2. Review of Literature

A comprehensive review of the literature upholds for conducting research in a methodical approach, offering extensive insights into relevant studies. Such reviews facilitate the development of innovative research ideas. This section presents detailed examinations of *Rhododendron arboreum* Sm. leaf and flower encompassing its phytochemical composition, antioxidant activity, computational analysis and *in vivo* studies.

2.1. Reviews on *Rhododendron arboreum* Sm. Leaf and Flower

Oral administration of flower extracts has demonstrated effective reduction in cholesterol levels and enhanced anti-diabetic properties. Previous studies have confirmed the antimicrobial efficacy of both aqueous and ethanolic extracts against *E. coli* and *S. aureus* (Sonar *et al.,* 2012)*.* Furthermore, the anthocyanidins found in the flower petals are known for their antioxidant, antidiabetic (Tsuda *et al.,* 2003) and anticarcinogenic (Hagiwara *et al.,* 2001) properties. This compound (anthocyanidins) has also demonstrated its uses in treating inflammation and UV-induced damage in liposomes (Min *et al.,* 2010).

In Homeopathic Materia Medica, the tincture made from dried leaves of *R. arboreum* is used to treat gout and rheumatism. Moreover, the Ayurvedic preparation "Asoka Aristha," containing *R. arboreum* leaf shows oxytocic, estrogenic and prostaglandin synthetase-inhibiting properties. The juice extracted from the leaves is applied to cots and beds to eliminate bed lice (Srivastava, 2012).

Traditionally, the plant is utilized for treating ailments like rheumatism, nosebleeds, fever, wound healing and amoebic dysentery (Madhvi *et al.,* 2019). Its leaves, flowers and bark are rich in various phytochemicals including phenols, saponins, proteins, steroids, tannins, coumarins, carbohydrates, carboxylic acids, ascorbic acid, anthocyanins, alkaloids, reducing sugars, flavonoids, terpenoids, anthraquinones, phlobatanins and glycosides (keshari *et al.,* 2018).

Rhododendron arboreum is a small tree or evergreen shrub belonging to the Ericaceae family. Originally found in Northern India particularly in the

Himalayan regions spanning from Kashmir to Bhutan, it also thrives in the elevated hills of Manipur and Assam (Ahmad *et al.,* 2020)**.** Due to its composition of flavonoids, tannins and saponins, *R. arboreum* exhibits antioxidant, hepatoprotective, anti-inflammatory, antidiabetic, anticancer and anti-diarrheal properties (Prakash *et al.,* 2007; Verma *et al.,* 2010).

As the national flower of Nepal and the state tree of Uttarakhand, this evergreen species holds significant cultural and economic value. In North India, tribal communities utilize it extensively for cooking and traditional medicinal purposes. Its flowers are particularly cherished in the hilly regions of Himachal Pradesh, where Local people utilize the sweet-sour tasting flowers for various culinary purposes such as making jellies, local brews, pickles, chutney and jams (Gautam *et al.,* 2020).

Osman *et al.,* 2020, investigated phytochemical analysis across 10 plants, 70% ethanolic extracts showed presence of steroids, terpenoids, flavonoids, tannins and phenols. Antioxidant activity (IC_{50}) ranged widely: 42.33 µg/mL to 1,519.76 µg/mL (DPPH method) and 31.60 µg/mL to 1,133.79 µg/mL (ABTS method). Total phenolic content varied: 8.72 ± 0.08 mg GAE/g to 91.67 ± 0.13 mg GAE/g.

Additionally, the juice extracted from these flowers has traditional medicinal uses including treating headaches, stomachaches, fevers, nosebleeds, diabetes and rheumatism. Fresh flowers are employed for addressing dysentery and diarrhea, whereas dried flowers are used to manage bloody dysentery (Bhatt *et al.,* 2022).

Li *et al.,* 2021, investigated the anthocyanin regulatory networks in five *Rhododendron* species using RNA-seq and *de novo* assembly generating 159,408 unigenes with 106,766 annotated. Flavonoid biosynthesis was the most abundant pathway and flower color-related genes varied between species: carotenoid and isoflavonoid biosynthesis in *R. molle* (yellow), flavonoid in *R. fortunei* (light pink), *R. mariesii* (pink), anthocyanin in *R. simsii* (red) and both carotenoid and flavonoid in *R. pulchrum* (purplish red). The species clustered into two groups with *R. molle* closely related to

R. simsii. The study offers insights for flower color improvement in *Rhododendron* species.

Agarwal & Rajput, 2022, conducted a comprehensive review highlighting the phytochemistry, medicinal applications and pharmacology of *R. arboreum*. They discussed traditional uses of *Rhododendron* in mountain communities, explored the potential for developing value-added products for rural tribal populations and underscored the medicinal benefits derived from its phytochemical properties.

Ahmad *et al.,* 2022, reviewed on *R. arboreum*, highlighting its rich mineral and nutrient content and traditional uses. The plant showed diverse pharmacological activities including anti-inflammatory, antimicrobial, antidiabetic, antioxidant and immunomodulatory effects due to its bioactive compounds like flavonoids, esters, steroids and terpenes.

Basnett & Ganesan, 2022, reviewed on *Rhododendron* species in the Indian Himalayas which possess significant medicinal value contributing to local healthcare practices. Various species are traditionally used for treating ailments due to their rich phytochemical content, including polyphenols, alkaloids and flavonoids which exhibit anti-inflammatory, antimicrobial and antioxidant properties. These plants are integral to the livelihoods of rural communities who rely on them not only for their healing benefits but also for their economic value in the production of natural medicines.

Zhang *et al.,* 2023, conducted a comprehensive RNA sequencing analysis of *R. liliiflorum* H. Lév, flower with distinctive white outer edges and yellow inner edges. Their study identified 219,221 transcripts and 180,677 unigenes from 48.52 Gb of clean reads. DEG analysis revealed 2310 upregulated and 3062 downregulated unigenes between the white (W) and yellow (Y) flower parts with 36 DEGs linked to flavonoid biosynthesis. Pathway enrichment indicated significant involvement in phenylpropanoid, flavonoid and isoflavone biosynthesis with key genes like dihydroflavonol-4- reductase (DFR) and chalcone synthase (CHS) influencing flower color variation. This research provides insights into the molecular mechanisms behind flower color formation in *Rhododendron.*

R. arboreum, native to the Himalayan region has garnered attention for its medicinal properties including antibacterial and antioxidant activities. Studies emphasize the potential of combining plant-derived compounds with antibiotics to combat resistance and discover novel therapeutic agents Mehta *et al.,* 2023, primarily focused on studying the antibacterial properties of flower and leaf extracted *R. arboreum* against pathogenic bacteria like *Bacillus cereus* and *Staphylococcus aureus*. The extracts were screened for agar well diffusion methods and tested for possible synergistic activity against conventional antibiotics (norfloxacin and ciprofloxacin) using various solvents for extraction of the extracts.

Sorokhaibam *et al.,* 2024, explored the intraspecific floral trait variations of *R. arboreum* Sm. in the Western Himalayas, focusing on two distinct elevational morphs: red morphs at lower elevations and pink morphs at higher elevations. The study revealed significant differences in floral traits such as phenology, floral dimensions, display, reward quality and pollinator interaction with pink morphs exhibiting entomophily and red morphs displaying ornithophily. Both morphs were self-compatible but self-pollination resulted in lower fruit set compared to cross- or open-pollination. Using simple sequence repeat (SSR) markers the study showed that the outcrossing rate in the red morph (tm = 0.82) was higher than in the pink morph (tm = 0.76) suggesting a shift in the pink morph toward a mixed-mating strategy. However, both morphs had comparable levels of biparental inbreeding. These findings indicate that differences in floral traits and pollinator interactions are influencing reproductive strategies and mating outcomes in these morphs.

2.2. Phytochemical Screening Approaches

Madhvi *et al.,* 2019; Srivastava, 2012, highlighted the pharmacological activities and medicinal importance of *R. arboreum* including its antidiabetic, anti-inflammatory, hepatoprotective and antioxidant properties supported by its rich composition of flavonoids, saponins and other phytochemicals.

The plant is utilized in both human and veterinary traditional medicine employing various parts (leaves, stems, roots, seeds and latex) and preparations (infusion, decoction, maceration, etc.) administered orally or topically (Félix *et al.,* 2014).

Rawat *et al.,* 2018 investigated *R. arboreum* leaves, analyzing phytoconstituents both qualitatively and quantitatively. Their research aimed to assess the immunomodulatory effects of these extracts using diverse *in vitro* techniques to evaluate intracellular and phagocytic killing capabilities of neutrophils. Their findings revealed that all extracts contained a range of biologically significant phytochemicals. Furthermore, they noted that the total phenolic and flavonoid content of the plant were comparable to those found in other medicinal plants.

Park *et al.,* 2018, conducted a metabolic profiling study on *R. schlippenbachii* Maxim. a garden plant with medicinal value due to its diverse metabolites. They analyzed two anthocyanins and 40 primary and secondary metabolites in flowers of different colors, finding that cyanidins were the major anthocyanins. Red flowers had the highest anthocyanin content at 1.02 ± 0.02 mg/g dry weight. Using principal component analysis on GC-TOFMS data the study revealed significant differences ($p < 0.0001$) in tricarboxylic acid cycle intermediates such as succinic acid, fumaric acid and malic acid in red flowers compared to flowers of other colors. The research used both gas chromatography time-of-flight mass spectrometry (GC-TOFMS) and high-performance liquid chromatography (HPLC) to explore metabolite interactions and phenotypic variations among white, violet and red flowers of *R. schlippenbachii*.

Mohammed & Mustafa, 2020, focused on extracting coumarins from red delicious apple seeds using various solvents and extraction techniques. Phytochemical screening detected coumarins in methanol and chloroform extracts. Four new furanocoumarins were isolated and their chemical structures were identified. These compounds demonstrated notable antibacterial and antifungal effects against standard strains. These findings propose that these novel furanocoumarins could serve as a basis for developing novel antimicrobial agents.

Gollo *et al.,* 2020, explored the phytochemical composition and biological effects of extracts from *in vitro* cultivated *Nidularium procerum* leaves a type of bromeliad. Various solvents were utilized to extract alkaloids, flavonoids, tannins, steroids, fatty acids, polysaccharides and antioxidants. A particular emphasis was placed on aqueous extracts for their non-toxicity and cost-effective extraction. The research outcomes offer valuable insights into the phytochemical makeup and potential therapeutic uses of this vulnerable bromeliad species.

Kumar *et al.,* 2020, investigated the potential cytotoxic, antibacterial and enzyme inhibitory activities of *R. arboreum* flower extract, relevant to traditional medicinal knowledge systems. They also conducted LC-MS analysis to identify the chemical composition revealing major constituents such as cis-retinoic acid, phytol, kempferol-3,4,7-tri-O-sulfate, 6-β-D-xylopyranosylluteolin and mucronulatol.

Madhvi *et al.,* 2020, aimed to assess the antioxidant activity and quantify phenol and flavonoid concentrations in *R. arboreum* leaf extracts. The study identified phenols, flavonoids, saponins, terpenoids, glycosides and coumarins in the extracts. Among the extracts tested the hydroethanolic extract (70% ethanol-water) demonstrated the highest phenolic content (135.10 ± 0.12 mg equivalent to gallic acid per gram of extract) surpassing the ethanol extract (102.3 ± 0.05 mg) and water extract (19.23 ± 0.13 mg). Conversely, the ethanol extract showed the highest flavonoid content (67.70 ± 0.10 mg equivalent to quercetin per gram of extract) compared to 31.53 ± 0.06 mg in the hydroethanolic extract.

Verma & Singh, 2020, conducted a field survey on the ethnomedicinal aspects of medicinal plants followed by phytochemical analysis and evaluation of their cytostatic potential focusing on their mode of action against Dalton's lymphoma (DL) cell line. The study found that *Murraya koenigii* induced greater apoptotic cell death in DL cells compared to *Annona reticulata*.

Zhang *et al.,* 2020, explored the flavonoid composition of *R. pulchrum* flowers, known for their vibrant colors and long flowering periods.

Using HPLC-ESI-MS/MS, they identified six key flavonoids: malva-3-arabinoside, myricetin 3-rhamnoside, quercetin-3-galactoside, quercetin-3-O-arabinoside, quercetin-3-rhamnoside and quercetin. The flavonoid content was highest at the bud stage, decreasing as the flowering process progressed. Their study highlights the high developmental and application value of *R. pulchrum* especially in ornamental cultivation and flavonoid extraction with HPLC-ESI-MS/MS proving useful for rapid quality detection.

Sharifi *et al.,* 2020, aimed to investigate the types of secondary metabolites, quantity and evaluate the antioxidant, cytotoxic, antifungal and antibacterial properties of methanolic, ethanolic and water extracts from the roots, leaves and flowers of *Nepeta juncea* Benth. Their findings indicated that the methanolic extract of the leaves exhibited the highest concentrations of total phenols (69.54 ± 0.31 mg gallic acid equivalents (GAE)/g dry weight), total flavonoids (41.37 ± 0.17 mg quercetin equivalents (QE)/g dry weight), anthocyanins (6.52 ± 0.21 mg cyanidin/100 g dry weight) and tannins (47.36 ± 0.33 mg catechin/g dry weight).

