conflict of interest.

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