The study focused on analyzing the methanolic extract of *Cynodon dactylon* (L.) Pers. rhizomes, identifying major fatty acids (palmitic acid, oleic acid, linoleic acid), alpha-tocopherol, sitosterol and hydroquinone as the primary phenolic compound (Savadi *et al.,* 2020). These results underscore the extract's antioxidant potential attributed to its specific phytochemical makeup.

Oikeh *et al.,* 2020, conducted a study on the phytochemical content and antimicrobial activities of ethanol extracts from fresh and dry *Citrus sinensis* (sweet orange) peels observing the zone of inhibition using disc diffusion method for antimicrobial activities against five (5) bacterial strains (*Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhimurium*) and three (3) fungal strains (*Candida albicans, Aspergillus niger* and *Penicillium notatum*). The results revealed that the fresh peel extract contained higher levels of total phenolics, flavonoids and tannins compared to the dry peel extract. Additionally, the fresh peel extract showed stronger antibacterial activity against tested bacterial strains and *Candida albicans*, whereas the dry peel extract exhibited greater inhibition against *Aspergillus niger* and *Penicillium notatum*. These findings suggest that drying plant materials before extraction may result in the loss of specific active phytochemical compounds.

The study by Țigu *et al.,* 2021, focused on conducting a phytochemical analysis and evaluating the *in vitro* effects of *Allium fistulosum* L. (Welsh onion) and *Allium sativum* L. (garlic) extracts on human normal and tumor cell lines. It was observed that both extracts demonstrated antioxidant and antiproliferative activities with the *A. sativum* extract displaying stronger effects on tumor cell lines in comparison to the *A. fistulosum* extract.

The phytochemical composition, biological activity and cell cytotoxicity of extracts from flower petals and leaves of *R. arboreum* were investigated by Winitchai *et al.,* 2021. Initial qualitative screening revealed varying compositions across different plant parts. Saponins and tannins were found in all extracts while anthraquinones were detected exclusively in flowers and terpenoids solely in leaves. Among the extracts, dry leaf exhibited the highest levels of total phenolic content (TPC) at 405.21 mg gallic acid equivalent per gram of dry extract and total flavonoid content (TFC) at 127.30 mg catechin equivalent per gram of dry extract followed by dry flower and fresh flower.

Using Gas Chromatography-Mass Spectrometry (GCMS), phytochemical analysis was conducted on both aqueous and methanolic extracts of *Moringa oleifera* leaves (Bhalla *et al.,* 2021) and identified fifty-four bio-components in the methanolic extract, which showed significantly higher levels compared to the aqueous extract. Major compounds detected included 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- (21.19%), Propionic acid, 2-methyl-, octyl ester (15.02%), Ethanamine, N-ethyl-N-nitroso- (5.21%) and 9,12,15-Octadecatrienoic acid, among others.

The phytochemical analysis of the ethanolic extract of *Sterculia quadrifida* R.Br. identified diverse bioactive compounds with potential health benefits. The study focused on characterizing the plant extract's composition, highlighting flavonoids, alkaloids, phenolics and other secondary metabolites (Siswadi & Saragih, 2021). These findings offer valuable insights into the medicinal properties and potential applications of *Sterculia quadrifida* R.Br. in natural medicine and pharmaceutical industries.

A study that aimed to utilize mandarin peel (*Citrus unshiu* Marc. var. Kuno) effectively through innovative green extraction methods (Šafranko *et al.,* 2021). Initially, supercritical CO_2 (SC-CO_2) extraction was employed to isolate aromatic and volatile components with limonene identified as the predominant volatile compound. Subsequently, the residual material left after SC-CO_2 extraction underwent subcritical water extraction (SWE) to extract polyphenolic compounds, primarily hesperidin, narirutin and rutin.

Sharma *et al.,* 2021, examined the impact of solvent on extraction efficiency, phytochemical content and antioxidant capacity across different components of *R. arboreum*. The methanolic extract from flowers exhibited the highest total phenolic content (TPC) at 107.46±8.74 mg/g GAE and total flavonoid content (TFC) at 254.26±29.33 mg/g RE. Assessments using radical scavenging assays revealed the lowest IC_{50} values in flower extracts (both methanolic and aqueous), highlighting the potential of these flowers as a natural antioxidant source.

Singh *et al.,* 2022, focused their study on the phytochemical analysis and characterization of corn silk (Zea mays, G5417). They identified various phytochemicals including phenols, flavonoids, alkaloids and quantified specific compounds such as ferulic acid, vanillic acid and p-coumaric acid.

Agidew, 2022, conducted a review focusing on relevant medicinal plants based on indigenous knowledge from Ethiopia. They reported that several traditional medicinal plants whose phytochemical properties remain unstudied are reported to have diverse therapeutic uses. These include treating conditions such as mastitis, boils, hemorrhoids, congestion, headaches, hepatitis, liver issues, vertigo, stomatitis, kidney ailments, anemia, coughs, fluxes and stomatitis in both animals and humans.

The phytochemical analysis of *Ficus religiosa* stem barks, revealed increasing concentrations of phenolic and flavonoid compounds with higher extraction quantities (Baliyan *et al.,* 2022). The study emphasized the free-radical scavenging and antioxidant properties of *F. religiosa* attributing these effects to its components including carbohydrates, tannins, phenolics, reducing sugars, proteins and flavonoids. Furthermore, it underscored the potential antioxidant activities of multiple polyphenols and flavonoids present in *F. religiosa*.

Prakash, 2023, emphasized the importance of validating herbal agents due to the rich source of secondary metabolites in plants, which exhibit diverse biological activities. The study focused on identifying bioactive compounds in the methanol extract of *R. arboreum* using GC-MS. The GC-MS analysis revealed ten phytochemical constituents with Limonene (18.48%), Caryophyllene (13.01%), 3-Heptanoic acid methyl ester (11.00%) and Phenol, 2-(2-methyl-2-propenyl) (5.52%) as the major components. These findings support the traditional use of *R. arboreum* in herbal medicine for treating bacterial and degenerative diseases validating its medicinal potential.

Sabharwal *et al.,* 2023, evaluated the antioxidant, antibacterial and anti-inflammatory properties of extracts derived from *Rhodobryum roseum*. The phytochemical analysis of the crude methanolic extract indicated the presence of terpenoids, alkaloids, phenols and flavonoids. GC-MS further identified phenolic acids, fatty acids and phytosterols as prominent components while LC-MS highlighted terpenoids and flavonoids as major bioactive compounds.

Hiremath *et al.,* 2024, assessed the mineral and phytochemical composition of *R. arboreum* flowers using various drying techniques. Sun-dried petals exhibited the highest levels of calcium (651±8.54 mg), iron (9±2.0 mg) and phosphorus (121±2.65 mg) per 100 g compared to hot air and microwave oven-dried samples. However, hot air-dried petals had the highest polyphenol content (290±2.91 mg GAE/100 g) while sun-dried samples showed the highest antioxidant activity (113±1.59 mg/100 g). The study revealed that different drying techniques significantly affect the mineral and phytochemical composition of *R. arboreum* flowers.

2.3. *In vitro* Antioxidant Evaluation: Free Radical Scavenging Potential

Compounds that can interrupt the chain reaction initiated by reactive oxygen species (ROS) are crucial for managing and preventing diseases caused by oxidative stress. Adegbola *et al.,* 2020, conducted a review focusing on the antioxidant potential of plants particularly highlighting the radical scavenging and antioxidant activities found in various species of the *Amaranthus* genus. They reported that *Amaranthus* family's underscores the importance source of natural antioxidants with therapeutic implications for combating oxidative damage-related diseases.

Chaves *et al.,* 2020, compared the sensitivity and discriminative capacity of different methods in assessing the antioxidant activity of extracts from various plant species. They selected 12 species with varying phenolic compound contents and evaluated their extracts using four methods: 2,2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay, ferric reducing antioxidant power (FRAP) assay, Trolox equivalent antioxidant capacity (ABTS) assay and reducing power (RP) assay. The study found that reducing power exhibited the highest sensitivity in distinguishing differences or similarities between species whereas ABTS demonstrated the lowest sensitivity among the methods studied.

Diniz *et al.,* 2020, reviewed the significance of spice plants in traditional medicine, focusing on *Cinnamomum zeylanicum, Mentha piperita, Ocimum basilicum*, *Origanum vulgare, Piper nigrum, Rosmarinus officinalis* and *Thymus vulgaris*. They explored bioactive compounds in their essential oils emphasizing antioxidant properties and potential applications. Their review stressed the need for using multiple *in vitro* methods to ensure reliable assessment of antioxidant activity.

A study aimed to evaluate the antioxidant activities of crude juices extracted from leaves of fig, guava, olive and pomegranate as well as from peels of ripe pomegranate fruits (Farag *et al.,* 2020). The assessment utilized three methods: DPPH assay, reducing power assay and metal chelating assay. Results showed that the crude juices extracted from pomegranate peels contained significantly higher amounts of polyphenols, flavonoids, tannins and anthocyanins compared to the crude juices from leaves of fig, guava, olive and pomegranate. This suggests that pomegranate peels are particularly rich sources of antioxidant compounds, highlighting their potential health benefits and supporting their traditional medicinal uses.

A study was evaluated for the biological activity of *R. arboreum,* methanol extract leaf (MEL) exhibited superior antioxidant activity in the lipid peroxidation inhibition assay (95.32±0.37%) compared to methanol extract flower (MEF) (77.09±4.17%), with IC_{50} values of 103.6 µg/ml for MEL and 271.17 µg/ml for MEF. In the nitric oxide scavenging assay, MEF demonstrated an activity of 94.46 ± 0.32% (IC_{50} 150.13 µg/mL), whereas MEL showed 83.71±0.74% activity (IC_{50} 179.52 µg/mL) (Gautam *et al.,* 2020).

A study investigated the phytochemicals, elemental composition, antioxidant activity and cytotoxicity of *R. arboreum* W.W. Sm (Tawng Za Lat Ni), which is used in traditional Chin ethnic foods (Khaing *et al.,* 2020). The study reported an IC_{50} value of 4.57 µg/mL for the flower extract of Tawng Za Lat Ni, indicating moderate antioxidant activity compared to the standard ascorbic acid (IC_{50} = 0.39 µg/mL), which exhibited higher antioxidant potency.

The antioxidant activity of methanol extracts from *R. arboreum*, prepared using 70:30 and 50:50 methanol ratios was assessed using DPPH and FRAP assays alongside antibacterial testing (Kumar *et al.,* 2020)**.** The extracts contained primary constituents such as cis-retinoic acid, phytol, kaempferol-3,4,7-tri-O-sulfate, 6-β-D-xylopyranosylluteolin and mucronulatol. These compounds demonstrated strong antioxidant and antibacterial properties, with an IC_{50} of 20.1 µg/mL compared to ascorbic acid and BHT and exhibited the highest FRAP value with an IC_{50} of 28.4 µg/mL.

A study identified phenols, flavonoids, saponins, terpenoids, glycosides and coumarins in the *R. arboreum* leaf extracts through phytochemical screening, where antioxidant activity was assessed using reducing power and DPPH radical scavenging assays, with the 70% ethanol extract demonstrating the greatest reducing power and DPPH radical scavenging activity, followed by the ethanol and water extracts (Madhvi *et al.,* 2020).

Mwamatope *et al.,* 2020, evaluated methanol extracts from leaves of *Senna singueana, Melia azedarach, Moringa oleifera* and barks of *Lannea discolor* for antioxidant activity using DPPH and FRAP assays. They found that all extracts demonstrated significant DPPH radical scavenging activity (42.72% to 89.09%) and varied FRAP values (68.47 to 166.3 mg TEACg−1 DW). The study reported all these plants contain phenolic aglycones with potential antioxidant and anticancer properties highlighting their therapeutic potential against oxidative stress-related diseases.

Nguyen *et al.,* 2020, assessed the antioxidant properties of *Annona muricata* leaves by determining their total phenolic and flavonoid contents. They used the ABTS and DPPH radical scavenging assays to measure antioxidant activity. The ethanol extract of *A. muricata* leaves showed total phenolic and flavonoid contents of 609.08±5.82 mg GAE/g and 209.52±1.88 mg QE/g respectively. The antioxidant activity with IC_{50} values of 20.75±0.28 µg/mL for DPPH and 12.84±0.21 µg/mL for ABTS scavenging activities.

The study by Sharma *et al.,* 2021, explored solvent effects on extraction yield, phytochemicals and antioxidant potential of *R. arboretum*. Cold maceration yielded the highest in aqueous leaf extract (10.78%) followed by methanolic flower extract (10.42%). Methanolic flower extract exhibited the highest TPC (107.46±8.74 mg/g GAE) and TFC (254.26±29.33 mg/g RE). Flowers (both methanolic and aqueous) showed the lowest IC_{50} values in DPPH, FRAP and ABTS assays.

The antioxidant activity of extracts from caraway (*Carum carvi* L.), coltsfoot (*Tussilago farfara* L.), dandelion (*Taraxacum officinale* F.H.Wigg.), lovage (*Levisticum officinale* L.), tarragon (*Artemisia dracunculus* L.) and white mulberry (*Morus alba* L.) are known for their high content of caffeic acid derivatives (Tajner *et al.,* 2020). Specifically, the water-ethanolic extracts of these plants were evaluated for radical cation scavenging activity (ABTS assay) and radical scavenging activity (DPPH assay). Their study demonstrated a positive correlation between the presence of caffeic acid derivatives and antioxidant efficacy, highlighting the potential of these extracts as natural sources of antioxidants beneficial for health and wellness.

Muflihah *et al.,* 2021, determined the antioxidant activity (AA), total phenolic content (TPC) and total flavonoid content (TFC) of selected Indonesian *Zingiberaceae* herbs using four chemical assays: DPPH radical scavenging, H_2O_2 scavenging, Folin–Ciocalteau (F-C) and $NaNO_2$-$AlCl_3$-NaOH assays. Their findings highlighted significant variations in AA, TPC and TFC among the herbs with *Curcuma longa* showing the highest AA. Analysis identified *curcumin* as a major antioxidant compound present in five highly active herbs with *C. longa* containing the highest amount. Pearson correlation analysis suggested that TPC, TFC and specifically *curcumin* contributed significantly to the observed antioxidant activity.

The study investigated the pharmacognostic characteristics and *in vitro* antioxidant, anti-inflammatory and anti-diabetic activities of the Himalayan *R. arboreum* flower (Sharma & Raju, 2021). According to findings from both hydro-alcoholic and aqueous extracts of the flower demonstrated significant antioxidant, anti-inflammatory and antidiabetic effects in a dose-dependent manner surpassing standard drugs. The experimental results underscore the flower's potential as an effective agent for antioxidant, anti-inflammatory and antidiabetic purposes.

Shresta *et al.,* 2021, studied the biochemical properties of crude extracts from fifteen Nepalese medicinal plants. They found that *Acacia catechu* exhibited strong antioxidant properties with an IC_{50} value of 1.3 μg/mL. Additionally, they also reported strong antioxidant activity of *Myrica esculenta, Syzygium cumini* and *Mangifera indica* extracts.

The antioxidant properties of *R. arboreum* were assessed using two radical scavenging assays namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethyl-benzthiazoline-6-sulfonic acid (ABTS) (Winitchai *et al.,* 2021). The results showed that all extracts demonstrated stronger inhibitory activity against the DPPH radical compared to the ABTS radical with IC_{50} values ranging from 24.65 to 48.15 μg/mL for DPPH and 65.19 to 76.36 μg/mL for ABTS. A positive correlation coefficient between the antioxidant assays and the total phenolic content (TPC) and total flavonoid content (TFC) of *Rhododendron* extracts indicated that the antioxidant potential is distributed across both components.

A study investigated the *R. arboreum's* impact of a rapid drying process on the extraction of phenolics, flavonoids, flavanols and antioxidant activity (measured using FRAP and DPPH assays), along with protein content (Barola *et al.,* 2022). Correlation analysis indicated a positive relationship between total phenolic content and ABTS and FRAP values.

Bhatt *et al.,* 2022, utilized UPLC-ion mobility quadrupole time of flight tandem mass spectrometry to investigate the presence of anthocyanins, non-anthocyanin flavonoids and phenolic acids as well as the free radical scavenging potential in the flowers of *R. arboreum.* The study reported the analysis of extracts, revealing total phenolic content (123.6 mg GAE/g), anthocyanin content (1.76% w/w) and assessed antioxidant activity through various assay against 2,2-diphenyl-1-picrylhydrazyl radical (IC_{50}: 102.06 and 96.92 µg/ml) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (112.25 and 45.59 µM TE/g).

Ilić *et al.,* 2022, investigated the impact of light intensity on essential oil yield of lemon balm, mint and sweet basil. They identified piperitenone oxide (12–14%) and 1,8 cineole (1.32–2.27%) as the main components of mint essential oil and noted linalool (9.06–10.2%), 1,8 cineole (1.26–8.06%) and α-trans bergamotene (1.21–1.47%) in basil essential oil. Basil leaves grown under shade exhibited the highest antioxidant activity with an EC_{50} value of 0.68 mg/mL after 20 minutes of incubation.

Vitale *et al.,* 2022, reviewed on all recent studies on herbal preparations for wound management, highlighting phytochemicals' diverse effects like anti-inflammatory, antimicrobial, antioxidant properties and their role in collagen synthesis, cell proliferation and angiogenesis. They suggest combining natural compounds with nanotechnology for more effective treatments, emphasizing the need for thorough safety evaluations in clinical trials before widespread clinical use.

Paul *et al.,* 2023, aimed to provide initial insights into the potential of *R. arboreum* flower (RAF) concerning its antioxidant properties and effects on oxidative stress markers in a neurodegenerative disease model crucial for advancing novel therapeutic drugs. The study revealed that RAF extract not only exhibited potent antioxidant activity but also demonstrated efficacy against oxidative stress-induced neurodegeneration in an *ex vivo* brain model. Additionally, analysis of oxidative stress biomarkers in models induced by H_2O_2 and $HgCl_2$ showed significant reductions upon treatment with RAF extract.

2.4. *In vitro* Cytotoxicity Assessment using MTT Assay and Brine Shrimp Lethality Test

Gautam *et al.,* 2020, investigated the activity of methanol extracts from *Rhododendron arboreum* leaves (MEL) and flowers (MEF). They found that both extracts exhibited significant inhibition of cancer cell growth in three cell lines: HeLa (human cervical cancer), MCF7 (breast cancer) and A549 (lung cancer). Specifically, MEL showed higher activity against HeLa (64.62±2.65%) and A549 (75.08±1.68%) compared to MEF (HeLa: 53.11±2.84%, A549: 45.92±2.43%). The EC_{50} values for MEL were 232.76 µg/mL in HeLa and 155.38 µg/mL in A549, while MEF exhibited EC_{50} values of 395.50 µg/mL in HeLa and 660.26 µg/mL in A549.

Verma & Singh, 2020, assessed the cytotoxicity of methanol extracts using the MTT reduction assay and investigated their impact on apoptosis in DL cells through fluorescence microscopy and flow cytometry. They explored the apoptotic mechanisms by measuring reactive oxygen species (ROS), mitochondrial membrane potential and expression levels of apoptosis- inducing proteins. Their study revealed that treatment with extracts from *Murraya koenigii* and *Annona reticulata* led to decreased mitochondrial membrane potential, cytochrome c release, elevated ROS levels and increased expression of caspases (3 and 9) indicating involvement of the mitochondrial pathway in apoptosis induction. Thus, the extracts significantly induced cytotoxicity in DL cells via apoptosis.

Alabi *et al.,* 2020, examined the effects of two recipes (*Allium ascalonicum, Anchomanes difformis, Antiaris africana, Bridellia scadens, Calliadra haematocephala,* Citrus medica, Garcinia kola, *Kigelia africana, Mangifera indica, Nauclear latifolia, Tricalysia macrophylla, Trichiliamonadelpha* and Xylopia aethiopica) made from Nigerian and African medicinal plants on breast cancer cells (MCF-7 and MDA-MB-231) and normal human fibroblasts. They assessed *in vitro* cytotoxicity and anti- proliferative effects using the MTT assay and evaluated cell cycle distribution and apoptosis through flow cytometry with propidium iodide staining. The study found that both recipes inhibited the growth and proliferation of cancer cells and fibroblasts in a dose-dependent manner ($p < 0.05$). Furthermore, both treatments induced G_0/G_1 cell cycle arrest as observed by flow cytometry.

Kim *et al.,* 2020, screened 239 traditional Chinese medicinal plant extracts for antibacterial activity against multidrug-resistant *Staphylococcus aureus* and evaluated cytotoxicity. They identified 74 extracts with strong antibacterial properties (inhibition zone ≥ 15 mm) and 18 extracts with low cytotoxicity (LC_{50} > 100 µg/mL). Promising extracts included *Rhus chinensis, Ilex rotunda, Leontice kiangnanensis, Oroxylum indicum, Isatis tinctorial, Terminalia chebula, Acacia catechu, Spatholobus suberectus, Rabdosia rubescens, Salvia miltiorrhiza, Fraxinus fallax, Coptis chinensis, Agrimonia pilosa* and *Phellodendron chinense.*

Aslany *et al.,* 2020, investigated green-synthesized silver nanoparticles (AgNPs) using *Artemisia ciniformis* leaf extract. The AgNPs inhibited growth of AGS human gastric carcinoma cells in a dose- and time-dependent manner. They induced apoptosis confirmed by flow cytometry, up-regulated pro-apoptotic genes (caspase-3, caspase-9, Bax), down-regulated anti-apoptotic gene (Bcl2) and inhibited cyclin D1 and MMP2 genes. Cell cycle analysis showed G_0/G_1 phase arrest in cancer cells.

Hashemi *et al.,* 2020, developed a green synthesis method for stable silver nanoparticles (AgNPs) using leaf extract from *Teucrium polium* a medicinal plant. The phytoconstituents *in T. polium* extract acted as both capping and reducing agents, ensuring stability of the AgNPs. They demonstrated that *T. polium*-AgNPs exhibit anticancer effects on the MNK45 human gastric cancer cell line.

Somaida *et al.,* 2020, examined the cytotoxicity of methanolic extracts from four Cameroonian plants (*Xylopia aethiopica, Imperata cylindrica, Echinops giganteus, Dorstenia psilurus*) on seven human cancer cell lines. The extracts inhibited cell proliferation in a dose-dependent manner, induced apoptosis in HeLa cells through caspase activation and sub-G_0/G_1 accumulation, disrupted mitochondrial membrane potential and inhibited cell migration. Genetic toxicity was minimal. Overall, they reported that extracts show promising anticancer activity by inducing tumor cell death mechanisms.

Ribera *et al.,* 2020, investigated the anticancer and antioxidant properties of blueberry plant extracts comparing untreated and methyl jasmonate (MeJA)-treated samples. They found that leaf extracts especially from MeJA-treated plants exhibited higher inhibition of AGS human gastric cancer cell viability and migration. These extracts also showed enhanced antioxidant properties, attributed mainly to increased anthocyanin content.

Rahimivand *et al.,* 2020, synthesized alginate nanogels containing *Artemisia ciniformis* extract and evaluated their apoptotic effects on AGS gastric cancer cells. Their study demonstrated that both the alginate nanogel-encapsulated extract and the free extract had anti-proliferative activity and induced apoptosis in AGS cells. The nanogel formulation showed enhanced apoptotic effects compared to the free extract with cell cycle analysis revealing G_0/G_1 phase arrest. Apoptosis induction occurred in a time- and dose-dependent manner, accompanied by up-regulation of pro-apoptotic genes and down-regulation of anti-apoptotic and metastatic genes highlighting the potential of the nanogel formulation as a promising therapeutic approach for gastric cancer.

Sowa *et al.,* 2020, evaluated the cytotoxicity, antioxidant properties and polyphenolic profiles of ethanolic extracts from *Tanacetum vulgare* L. (tansy), *Achillea millefolium* L. (yarrow) and *Solidago gigantea* Ait. (goldenrod). Using the xCELLigence system and MTS assay (colorimetric assay) with the porcine intestinal epithelial cell line (IPEC-1) they found strong cytotoxic effects from tansy and yarrow extracts (125–1000 µg/mL) while goldenrod extracts (125 µg/mL) exhibited a supportive effect on cell proliferation. Their findings highlight the differential effects of these plant extracts on cell viability and suggest potential therapeutic applications based on their cytotoxic or supportive properties.

Tang *et al.,* 2020, studied the anticancer effects of MN-ZnONPs on AGS cells. They found that MN-ZnONPs induced apoptosis by increasing ROS production, decreasing mitochondrial membrane potential (MMP), causing apoptotic morphological changes observed with AO/EtBr staining, enhancing lipid peroxidation (TBARS), reducing antioxidant levels (SOD, GSH, CAT) and inducing cell cycle arrest. The nanoparticles exhibited spherical shape, crystalline nature, and various functional groups. Expression analysis showed modulation of apoptosis-related genes (Bax, caspase-9, caspase-3, Bcl-2) indicating their potential in gastric cancer treatment.

Osman *et al.,* 2020, investigated the correlation between antioxidant activity and cytotoxicity across 10 plants. Pearson's correlation coefficients indicated a strong relationship between antioxidant activity and phenolic content, moderate correlations with brine shrimp cytotoxicity and weaker correlations with cytotoxicity against carcinogenic liver and breast cells as well as antimicrobial activity.

Yun *et al.,* 2020, aimed to develop ethanolic extract of *Vitex negundo*-loaded gold nanoparticles (VN-AuNPs) and assess their anticancer effects on AGS cells. They analyzed cell viability, apoptotic morphological changes using TUNEL, AO/EtBr and Hoechst staining, alterations in mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) production. Additionally, they investigated the expression of apoptosis-related genes such as caspase-3, Bcl-2, Bcl-XL, Bax and caspase-9 using Western blotting and RT-PCR techniques. The study highlighted that VN-AuNPs induced apoptosis in AGS cells through cytotoxic effects, MMP alterations, ROS generation and nuclear and apoptotic morphological changes observed via TUNEL, AO/EtBr and Hoechst assays.

Wu *et al.,* 2020, investigated the potential anti-cancer effects of germacrone (*Rhizoma curcuma*) in the gastric cancer cell line BGC823. They assessed cell viability, proliferative activity and performed cell cycle analysis. Hoechst 33258 and Annexin V/PI double staining were used to detect apoptosis. Their findings showed that germacrone exhibited significant cytotoxicity against BGC823 cells as measured by MTT assay. Germacrone induced cell cycle arrest at the G2/M phase by downregulating cyclin B1, cdc2 and cdc25c expression. Additionally, germacrone treatment increased caspase-3 activity and promoted PARP cleavage, indicating induction of apoptosis. Their results suggest that germacrone inhibits cell proliferation in gastric cancer cells through G2/M phase cell cycle arrest and apoptosis induction.

Ilaghi *et al.,* 2021, studied the leishmanicidal activity of leaf extracts from *Quercus velutina, Cynanchum procera* and *Nicotiana tabacum* on *Leishmania tropica*. They tested various concentrations against both promastigote and amastigote stages, finding significant inhibition ($p < 0.001$) of parasite proliferation. *Q. velutina* showed the lowest IC_{50} values in both stages and reduced the number of amastigotes per macrophage effectively. The study underscores the potential of these extracts in combating *L. tropica* infections by inducing apoptosis in promastigotes.

Daniyal *et al.,* 2021, analyzed the antitumor activity of Heilaohulignan C (natural compound isolated from *Kadsura coccinea*) (B-6) on human gastric carcinoma cells (BGC-823). Their study demonstrated cytotoxicity against BGC-823 cells using MTT assay, increased cell death with high doses of B-6 shown by Calcein AM/Propidium Iodide staining and induction of apoptosis confirmed by flow cytometry. B-6 was found to exert pharmacological activity against gastric cancer through the p53 and mitochondrial-dependent apoptotic pathways, without toxicity to normal tissues.

Tesfaye *et al.,* 2021, screened the cytotoxic activities of 80% methanol extracts from 22 plants against human peripheral blood mononuclear cells (PBMCs) and various cancer cell lines including MCF-7 (breast), A427 (lung), RT-4 (bladder) and SiSo (cervical). Extracts from *Euphorbia schimperiana, Acokanthera schimperi, Kniphofia foliosa* and

Kalanchoe petitiana demonstrated strong antiproliferative effects with IC_{50} values ranging from 1.85 ± 0.44 to 17.8 ± 2.31 µg/mL against these cell lines. Additionally, they reported that extracts showed potent activity against other cancer cell lines such as LCLC-103H, DAN-G, A2780, KYSE-70, HL-60 and U-937, with IC_{50} values ranging from 0.086 to 27.06±10.8 µg/mL.

Mehrbod *et al.,* 2021, investigated on Iranian medicinal plant extracts and fractions for activity against influenza A virus (IAV). Extracts from *Glycyrrhiza glabra, Myrtus communis, Melissa officinalis, Hypericum perforatum, Tilia platyphyllos, Salix alba* and *Camellia sinensis* were tested after extraction with 80% methanol and fractionation with chloroform and methanol. Cytotoxicity was evaluated using the MTT assay on MDCK cells and effective concentrations (EC_{50}) were determined. Their findings showed that effective samples against IAV, particularly in combined treatments before viral penetration, included crude extracts of *G. glabra, M. officinalis* and *S. alba*, methanol fractions of *M. communis and M. officinalis* and chloroform fractions of *M. communis and C. sinensis* (fermented). These extracts and fractions were specifically rich in flavonoids, tannins, steroids and triterpenoids.

Salawu *et al.,* 2021, studied medicinal plants used for cancer management in Ilorin (city in Nigeria), assessing their growth inhibitory and cytotoxic effects. Nineteen plants from twelve families were identified containing various secondary metabolites. Extracts from *Detarium microcarpum* bark and *Prosopis africana* fruit showed significant cytotoxicity with *Detarium microcarpum* displaying higher potency against Hep-2C cancer cells (IC_{50} 0.08±0.00 µg/mL) than cyclophosphamide (2.69±0.25 µg/mL). The potential of *Detarium microcarpum* and *Prosopis africana* in developing new anticancer treatments were reported.

Devanesan & Alsalhi, 2021, synthesized silver nanoparticles (AgNPs) using *A. esculentus* flower extracts and characterized them with UV-vis spectrometry, FTIR, XRD, SEM, TEM and EDX. They evaluated cell viability using an MTT assay and found variable MIC and MBC values across bacterial strains. The synthesized AgNPs showed IC_{50} values comparable to standard drugs for tested cell lines, with smaller size and potent antibacterial, cytotoxic and apoptotic effects at low concentrations.

Ebrahimzadeh *et al.,* 2021, synthesized silver nanoparticles (AgNPs@SSE) using *Scrophularia striata* leaf extract and evaluated their cytotoxicity against cancer cell lines. The study showed significant cytotoxic effects of AgNPs@SSE at 150 µg/mL concentration, reducing viability to 6.875% in AGS (human gastric cancer) cells and 6.47% in MCF-7 (human breast cancer) cells. In contrast, normal fibroblast cells (HFF) exhibited a lower inhibition rate of about 33.1%.

El-Huneidi *et al.,* 2021, investigated the effects of carnosic acid on human gastric cancer cell lines (AGS and MKN-45). They utilized cytotoxicity assays, Annexin V-FITC/PI staining, caspase activation assays, cell cycle analysis and Western blotting to assess its impact. Carnosic acid inhibited cell proliferation and survival in a dose-dependent manner. Mechanistically, it suppressed phosphorylation/activation of Akt and mTOR, key proteins in cancer cell proliferation and survival pathways. Additionally, carnosic acid promoted apoptosis as evidenced by enhanced PARP cleavage and reduced survivin expression. This suggests that carnosic acid exerts antitumor activity against gastric cancer cells by targeting the Akt-mTOR signaling pathway.

Grover *et al.,* 2021, extracted turmeric rhizomes using different solvents and found that ethanolic extracts exhibited strong cytotoxicity against SCC-29B oral cancer cells (IC_{50} value of 11.27 µg/mL) while showing minimal cytotoxicity against Vero cells. Comparatively, curcuminoids showed similar cytotoxicity against SCC-29B but exhibited cytotoxic effects on healthy cells at lower doses. Their study suggests that ethanolic turmeric extracts may offer effective cytotoxicity against oral cancer cells with potentially lower toxicity to healthy cells compared to curcuminoids.

Khodavirdipour *et al.,* 2021, examined the potential anti-cancer properties of an ethanolic extract from *Syzygium cumini* (SCE) on the HT-29 colorectal cancer cell line. The study investigated the impact of SCE exposure on apoptosis induction and proliferation rates in HT-29 cells using MTT assays. Additionally, methods such as DNA damage assessment and a scratch test were employed to assess metastasis and cell migration capacities following treatment with SCE. They reported that results from the MTT assay indicated a significant suppression of HT-29 cell growth by SCE.

Ali *et al.,* 2021, investigated the anticancer properties of *Artemisia aucheri* Boiss extract against HT29 colon cancer cells compared to HEK293 normal cells. They employed MTT and annexin tests to assess cell viability and apoptosis induction by the extract. The study found that the *A. aucheri* extract exhibited the highest cytotoxic effect at 1000 µg/mL (80.63 ± 3.66) showing a dose-dependent response compared to the control in both cell lines ($p < 0.001$). Furthermore, the survival rate of HT29 (IC_{50} = 57.88 µg/mL) and HEK293 (IC_{50} = 295 µg/mL) cells decreased with increasing concentrations of *A. aucheri*, with the lowest cell viability observed at 1000 µg/mL. Overall, the cytotoxic effects of the *A. aucheri* extract were dose-dependent in both cell types.

Elizondo *et al.,* 2022, evaluated the cytotoxic, antioxidant and anti-hemolytic activities of 15 plants traditionally used in Mexico. Methanol extracts were prepared using the Soxhlet method, and their cytotoxicity was assessed against human hepatocellular carcinoma (HEP-G2) and monkey kidney epithelial (Vero) cells using the MTT assay. They found that the extract from *Ruta chalepensis* exhibited an IC_{50} of 1.79 µg/mL against HEP-G2 cells and 522.08 µg/mL against Vero cells, resulting in a selectivity index (SI) of 291.50. Thus, suggested that *R. chalepensis* methanol extract selectively targets HEP-G2 cancer cells while sparing non-tumorigenic Vero cells.

Canga *et al.,* 2022, reviewed 23 articles from 2017 to 2021 on African plant extracts' cytotoxic activity against cancer cell lines, focusing on breast (MCF-7, MDA-MB-231) and colorectal (HCT-116, Caco-2) cancers. Extracts, mainly from ethanol, methanol and water showed varied IC_{50} values (<20 µg/mL to >200 µg/mL). Despite water being commonly used, some studies found it less effective. Apoptosis and cell cycle arrest were highlighted as mechanisms of cytotoxicity. Many extracts met the NCI (National Cancer Institute) criteria for cytotoxicity (IC_{50} < 30 µg/mL), suggesting African plants as promising sources of cytotoxic agents.

Zhou *et al.,* 2022, studied the effects of gedunin (*Azadirachta indica*) on AGS cells, the study found that it inhibited cell viability (IC_{50} = 20 µM) increased ROS generation and induced apoptosis observed through AO/EtBr staining. Gedunin also reduced mitochondrial membrane potential (MMP) and upregulated Bax while downregulating Bcl-2 expression. Additionally, caspase-3 and caspase-9 activities were increased in gedunin-treated AGS cells, indicating activation of apoptotic pathways via oxidative stress. This finding suggests gedunin as a potential anticancer agent targeting gastric cancer cells by influencing ROS levels and apoptotic mechanisms.

Cheshomi *et al.,* 2022, investigated ellagic acid (EA) (pomegranate) anti-cancer effects on AGS (human gastric adenocarcinoma) cells and in immunocompromised mice with human gastric tumors. They used MTT assays, apoptosis analysis, scratch assays, gelatin zymography and RT-PCR to assess EA impact from pomegranate juice and peel extract. EA inhibited cell proliferation and migration and reduced MMP-2 and MMP-9 expression and activity, suggesting potential therapeutic benefits against gastric cancer.

Wang *et al.,* 2022, investigated the anticancer effects of polyphenols (quercetin and fisetin) on human gastric adenocarcinoma AGS cells and the impact of their noncovalent interaction with proteins like bovine serum albumin and casein. The study revealed that both polyphenols significantly reduced cell viability and induced anticancer effects. However, when quercetin and fisetin interacted with these proteins, their anticancer activity was reduced. This interaction led to less cell growth inhibition, decreased mitochondrial membrane potential loss, lower reactive oxygen species formation and reduced cell apoptosis, suggesting that protein binding weakens the anticancer potential of polyphenols.

Carmo *et al.,* 2023, investigated how *Geissospermum sericeum* (stem barks) exerts antitumor activity on the ACP02 cell line (human gastric adenocarcinoma) and elucidated the mechanism of cell death. They characterized ethanol extracts and fractions (neutral and alkaloid) using thin-layer chromatography and HPLC-DAD, identifying geissoschizoline N4-methylchlorine through NMR. Cytotoxicity was assessed in HepG2 and Vero cells using MTT with ACP02 cells used for anticancer evaluation.

The alkaloid fraction and geissoschizoline N4-methylchlorine exhibited significant inhibitory effects (IC_{50} 18.29 µg/mL and 12.06 µg/mL, respectively). Fractionation enhanced activity with selective cytotoxicity against ACP02 cells, suggesting geissoschizoline N4-methylchlorine as a potential caspase inhibitor in gastric cancer apoptosis pathways.

Devi *et al.,* 2024, aimed to investigate the phytochemical constituents, antioxidant properties and cytotoxic effects of *Phlogacanthus thyrsiformis* extract against the DL cell line. Their study revealed substantial quantities of total phenols and total flavonoids, indicating strong antioxidant abilities with close IC_{50} values comparable to ascorbic acid. Effective growth inhibition of DL cells was demonstrated across various treatment concentrations, while normal cells remained unaffected. The observed cytotoxic effects were attributed to the extract's apoptotic-inducing properties, evidenced by dose-dependent apoptotic cell death.

2.5. *In vivo* Animal Study using C57BL/6 Mice Model for Gastric Cancer Treatment

In a study conducted from July to September 2017 at Alborz University of Medical Sciences (Iran), Asgari *et al.,* 2020, evaluated 70 mice infected with *Helicobacter pylori* using the standard strain (ATCC 43504). Mice were treated with various *Lactobacillus* species and drugs and the anti-*H. pylori* effects were assessed through stool antigen tests and tissue staining. Results from ELISA and histological analyses showed reduced inflammation. The highest antimicrobial effect was observed in mice treated with *Lactobacillus rhamnosus* alone or all three *Lactobacillus* strains together. The study suggests that probiotics, including *L. acidophilus*, *L. plantarum* and *L. rhamnosus*, are effective in reducing *H. pylori* infection in this mouse model.

Chen *et al.,* 2020, investigated the role of probiotics as an adjuvant during chemotherapy for pancreatic cancer, a significant lethal and chemo-resistant disease. Using an LSL-KrasG12D/−-Pdx-1-Cre mouse model of pancreatic ductal adenocarcinoma (PDAC) the study examined the effects of a four-week multi-strain probiotic treatment (*Lactobacillus paracasei*

GMNL-133 and *Lactobacillus reuteri* GMNL-89) alongside gemcitabine therapy. After inducing pancreatitis in the mice via caerulein injections, they were treated with gemcitabine and probiotics. Results showed that combined treatment led to a lower grade of pancreatic intraepithelial neoplasia (PanIN) formation and reduced expression of vimentin and Ki-67. Additionally, levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were lower in mice receiving the combination therapy. Significantly, high-dose probiotics alone also inhibited PanIN changes and serum liver enzyme elevations. These findings suggest that probiotics can enhance the effectiveness of standard chemotherapy and improve patient tolerance.

Chen *et al.,* 2020, reported on the limitations of open orthotopic mouse models for colorectal cancer, which require advanced surgical skills and can cause trauma due to laparotomy. This study evaluates a minimally invasive, endoscopy-guided model that eliminates the need for laparotomy. The study injected varying concentrations of murine colorectal cancer cell lines CT26 and MC38 into the colorectal wall of BALB/C and C57BL/6J mice. The results showed consistent tumor growth accompanied by tumor-infiltrating lymphocytes, lympho-vascular invasion and early spontaneous metastases to lymph nodes, peritoneum and liver. The learning curve analysis indicated that this model is quick to establish and easy to learn allowing for intra-individual follow-up endoscopies. The use of specific cell lines and concentrations enables controlled local tumor growth and metastatic formation within short observation periods, facilitating studies on metastasis mechanisms and immune interactions.

Fujimori *et al.,* 2020, developed an immunocompetent mouse model of fibrous peritoneal metastasis (PM) that mirrors the clinical immune environment of gastric cancer. They used the C57BL/6 J mouse model with the YTN16 gastric cancer cell line either alone or co-inoculated with the myofibroblast cell line LmcMF which expresses α-SMA. Immunohistochemical analysis revealed significantly fewer CD8+ T cells and more CD163+ M2 macrophages in PM lesions compared to primary lesions. Azan staining showed that tumors co-inoculated with YTN16 and LmcMF were more fibrous and exhibited a more invasive growth pattern than tumors with YTN16 alone. The co-inoculated tumors also had reduced CD8+ cell infiltration and increased M2 macrophage infiltration. This model closely mirrors the human tumor microenvironment of PM with fibrosis and supports the development of new treatments targeting cancer-associated fibroblasts (CAFs).

Hsu *et al.,* 2020, successfully developed an orthotopic immunocompetent gastric cancer model using C57BL/6 mice. This model closely mirrors the biological characteristics of human gastric cancer. Employing CRISPR/Cas9 technology, they induced a genetic knockout of the STK24 (Serine/threonine-protein kinase 24) gene in tumor cells at the genomic DNA level. The STK24 gene knockdown led to increased tumor growth in this orthotopic model. Additionally, they established the first orthotopic transplantable gastric cancer model in syngeneic inbred mice. This study further reported the crucial role of STK24 in immune regulation during gastric cancer tumorigenesis.

Lee *et al.*, 2020, studied the effects of *Pediococcus acidilactici* J9 on *H. pylori* binding to human gastric cells and assessed its toxicity in C57BL/6 mice. Mice were divided into four groups and given daily oral doses of *P. acidilactici* J9 at 500, 1000, or 2000 mg/kg/day for two weeks. No adverse effects, including changes in body weight, organ weights, or clinical signs were observed.

Li *et al.,* 2020, explored the anti-tumor effects of AGS-30, focusing on its impact on angiogenesis. AGS-30 decreased VEGF expression and blocked microvessel sprouting in rat aortic rings, blood vessel formation in zebrafish embryos and tumor growth in mouse models. These effects were more pronounced than those observed with andrographolide, the parent compound of AGS-30. AGS-30 shows promise as a potent anti-cancer agent due to its ability to inhibit both tumor cell growth and angiogenesis.

Lin *et al.,* 2020, investigated the role of miR-370-3p in ulcerative colitis-associated colorectal cancer (UC-CRC) using an AOM/DSS-induced mouse model. They found that miR-370-3p treatment improved body weight and survival rates while inhibiting tumorigenesis in UC-CRC mice.

Mechanistically, miR-370-3p decreased the inflammatory response by targeting TLR4, leading to reduced levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and tumor-associated proteins (p53, β-catenin, Ki67). Additionally, miR-370-3p inhibited epithelial-mesenchymal transition (EMT) by increasing E-cadherin and decreasing N-cadherin and Vimentin expression. Overall, the study reports that miR-370-3p is a promising therapeutic target for UC-CRC by alleviating inflammation and EMT.

Liu *et al.,* 2020, studied the effects of exosomes from gastric cancer cell lines MKN-28, MKN-45 and SGC-7901 on immune function. They found that these exosomes altered gene expression and cytokine levels in CD8+ T cells, inducing cell cycle arrest and apoptosis. Systemically injected fluorescently labeled exosomes localized primarily to the lungs in C57BL/6 mice. The study suggests that exosomes from MKN-45 and MKN-28 cells create an immunosuppressive environment, aiding metastatic niche formation in the lungs.

Ribera *et al.,* 2020*, In vivo* studies in *Mongolian gerbils* did not show significant differences in *Helicobacter pylori* colonization or inflammation levels between groups fed with blueberry leaf extract and control groups. Overall, they reported that MeJA treatment enhanced the anticancer effects of blueberry leaf extracts by boosting antioxidant compounds and suppressing cancer cell viability and migration, primarily through modulation of MAPK pathway-related proteins.

Saadat *et al.,* 2020, explored the anti-cancer effects of extracellular polysaccharides (EPSs) from *Kluyveromyces marxianus* and *Pichia kudriavzevii* on various colon cancer cell lines. Their research showed that these EPSs induce apoptosis as confirmed by DAPI and Annexin V/PI assays but do not operate through ferroptotic pathways. Instead, they inhibit the AKT-1, mTOR and JAK-1 pathways, suggesting that these probiotic yeast EPSs could serve as novel molecular-targeted therapies for colorectal cancer (CRC).

Silva *et al.,* 2020, conducted a comprehensive study on the hematological and biochemical parameters of four mouse lineages-Swiss, BALB/c, C57BL/6 and B6D2F1 to establish reference values crucial for validating scientific research. The study involved 160 mice (20 males and 20 females per lineage) and utilized standardized analysis methods, including the pocH-100iV Diff™ for 19 hematological parameters and VITROS® 350 for 12 biochemical parameters. The results, presented as means and standard deviations, revealed variations compared to previous studies, potentially attributed to methodological differences or individual variability. The findings underscore the importance of understanding physiological parameters in laboratory animals and highlight the need to consider factors like gender, lineage and genotype when selecting experimental models.

Taniura *et al.,* 2020, examined the anticancer effects of 5-fluorouracil (5-FU) and oxaliplatin (L-OHP) combined with cyclophosphamide (CP) in two mouse models of colon cancer (CT26 and MC38). In the CT26 model, this combination significantly suppressed tumor growth compared to either treatment alone without causing significant body weight loss. However, efficacy was reduced in nude mice. Cured mice developed protective immunity against CT26, generating CT26-specific cytotoxic T cells (CTLs) from splenic cells. Analysis of tumor-infiltrating immune cells revealed an increase in CD8+ T cells and changes in myeloid-derived suppressor cell (MDSC) populations with the combination therapy. In the MC38 model, the triple therapy showed significant effects which were enhanced by adding anti- PD-1 antibody, leading to increased cured mice and MC38-specific CTLs. Overall, the study indicates that the effectiveness of the 5-FU/L-OHP and CP combination relies on host T cells and can be boosted by immune checkpoint blockade.

Bali *et al.,* 2021, investigated how different genetic backgrounds affect the gastric microbiome in mice. They studied wild type (WT), MyD88-deficient (Myd88$^{-/-}$), TRIF-deficient (TrifLps2) and MyD88- and TRIF- deficient (DKO) mice after infection with *H. felis*. All genotypes showed reduced microbial diversity post-infection with a significant decrease in Campylobacterales in Myd88$^{-/-}$ mice. Additionally, an increase in *Lactobacillales* was noted in infected Myd88−/− and DKO mice compared to TrifLps2 and WT mice. This rise in *Lactobacillales,* associated with

Helicobacter infection and gastric cancer progression, suggests that genetic variations can influence the gastric microbiome and its susceptibility to cancer.

Kumar *et al.,* 2021, aimed to evaluate the anti-metastatic properties of synthesized chalcones Ch-1 and Ch-2 in cancer, focusing on metastasis as a leading cause of mortality. *In vivo* study of Ch-1 (100 mg/kg) reduced metastatic lung nodules by 77.32% in mice, surpassing Quercetin's 56.5% reduction. Ch-1 also improved the hematological profile, indicating its potential as an anticancer agent.

Ma *et al.,* 2021, investigated the effects of LPEPS (*Lactiplantibacillus plantarum*-12) on colon cancer in C57BL/6 mice treated with azoxymethane/dextran sulfate sodium salt (AOM/DSS). The study reported that daily oral administration of LPEPS for 85 days improved colon health by increasing tight-junction protein expression, reducing colon shortening and tumor burden and lowering levels of pro-inflammatory factors while boosting anti-inflammatory IL-10. LPEPS also restored gut microbiota balance and altered fecal metabolites related to lipid and amino acid metabolism. Overall, LPEPS alleviated colon cancer symptoms by enhancing gut barrier function, modulating gut microbiota and affecting key inflammatory and apoptotic pathways.

Pereira *et al.,* 2021, investigated the effects of low-level light therapy using red light-emitting diode (LED) irradiation on breast cancer-bearing mice. In this study, 4T1 cells were inoculated into the mammary fat pad of female BALB/c mice, and the effects of three different energy levels (1.2, 3.6, and 6.0 J) were evaluated when tumor volume reached 100 mm^3. Control groups included untreated animals and healthy mice. Monitoring tumor volume and total blood counts, the results revealed that single irradiation did not increase tumor volume and led to improved clinical conditions in irradiated groups compared to controls, which exhibited significant decreases in platelet and red blood cell levels. Significantly, the 3.6 J energy level not only arrested the neutrophil-lymphocyte ratio but also promoted longer survival and fewer metastatic nodules in the lungs. The findings suggest that while a single red LED irradiation does not impact disease progression, the intermediary dose merits further investigation due to its potential for enhancing outcomes in breast cancer treatment.

Rahman *et al.,* 2023, examined the effects of non-electrolyzed hypochlorous acid (HOCl) water on *H. pylori* infection in C57BL/6 mice over 10 weeks. The study involved three groups: a normal control group receiving purified water a group infected with *H. pylori* receiving purified water and a group infected with *H. pylori* receiving HOCl water. The results showed that HOCl treatment significantly inhibited white blood cell (WBC) counts and differential counts (neutrophils, lymphocytes, monocytes and eosinophils) compared to the purified water group post-infection. Additionally, reactive oxygen species and calcium activity decreased in the HOCl group, while glutathione peroxidase (GPx) activity increased. Cytokine levels, including granulocyte-macrophage colony-stimulating factor, IL-1β and TNF-α, were lower in the stomach lysates of the HOCl group. The expression levels of pro-apoptotic markers (Bax, MMP-3, MMP-9, TLR-4) decreased, while the anti-apoptotic marker Bcl-2 increased in the stomach lysate after HOCl treatment. Overall, the study suggested that non-electrolyzed HOCl water possesses anti-oxidative, anti-inflammatory and anti-apoptotic effects in *H. pylori*-infected mice, highlighting its potential as a therapeutic agent.

Seyed *et al.,* 2021, investigated the use of mesenchymal stem cells (MSCs) loaded with oncolytic reovirus for cancer treatment in a C57BL/6 mouse model. The study aims to improve the delivery and efficacy of oncolytic viruses, which face challenges in clinical applications. C57BL/6 mice were inoculated with TC-1 cell lines to establish tumours and treated with either reovirus, MSCs loaded with reovirus, MSCs alone or PBS as a control. The study monitored tumor growth, apoptosis rates and cytokine release over two months. Results showed that reovirus infection did not significantly impair the migration capacity of MSCs, particularly at multiplicities of infection (MOI) 1 and 5. Furthermore, MSCs effectively delivered the oncolytic virus to the tumor site, resulting in significant tumor growth inhibition and increased secretion of IFN-γ. The study concludes that using MSCs as carriers for oncolytic reovirus enhances viral delivery and antitumor activity, demonstrating potential for broader cancer applications.

Tanaka *et al.,* 2021, examined the effects of bovine lactoferrin (bLF) on colorectal cancer in a mouse model related to inflammatory bowel disease (IBD). Patients with ulcerative colitis or Crohn's disease are at increased risk for colorectal cancer and prior studies have shown that bLF may inhibit colon carcinogenesis and promote remission in Crohn's disease. In this study, C57BL/6 mice were divided into four groups: no treatment, bLF only, azoxymethane plus dextran sulfate sodium (AOM + DSS) and AOM + DSS + bLF. Results indicated that mice treated with AOM + DSS + bLF had improved fecal scores, fewer colon lesions and less weight loss compared to those without bLF. However, there were no significant differences in tumor burden between the two AOM + DSS groups.

Tong *et al.,* 2021, reported the significant impact of gastrointestinal cancer, a leading cause of cancer mortality characterized by a high number of cases across various affected sites. They emphasize the association between high-fat diets and an increased risk of digestive system diseases and tumours, suggesting that such diets may accelerate cancer development through mechanisms involving inflammation and altered metabolism. The authors note the emerging role of the gut microbiome in cancer research, emphasizing its potential effects on cell damage and tumor immune microenvironment changes, which can contribute to gastrointestinal tumor progression. They propose that high-fat diets induce gastrointestinal tumours by disrupting the balance of intestinal microbes, ultimately influencing cancer development.

Wang *et al.,* 2021, reported on gastrointestinal cancer as a major global health issue characterized by high morbidity and mortality. The authors emphasize the potential of natural killer (NK) cells in cancer therapy due to their ability to rapidly target and destroy tumor cells without prior activation. NK cells also enhance adaptive immune responses through cytokine secretion and immunological memory, making them promising anti-cancer agents. Despite challenges like low infiltration and impaired function in tumours, strategies such as adoptive transfer, immune checkpoint inhibitors and cytokine treatments aim to enhance NK cell effectiveness. Preclinical data show promising results for NK cell applications in gastrointestinal cancer therapy.

Wang *et al.*, 2021, reviewed the role of chronic inflammation as a risk factor for gastrointestinal cancer, emphasizing the influence of nonsteroidal anti-inflammatory drugs (NSAIDs) in suppressing cancer incidence, growth and metastasis. The review highlights the cyclooxygenase-2 (COX-2) and its downstream bioactive lipid product, prostaglandin E2 (PGE2), as critical links between inflammation and cancer development. While this pathway has been studied extensively over the past two decades, recent research has begun to explore its effects on host defenses against transformed epithelial cells.

Zhang *et al.,* 2021, developed *in vitro* models with overexpression and knockdown of miR-375 in cells infected with *Helicobacter pylori* to study its effects. They analyzed dendritic cell (DC) maturity and cytokine expression (IL-6, IL-10, VEGF) and examined changes in the JAK2-STAT3 signaling pathway. *In vivo*, they assessed CD4+ and CD8+ T cell counts and tumor sizes using subcutaneous tumor models. The study found that *H. pylori* down regulates miR-375, affecting cytokine expression through the JAK2-STAT3 pathway and impairing DC maturation. Their findings were supported by *in vivo* results, showing that miR-375 reduces tumor size and that immature DCs lead to decreased CD4+ and CD8+ T cell numbers.

Lactobacillus plantarum ZJ316, which survives well in simulated gastrointestinal conditions has demonstrated significant anti-*H. pylori* effects. Zhou *et al.,* 2021, utilized a C57BL/6 mouse model to show that this strain not only prevents and treats *H. pylori*-induced gastritis but also significantly reduces levels of interferon γ (IFN-γ) and interleukin 6 (IL-6) while increasing interleukin 10 (IL-10) and repairing mucosal damage. Additionally, 16S rRNA gene sequencing revealed that *L. plantarum* ZJ316 effectively reduces *H. pylori* abundance. Their study suggests that *L. plantarum* ZJ316 is a promising candidate for preventing and treating *H. pylori*-induced gastritis by modulating the gastric microbiota and alleviating mucosal inflammation.

Cruz *et al.,* 2022, explored a nutritional strategy to mitigate the side effects of antineoplastic agents by combining cisplatin treatment with watermelon pulp juice supplementation in C57BL/6 mice with melanoma. Watermelon, known for its rich content of vitamins, minerals and antioxidants like lycopene, has been recognized for its therapeutic benefits in various health conditions. The study analyzed several parameters, including animal survival, bone marrow genotoxicity, serum creatinine and urea levels and histopathological features of tumor tissue. The findings indicated that while watermelon pulp juice did not exhibit antitumor effects, it effectively reduced the toxicity associated with cisplatin treatment, evidenced by an increased number of bone marrow cells and decreased serum creatinine and urea levels. This research suggests that watermelon pulp juice may serve as a viable option for alleviating the adverse effects of chemotherapy agents.

Kawanabe *et al.,* 2022, found that exopolysaccharides (EPS-R1) from *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1 stimulate CCR6+ CD8+ T cells in both mice and humans. In mice, EPS-R1 enhanced the effectiveness of anti–CTLA-4 and anti–PD-1 antibodies against tumours expressing CCL20, increasing CCR6+ CD8+ T cells that produce IFNγ and boosting immune responses. Significantly, EPS-R1 also had an adjuvant effect in germ-free mice. The induction of CCR6 expression was linked to the phosphorylated structure of EPS-R1 and its interaction with a lysophosphatidic acid receptor on CD8+ T cells. Overall, consuming EPS-R1 promotes CCR6+ CD8+ T cells in Peyer's patches and enhances the effectiveness of immune checkpoint therapies in tumors producing CCL20.

Li *et al.,* 2022, investigated on *H. pylori* infection that affects microRNAs (miRNAs) and their role in gastric carcinogenesis using a mouse model. They found that *H. pylori* infection inhibits autophagy in the gastric mucosa and decreases levels of miR-1298-5p in human and mouse gastric tissues. Bioinformatics, miRNA pull-down assays and luciferase reporter assays identified mitogen-activated protein kinase kinase 6 (MAP2K6) as a direct target of miR-1298-5p. This miRNA regulates autophagy and gastric cancer cell viability and motility through the MAP2K6/p38 MAPK pathway. The study suggests that *H. pylori* infection promotes tumor growth by disrupting miR-1298-5p expression and highlights the miR-1298-

5p/MAP2K6/p38 MAPK axis as a potential target for managing *H. pylori*-related gastric cancer.

Morningstar *et al.,* 2022, investigated the role of the *H. pylori* TNFα inducing protein (Tipα) in promoting inflammation and associated disease. In their study, mice were infected with either the wild-type Sydney strain (SS1) or a mutant strain lacking Tipα (Δtipα) for one to four months. Results showed that mice infected with the Tipα-expressing SS1 strain had significantly higher levels of TNFα and KC, leading to more severe histological gastritis and hyperplasia than those infected with the Δtipα strain. Microarray analysis of gastric epithelial cells treated with recombinant Tipα indicated activation of the NFκB pathway. These findings suggest that Tipα is crucial in driving *H. pylori*-induced inflammation.

Pappas *et al.,* 2022, reported on a new type of nanomotor aimed at treating *H. pylori* a significant cause of gastric cancer. This nanomotor has a bottle-shaped container that carries clarithromycin, nano calcium peroxide (CaO_2) and platinum nanoparticles (Pt NPs). It works by neutralizing gastric acid through a reaction with CaO_2, producing hydrogen peroxide (H_2O_2) which is then broken down into oxygen (O_2) by Pt NPs. This reaction helps propel the nanomotor and release the drugs. In tests with animals, a dose of 15 mg significantly lowered the *H. pylori* levels by 2.6 times compared to the control group and restored normal stomach acidity within one day, showing promise for treating *H. pylori* infections.

Tan *et al.,* 2022, explored the mechanisms behind the low radio sensitivity of colorectal cancer (CRC) and the associated toxicity to surrounding normal tissues. The study identified that GSDME is silenced in CRC cells but highly expressed in adjacent normal tissues. GSDME plays a crucial role in radiation-induced pyroptosis in both CRC and normal epithelial cells via a caspase-3-dependent pathway enhancing the sensitivity of radioresistant CRC cells to radiation. In homograft models, tumors expressing GSDME showed significantly reduced volume and weight following radiation treatment compared to GSDME-knockout tumors. The study also found that GSDME-mediated pyroptosis recruited and activated natural killer (NK) cells, boosting antitumor immunity. Moreover, GSDME-knockout mice exhibited protection from radiation-induced weight loss and damage to intestinal, stomach, liver and pancreatic tissues compared to wild-type controls. The findings highlight GSDME's pivotal role in CRC radiosensitivity and radiation-related toxicity, linking radiation to pyroptosis for the first time.

Tripathi *et al.,* 2022, investigated the radio-mitigative effects of melatonin (N-acetyl-5-methoxytryptamine) in C57BL/6 mice following whole-body irradiation (WBI). Melatonin, administered orally at 100 mg/kg post-irradiation for seven days significantly improved survival rates to 60% after lethal (9 Gy) and 90% after sub-lethal (7.5 Gy) WBI. It alleviated radiation-induced myelosuppression, pancytopenia and restored bone marrow and spleen cellularity. Melatonin also promoted intestinal recovery, reducing damage to villi and crypts. These results highlight melatonin's potential as a protective agent in radiation-induced injuries supporting its use in cancer treatment and radiotherapy.

Lee *et al.,* 2023, examined immunophenotypes and responses to immune checkpoint inhibitors (ICIs) using murine gastric cancer cell lines YTN3 and YTN16 in immunocompetent C57BL/6J mice. They established subcutaneous tumours with these cell lines and treated the mice with either an IgG isotype control or an anti-PD-L1 neutralizing antibody. Tumor-infiltrating immune cells were analyzed via immunohistochemistry on formalin-fixed paraffin-embedded tissue. The study found that YTN16 tumours which had higher levels of immunosuppressive genes like CCL2 and M-CSF exhibited more macrophages and exhausted T cells compared to YTN3 tumours. While YTN3 tumours showed significant regression with PD-L1 inhibition, YTN16 tumours did not respond to IgG isotype control or an anti-PD-L1 neutralizing antibody. Although PD-L1 inhibitor treatment increased T cell infiltration in both tumor types, it did not reduce the proportion of exhausted immune cells in the non-responder group.

Shen *et al.,* 2023, investigated the effects of various *Lactobacillus* strains on *H. pylori* colonization and gastric mucosa inflammation. They tested six probiotic strains- *Lactobacillus acidophilus* NCFM, *Lactobacillus acidophilus* La-14, *Lactiplantibacillus plantarum* Lp-115, *Lacticaseibacillus* *paracasei* Lpc-37, *Lacticaseibacillus rhamnosus* Lr-32 and *L. rhamnosus* GG reduced on *H. pylori* adhesion in AGS cells using fluorescence microscopy and urease activity assays. In *in vivo*, male C57BL/6 mice were divided into groups treated with various combinations of *L. acidophilus* NCFM and *L. plantarum* Lp-115 for six weeks. The study found that all *Lactobacillus* strains reduced *H. pylori* adhesion and inflammation with *L. acidophilus* NCFM and *L. plantarum* Lp-115 showing the most significant effects. These probiotics decreased mRNA and protein levels of IL-8 and TNF-α reduced *H. pylori* colonization and lowered inflammatory cell infiltration in the gastric mucosa. The combined treatment with *L. acidophilus* NCFM and *L. plantarum* Lp-115 notably reduced inflammation and *H. pylori* colonization with decreased IFN-γ expression in treated mice.

Xu *et al.,* 2023, developed a novel orthotopic model for anaplastic thyroid carcinoma (ATC) in C57BL/6 mice, focusing on tumor immunity. They used adapted TBP3743 cells, created through *in vivo* passaging in C57BL/6 mice and established three models: B6129SF1 mice with original TBP3743 cells (original/129), B6129SF1 mice with adapted cells (adapted/129) and C57BL/6 mice with adapted cells (adapted/B6). The adapted cells maintained similar *in vitro* properties to the originals. The adapted/129 model showed increased tumor proliferation and oncogenic properties compared to the original/129. In contrast, the adapted/B6 model had smaller tumours, lower proliferation but stronger immune responses with higher CD8+ T cell and monocytic-myeloid-derived suppressor cell fractions. This model demonstrated more aggressive behaviour and robust immune responses compared to the original B6129SF1 model.

Dinis *et al.,* 2024, conducted a systematic review of international guidelines on managing gastric intestinal metaplasia (GIM) to offer a unified perspective for clinicians. They analyzed 16 guidelines globally, finding broad agreement on the need for endoscopic surveillance in high-risk GIM cases such as those with corpus-extended GIM or persistent *H. pylori* infection. While most guidelines upfolded for *H. pylori* eradication to prevent gastric cancer, recommendations on surveillance intervals and histological staging vary. The study emphasized the need for standardized guidelines particularly in screening populations, endoscopy quality metrics and additional non-endoscopic interventions for gastric cancer prevention beyond *H. pylori* eradication.

Du *et al.,* 2024, explored strategies to improve immune checkpoint inhibitor (ICI) treatment for peritoneal dissemination in gastric cancer (GC). They evaluated the effectiveness of anti-PD1, anti-CTLA4 and their combination using the YTN16 peritoneal dissemination model, analyzing the tumor microenvironment (TME) through immunohistochemistry, flow cytometry and bulk RNA sequencing. While dual ICI treatment showed therapeutic promise in some mice, its efficacy was limited by CD8+ T cell exhaustion in resistant cases. Pathway analysis revealed abnormal activation of the JAK-STAT pathway in resistant tumours along with increased infiltration of immunosuppressive cells like macrophages, neutrophils and Tregs. Introducing a Janus kinase inhibitor (JAKi) improved CD8+ T cell function and reconfigured the immunosuppressive TME enhancing the effectiveness of dual ICI therapy. Thus, dual ICI treatment increases tumor-specific CD8+ T cell infiltration and combining it with JAKi can overcome ICI resistance by modifying the TME.

Hernández *et al.*, 2024, explored the impact of breeding nuclei on prostate cancer development and intratumoral macrophage populations in C57BL/6 mice which are essential for immune-oncology research. An isotransplantation experiment using TRAMP-C1 prostate adenocarcinoma cells was conducted on C57BL/6J mice from two different breeding nuclei (nA and nB). The results showed that 54% of the BL/6nB mice exhibited tumor implantation after a 69-day growth period while the BL/6nA group reached 100% implantation within 28 days. Despite no significant differences in total macrophage populations across various tumor regions, the BL/6nB tumours displayed a higher proportion of M1 macrophages (11–27%) compared to BL/6nA (4–15%) alongside a lower prevalence of non-polarized macrophages. The M1:M2 average ratio was 1:8 for group A and 1:4 for group B, indicating a more favorable M1 profile in the BL/6nB tumours. The findings highlight significant differences in tumor progression and macrophage populations among mice from the same substrain, underscoring the importance of considering breeding source variations to control variables in murine cancer models.

Li *et al.,* 2024, investigated NKG2A as a potential immunotherapeutic target in gastric cancer and explored new combination strategies for immune checkpoint inhibitors. Using single-cell RNA sequencing and advanced analytical models, the study examined immune checkpoint expression in CD8+ T cells and identified patterns of resistance to anti-PD-1 therapy. They discovered that co-expression of NKG2A and PD-1 significantly inhibits CD8+ T cell proliferation. Two distinct immune checkpoint expression patterns (ICEPs) were identified: ICEP1 (CD8+ T cells co-expressing PD-1 with other checkpoints) and ICEP2 (CD8+ T cells expressing only NKG2A or with other checkpoints). ICEP2 was linked to resistance to anti-PD-1 therapy, potentially due to the recruitment of LGMN+ macrophages via the CXCL16-CXCR6 signaling pathway. These findings highlight NKG2A as a promising target for gastric cancer immunotherapy and suggest novel approaches for combining immune checkpoint inhibitors to overcome therapy resistance.

Li *et al.,* 2024, investigated the role of circular RNAs (circRNAs) in gastric cancer (GC) progression and their impact on immune escape and immunotherapy efficacy. They identified differential circRNA expression through sequencing revealing that circRHBDD1 is significantly overexpressed in GC tissues. High levels of circRHBDD1 correlate with poor overall and disease-free survival in patients. Functional experiments demonstrated that circRHBDD1 promotes PD-L1 expression and hinders CD8+ T cell infiltration. Mechanistically, circRHBDD1 interacts with IGF2BP2 inhibiting its degradation and enhancing PD-L1 mRNA stability via m6A modification. The study also developed PLGA-PEG nanoparticles to deliver circRHBDD1 siRNA showing that combining this treatment with anti-PD-1 improves cancer immunotherapy effectiveness *in vivo*. These findings suggest that targeting circRHBDD1 could enhance immune responses in GC offering a promising avenue for future therapeutic strategies.

Ma *et al.,* 2024, utilized advanced omics technologies such as proteomics and transcriptomics to classify gastric cancer (GC) into distinct subtypes, focusing on diffuse gastric cancer (DGC) and intestinal-type gastric cancer (IGC). They identified three DGC subtypes through proteomic analysis of phosphorylated proteins with the Ph1 subtype showing the best prognosis due to higher levels of tumor-infiltrating lymphocytes (TILs) and stromal cells. ARID1A mutations were found to have different prognostic impacts: detrimental for DGC but beneficial for IGC. The study highlights the importance of molecular profiling for guiding treatment and suggests that integrating omics data can lead to more personalized and effective treatment strategies advancing precision oncology in GC management.

Wu *et al.,* 2024, reviewed on the role of long non-coding RNAs (lncRNAs) particularly RNF144A-AS1 in cancer biology emphasizing their involvement in tumor progression and chemoresistance. Their study highlights how RNF144A-AS1 stabilizes the TAF15 protein promoting malignant behaviours in various cancers including skin cutaneous melanoma and glioblastoma. The study reported that RNF144A-AS1 acts as a critical regulator of gene expression and cellular pathways contributing to the aggressive nature of tumours and their resistance to therapies. This review underscores the potential of targeting lncRNAs like RNF144A-AS1 for developing novel therapeutic strategies in cancer treatment.

2.6. *In silico* Study and Network Pharmacology: Molecular Docking and Computational Analysis

Chong *et al.,* 2020, conducted a bioinformatics study to identify key genetic markers associated with gastric cancer (GC) for early detection and prevention. By analyzing differentially expressed genes (DEGs) from GSE54129 and GSE13911 datasets using GEO2R, they identified 1034 DEGs. Gene enrichment analysis revealed that these DEGs were primarily involved in the plasma membrane, cell adhesion and PI3K-Akt signaling pathway. A protein-protein interaction (PPI) network was constructed, identifying 18 candidate genes through topological and module analysis. Survival analysis showed that high expressions of FN1, TIMP1, SPP1, APOE and VCAN were linked to poor overall survival in GC patients, highlighting these genes as key markers in GC.

Li *et al.,* 2020, aimed to identify key prognosis-related genes for gastric cancer (GC) in the Chinese population through bioinformatics analysis. Using four microarray datasets from the GEO database, they identified 240 differentially expressed genes (DEGs) including 80 upregulated and 160 downregulated genes. Functional enrichment analysis revealed that upregulated genes were primarily involved in extracellular matrix (ECM) organization and structural constituents while downregulated genes were associated with digestion and oxidoreductase activity. The KEGG analysis highlighted pathways like ECM-receptor interaction, focal adhesion and the PI3K-Akt signaling pathway for upregulated genes. Four genes- COL1A1, COL5A2, COL12A1 and VCAN were identified as potential biomarkers for GC, providing insights into the disease's molecular mechanisms in the Chinese population.

Wang *et al.,* 2020, conducted a comprehensive bioinformatics study to investigate the molecular mechanisms underlying gastric cancer (GC). By analyzing differentially expressed genes (DEGs) from TCGA and GEO datasets, they identified key hub genes involved in GC progression including SERPINH1, COL1A1 and others. The study highlighted SERPINH1 as a core therapeutic biomarker, demonstrating its role in promoting the proliferation, migration and cell cycle regulation of GC cells. This study emphasizes the potential of SERPINH1 as a critical target for diagnostic and therapeutic strategies in GC.

Yang *et al.*, 2020, investigated the potential mechanisms of quercetin in treating gastric cancer (GC) using network pharmacology approach. They identified quercetin’s targets and pathways through database mining and analyzed these targets relevance to GC using Gene Ontology (GO) and KEGG pathway enrichment. Molecular docking further explored quercetin's interactions with key targets. The study identified 36 genes associated with quercetin and 15 overlapping GC-related targets which were linked to 319 GO biological processes and 10 significant pathways. Six hub targets- AKT1, EGFR, SRC, IGF1R, PTK2 and KDR were found to be associated with poor overall survival in GC patients. Molecular docking confirmed that quercetin binds effectively to these targets.

Yu *et al.,* 2020, identified key genes and pathways involved in gastric cancer (GC) progression using bioinformatics analysis of the GSE103236 dataset. They found 161 differentially expressed genes (DEGs) and constructed a protein-protein interaction (PPI) network, identifying hub genes like COL1A1, TIMP1, SPP1, MMP3, BGN, APOE and LOX. SPP1 and MMP3 were significantly associated with poor overall survival. Stage-specific correlations were found for COL1A1, BGN and LOX. Methylation of SPP1 and MMP3 showed prognostic significance. APOE was highly correlated with immune cell infiltration. Therefore, this study reported insights into molecular mechanisms for personalized therapeutic approaches in GC.

Zhou *et al.,* 2021, developed a prognostic model for gastric cancer (GC) using bioinformatics to improve outcome predictions and treatment optimization. Gene expression and clinical data were obtained from GEO, GTEx and TCGA databases. The study identified a four-gene signature and constructed a reliable prognostic model validated both internally and externally. Additionally, a nomogram was created to predict patient prognosis with high accuracy as confirmed by a calibration curve. The four genes were linked to tumor development and are considered potential therapeutic targets making the model beneficial for GC patient prognosis and treatment planning.

Alatan *et al.,* 2021, conducted an integrated bioinformatics analysis to identify potential biomarkers and molecular targets for gastric adenocarcinoma (GAC). Using gene expression profiles from the GEO database, they screened differentially expressed genes (DEGs) and identified 15 hub genes through protein-protein interaction (PPI) network analysis. Gene ontology (GO) and KEGG pathway enrichment analysis highlighted the role of extracellular matrix (ECM) proteins in GAC. Among the hub genes- BGN, VCAN, COL1A1 and TIMP1 were found to be associated with poor prognosis, suggesting their potential as prognostic biomarkers and therapeutic targets for GAC.

Almatroodi *et al.,* 2021, reported that quercetin impacts cell signaling pathways in cancer therapy by modulating key molecular mechanisms crucial for cancer progression and survival. It affects pathways such as PI3K/Akt/mTOR, MAPK and Wnt/β-catenin all of which are vital to cell proliferation, survival and apoptosis. By inhibiting these pathways, quercetin induces apoptosis in cancer cells, reducing tumor growth and metastasis. Its anti-inflammatory properties also lower the production of pro-inflammatory mediators that contribute to cancer development. Additionally, quercetin inhibits VEGF signaling which helps prevent angiogenesis a key factor in tumor growth. These studies highlight quercetin's potential as a complementary or alternative therapeutic agent in cancer management, targeting cancer cells while sparing normal cells from toxicity.

Divyashri *et al.,* 2020, investigated mango ginger (*Curcuma amada* Roxb.) for potential inhibitors against *H. pylori* using molecular docking against crystal structures of selected drug targets from the Protein Data Bank. They screened 130 compounds known for activity against *H. pylori* and selected 15 for ADMET and Lipinski's rule of five evaluations all showing promising results. Molecular dynamics simulations identified gentisic acid as a promising compound targeting shikimate kinase of *H. pylori* reporting mango ginger compounds as potential therapeutics against *H. pylori* infections.

Kalimuthu *et al.,* 2021, explored bioactive compounds from Rasam, a South Indian dish, against oxidative stress-induced malignancies. They identified 66 compounds and conducted molecular docking against MAPK6 comparing with PD-173955. Four compounds- Assafoetidinol A, Naringin, Rutin and Tomatine showed promising binding energies (-6.1 to -10.6 kcal/mol) and were stable in MAPK6 binding sites during molecular dynamics simulations. Therefore, they reported that all the compounds hold potential as lead candidates for combating oxidative stress-induced human malignancies.

Laloo *et al.,* 2021, aimed to identify phytoconstituents responsible for gastric anti-secretory effects through *in silico* molecular docking techniques. The study focused on EC's (ellagic acid) inhibition of H^+, K^+ATPase enzyme. *In silico* molecular docking indicated that EC exhibits a strong inhibitory effect (binding energy of -8 kcal/mol) towards H^+, K^+ATPase attributed to multiple hydrogen bond interactions (with Asp 137, Val 341, Glu 795 and Leu 811) and π-π stacking (with Tyr 799) with amino acids in the enzyme's active site.

Lu *et al.,* 2021, studied the active ingredients and mechanisms of *Sparganii rhizoma* (SL) in treating gastric cancer (GC) using HPLC-Q-TOF–MS/MS and network pharmacology. They identified 41 compounds from SL and determined seven key active ingredients and ten hub genes through target prediction, gene function annotation and molecular docking. The main ingredients included acacetin, sanleng acid and caffeic acid while the hub genes were PIK3R1, PIK3CA, SRC, MAPK1, AKT1, HSP90AA1, HRAS, STAT3, FYN and RHOA. Analysis of mRNA and protein levels in GC patients showed that SL may affect GC treatment by modulating PI3K-Akt and other signaling pathways, influencing processes such as cell proliferation, apoptosis, migration and angiogenesis.

Ma *et al.,* 2021, aimed to identify potential biomarkers and prognosis-related hub genes associated with gastric cancer (GC). Using the GSE79973 dataset from GEO, they identified 1269 up-regulated and 330 down-regulated differentially expressed genes (DEGs). A protein-protein interaction (PPI) network was constructed and 11 hub genes were selected through CytoHubba analysis. Functional enrichment revealed that these genes were linked to cellular senescence, the cell cycle and p53 signaling pathways. Kaplan-Meier analysis and further validation identified seven key hub genes- C3, CDK1, FN1, CCNB1, CDC20, BUB1B and MAD2L1 as potential prognostic biomarkers and therapeutic targets for GC.

Mehta *et al.*, 2021, investigated the binding affinity of major phytocompounds from 35 medicinal plants of the North Western Himalayas with the RamR protein (PDB ID 6IE9) of *Salmonella typhimurium*. They used AutoDock 1.5.6 to prepare proteins and ligands followed by molecular docking with AutoDock Vina and 100 ns molecular dynamics (MD) simulations. Drug likeness and toxicity predictions were assessed using Molinspiration and ProTox II online servers. Among the phytocompounds evaluated beta-sitosterol demonstrated the highest binding affinity with RamR protein and showed non-toxic properties. MD simulation data indicated stability of the beta-sitosterol-RamR complex, suggesting its potential as an antimicrobial agent targeting *Salmonella typhimurium.*

Omoboyowa *et al.,* 2021, investigated the anti-ulcerative effects of the ethyl acetate extract from *Abrus precatorius* seeds in rats with HCl/EtOH-induced ulcers. They identified flavonoids as the major bioactive compounds responsible for interacting with pro-inflammatory targets. ADME/Tox screening and drug-likeness prediction analyses confirmed the safety of these compounds. The study reported that *A. precatorius* seeds have potential which act as a protective agent against ulcers by modulating inflammatory responses.

Qazi & Raza, 2021, conducted an *in-silico* analysis of Indian Ayurvedic plants to evaluate their anticancer efficacy against the ovarian cancer proliferation target receptor POTEE. They screened structures of 100 phytochemicals from 11 Ayurvedic plants based on ADME criteria followed by molecular docking and interaction analysis. Six phytochemicals (cedeodarin, deodarin, hematoxylin, matairesinol, quercetin and taxifolin) exhibited high affinity for POTEE with binding affinities ranging from -7.7 to -8.1 kcal/mol. These compounds were further analyzed using all-atom replica exchange molecular dynamics simulations for 50 ns showing stable RMSD values. The study concludes that phytochemicals from *Cedrus deodara* and *Asparagus racemosus* in Indian Ayurvedic plants have potential inhibitory effects against ovarian cancer proliferation mediated by POTEE.

Sun *et al.,* 2021, analyzed gene expression profiles and clinical data from TCGA to identify differentially expressed genes (DEGs) related to gastric cancer (GC). Using survival analysis, they filtered 238 strong DEGs including 140 up-regulated and 98 down-regulated genes. Functional enrichment analysis revealed that up-regulated DEGs were linked to processes like cytokine activity and systemic lupus erythematosus while down-regulated DEGs were associated with immune response and metabolism. Through protein-protein interaction (PPI) network analysis, eight hub genes including CCR8 and CCL25 were identified as potential prognostic biomarkers and future treatment targets for GC.

Wu *et al.,* 2021, explored the diagnostic and prognostic potential of oxidative stress (OS) genes in gastric cancer (GC) by analyzing transcriptome data from The Cancer Genome Atlas (TCGA) and validating their findings using the Gene Expression Omnibus (GEO) database. They identified 11 prognosis-associated OS genes and constructed a prognostic model which showed better diagnostic accuracy than traditional clinicopathological features and was significantly correlated with overall survival in GC patients. A nomogram based on the model's risk score demonstrated strong predictive ability. Additionally, Weighted Gene Co-expression Network Analysis (WGCNA) identified 13 OS-related hub genes associated with GC progression. Functional and Gene Ontology (GO) analyses revealed that these genes were involved in key pathways like cell adhesion and extracellular matrix remodeling, shedding light on the mechanisms by which OS genes contribute to GC progression.

Chivu *et al.,* 2022, conducted a bioinformatics analysis using their data along with two GEO microarray profiles to identify key biomarkers for gastric cancer (GC). They found 40 differentially expressed genes, with nine hub genes- COL12A1, COL5A2, COL10A1, BGN, CTHRC1, THBS2, SPP1, FAP and SULF1, highlighted through PPI network and MCODE analysis. These genes were associated with collagen assembly, degradation, cell adhesion and their high expression was linked to advanced pathological T stages. Moreover, the upregulation of genes like BGN, THBS2 and CTHRC1 correlated with poor overall survival and increased immunosuppressive M2 macrophage infiltration making them potential biomarkers for GC progression and targets for therapeutic intervention.

Das *et al.,* 2022, employed *in-silico* docking methods to identify potential inhibitors from *Houttuynia cordata* against overexpressed HER2 (breast cancer) and VEGFR2 (stomach cancer) genes. They screened 100 biologically active phytochemicals from *H. cordata* and docked them against the ligand-binding pocket of HER2 and VEGFR2 kinase domains. The results revealed that the selected phytochemicals including β-sitosterol and quercetin which exhibited higher binding affinity than the natural ATP ligand. These compounds formed strong interactions including hydrogen bonds and hydrophobic interactions, reporting their potential as effective inhibitors targeting HER2 and VEGFR2 receptors in cancer therapy.

He *et al.,* 2022, investigated the potential therapeutic strategy of disrupting the interaction between the CagA oncoprotein of *H. pylori* and phosphatidylserine in host cells using small molecules. They screened natural compounds from the ZINC database using bioinformatics tools focusing on physicochemical properties, ADMET characteristics and drug-like features. Three compounds- ZINC153731, ZINC69482055 and ZINC164387 showed high binding affinity to the CagA protein with binding energies of −11.53, −10.67 and −9.21 kcal/mol respectively, exceeding that of the control compound (−7.25 kcal/mol). Molecular dynamics simulations confirmed the stability of ZINC153731 and ZINC69482055 near the CagA binding pocket over 100 ns, suggesting their potential as lead compounds for further therapeutic development against gastric cancer progression facilitated by *H. pylori* infection.

Kaur *et al.,* 2022, conducted a study to explore the anticancer potential of phytoconstituents from *Foeniculum vulgare* Mill. against a breast cancer target protein (PDB ID: 6CHZ) using molecular docking. They utilized Autodock/Vina software for docking, Protox II server for toxicity prediction and Molinspiration for drug likeness assessment. Among 40 selected phytoconstituents α-pinene and D-limonene demonstrated the best binding energies (-6 and -5.9 kcal/mol respectively) with the breast cancer target. Both α-pinene and D-limonene met toxicity parameters and showed stability during 100 ns MD simulations with 6CHZ. The study reported α-pinene and D-limonene as potential therapeutic agents for treating breast cancer.

Paul & D'Souza, 2023, conducted *in-silico* docking analysis using the Lamarckian genetic algorithm to evaluate 12 selected phytochemicals. They assessed inhibition constants, binding energies and ADMET properties focusing on their potential inhibitory effects compared to commercial drugs. Rutin exhibited the highest binding affinity during molecular docking with (Catechol-O-methyltransferase) COMT reporting its strong potential as an inhibitor.

Saleem *et al.,* 2022, conducted a study to evaluate the bioactive compounds from *Thymus vulgaris*. Their GC-MS analysis identified 31 compounds in the methanolic crude extract with thymol, carvacrol, p-cymene and eugenol as major constituents. HPLC analysis of the ethyl acetate and n-butanol fractions revealed significant concentrations of flavonoids and phenolic acids. *In silico* analysis focused on chlorogenic acid which showed promising anticancer activity against gastric cancer genes, highlighting its potential for drug development in cancer therapy.

Zhou *et al.,* 2022, used network pharmacology to explore the mechanism of action (MoA) of Moluodan (MLD) in treating chronic atrophic gastritis (CAG). Their approach combined computational predictions with experimental validation revealing that MLD targets a wide array of biomolecules associated with various biological functions. The study found that MLD's MoA includes modulation of inflammation, immune responses, cell proliferation, apoptosis, differentiation and metabolism aligning with mechanisms of Western medicines for CAG. This integrated framework offers a novel and comprehensive method for understanding the therapeutic effects of traditional medicines like MLD.

Al-Rajhi *et al.,* 2023, analyzed phenolic compounds and flavonoids in *Acacia nilotica* fruits via HPLC. They conducted molecular docking of ferulic acid with the crystal structure of *H. pylori* protein (4HI0) to assess binding interactions. Their docking study revealed that ferulic acid effectively inhibited the 4HI0 enzyme of *H. pylori* showing a favorable binding mode with a low energy score of -5.58 kcal/mol. The interaction was primarily mediated by ferulic acid's O 29 atom which formed key interactions with the active site residue SER 139 indicating its potential as an antibacterial agent targeting *H. pylori*.

Ali *et al.,* 2024, conducted a study to inhibit HMG-CoA reductase using 15-oxoursolic acid, employing both *in vivo* and *in silico* assessments from stem bark of *R. arboreum. In silico* studies revealed that 15-oxoursolic acid exhibited significant interaction with HMG-CoA reductase with a binding energy of -9.26805 kcal/mol. The study concluded that 15-oxoursolic acid could effectively reduce total cholesterol, triglycerides and LDL levels in rats with high-fat diet-induced hyperlipidemia. The study reported its potential as a novel medication with significant hypolipidemic properties.

Carmo *et al.,* 2023, explored the antitumor activity of *Geissospermum sericeum* on the ACP02 cell line (human gastric adenocarcinoma) and elucidated the mechanism of cell death. Molecular modeling studies indicated that geissoschizoline N4-methylchlorine could effectively bind to the active sites of caspases 3 and 8 with favorable energetics. Fractionation of the plant extract enhanced its activity demonstrating significant selectivity for ACP02 cells. They reported that Geissoschizoline N4-methylchlorine emerged as a promising candidate for caspase inhibitors involved in apoptosis in gastric cancer, highlighting its potential therapeutic value.

Mehta *et al.,* 2023, explored the phytochemicals of *R. arboreum* using data from PubChem and reconstructed a target protein using the RCSB Protein Data Bank. They employed AutoDock Vina software to dock these phytochemicals with penicillin binding protein (3VSL) validating microbe interactions and bioactive molecules. Lipinski's Rule of 5 and drug-likeness analysis were conducted using Molinspiration and Swiss ADME servers respectively. Protox II and AdmetSAR tools were used to assess phytoconstituent toxicity. *In silico* data identified epifriedelanol and campanulin as promising antibacterial agents from *R. arboreum.*

Sehim *et al.,* 2023, used GC-MS analysis to confirm the presence of diverse bioactive components within a methanol extract of hibiscus. They employed molecular docking via the MOE-Dock tool to assess the binding interactions of n-Hexadecanoic acid, hexadecanoic acid-methyl ester and oleic acid, 3-hydroxypropyl ester with crystal structures of *E. coli* (MenB) (PDB ID: 3T88) and a cyclophilin from a colon cancer cell line (PDB ID: 2HQ6). The study reports its insights into the potential inhibitory effects of these compounds using molecular modeling techniques, reporting therapeutic implications for *E. coli* infections and colon cancer treatment.

Guo *et al.,* 2024, examined the pharmacological mechanisms of *Rhododendron molle* flower (RMF) in rheumatoid arthritis using metabolomics and network pharmacology. RMF alleviated RA symptoms by reducing pro-inflammatory factors IL-1β, IL-6, and TNF-α and mitigating bone destruction in CIA rats. The study identified four key targets- AKR1B1, TPH1, CYP1A1 and CYP1A2 and also the associated metabolites including

D-glucose, D-mannose, L-tryptophan, 11-deoxycorticosterone and 17α-hydroxyprogesterone. These targets and metabolites were linked to steroid hormone biosynthesis, tryptophan metabolism and galactose metabolism. This study provided a foundation for advancing the clinical application and development of RMF as a therapeutic drug.

Jangwan *et al.,* 2024, investigated the molecular mechanisms of *R. arboreum* flower (RAF) for treating non-alcoholic fatty liver disease (NAFLD) using network pharmacology and molecular docking. They assessed drug-likeness, potential side effects and ADMET profiles of RAF bioactives analyzed protein interactions with STRING and performed GO analysis. The study identified five active compounds and 30 target proteins revealing that RAF primarily affects fatty acid biosynthesis, lipid metabolism and stress response in NAFLD. Molecular docking and simulations showed rutin as having the best binding affinity. This study reported the valuable insights into RAF's mechanisms and potential for NAFLD treatment.

Mirazimi *et al.,* 2022, highlight that quercetin has demonstrated significant therapeutic potential in the treatment and prevention of gastrointestinal (GI) cancers including pancreatic, gastric, colorectal, esophageal, hepatocellular and oral cancers. Quercetin exhibits various mechanisms of action such as inducing apoptosis, promoting cell cycle arrest and exerting antioxidant properties. It increases pro-apoptotic molecules like P53 and BAX while decreasing anti-apoptotic proteins enhancing cell death in cancer cells. Additionally, it regulates the cell cycle by activating p21 and inhibiting proteins involved in cell proliferation causing G_1 phase cell cycle arrest. Quercetin's ability to inhibit carcinogenesis and metastasis underscores its potential as a natural therapeutic agent against GI tumors. However, the study reported that further studies are necessary to fully elucidate the underlying mechanisms and assess any potential adverse effects associated with its use.

Ozturk *et al.,* 2024, aimed to address the shortcomings of chemotherapy by evaluating the antiproliferative effects of *Plantago major* herbal extract on NIH/3T3 fibroblast cells and gastric (AGS) and colorectal (Caco-2) cancer cell lines using XTT cell viability assays. Molecular docking analysis revealed that active compounds in *P. major* extract including apigenin, aucubin, baicalein, caffeic acid and luteolin exhibited high binding affinities towards EpCAM proteins overexpressed in gastric and colorectal cancer cells. This study reported potential therapeutic benefits of *P. major* extract in targeting EpCAM-associated cancers with enhanced specificity and efficacy.

Zúñiga *et al.,* 2024, reported on quercetin (QRC) that has significant potential as a co-adjuvant factor in the treatment of gastric cancer (GC). It demonstrates that QRC can inhibit GC cell proliferation and induce apoptosis suggesting its effectiveness in targeting cancerous cells. Additionally, the study identified over 30 potential biological targets of QRC, many of which were linked to cancer survival and immune system modulation. Significantly, while most hub genes were not associated with improved survival in GC, the ability of QRC to alter immune cell infiltration presents a promising avenue for enhancing pharmacological treatments. Overall, the study reports for further exploration of QRC as a safer and complementary therapeutic option in GC management.