1. Introduction

Medicinal plants are an invaluable resource for discovering natural compounds used in both modern drug development and traditional healing practices. They provide powerful therapeutic benefits and hold a significant place in diverse medical traditions across the globe. The trial-and-error approach served as an initial method of using plant benefits to care for illnesses and boost human health. People used this traditional medical knowledge over multiple generations which resulted in standardized systems across worldwide traditional medical practices (Salmerón *et al.,* 2020).

The World Health Organization recorded that eight out of ten individuals living in low and middle-income countries depend on traditional plant-based medical treatments to address their healthcare requirements. Globally, up to 80% of the people depend on some form of ancient medicine, with 70-95% of people in developing countries depending on medicinal plants for their primary healthcare needs. In recent years, the use of these plants as remedies has seen a significant rise, largely due to their cost-effectiveness, widespread availability and the deep-rooted trust they have gained through generations of use. This enduring reliance underscores their vital role in both traditional practices and contemporary health solutions (Rabelo & Costa, 2018).

Traditional ethnomedicinal knowledge and indigenous practices act as essential guidance for modern pharmaceutical research and development. Among 3,50,000 plant species, 14% to 28% of vascular plant species exhibits medicinal properties, nearly 74% of bioactive compounds were identified from these plants (Vaou *et al.*, 2021). Medicinal herbs are prosperous sources of secondary metabolites such as terpenoids, phenols, steroids, flavonoids, tannins and aromatic compounds. These bioactive components are vital for defence and adaptation and play a main part in the pharmaceuticals, cosmetics and food industries. Over 21,40,000 secondary metabolites including 29,000 terpenoids, 12,000 alkaloids and 8,000 phenolics with ongoing discoveries expanding these categories (Pant *et al.,* 2021).

The Medicinal herbs are significant in drug discovery because they usually contain complex mixtures of compounds and are often synergistic.

Unlike molecules like synthetic drugs that bind only to their specific targets, the various phytochemicals found in medicinal plants can interact with several biological targets, enabling comprehensive and multifaceted therapeutic effects (Gorlenko *et al.,* 2020). The compounds like alkaloids, flavonoids and polyphenols show potent antioxidant, antimicrobial, anticancer and anti-inflammatory properties (Othman *et al.,* 2019).

1.1. Gastric Cancer

Several interacting environmental factors together with host-specific conditions lead to a complex carcinogenesis process that forms gastric cancer. Gastric carcinogenesis is a persistent disease caused by *Helicobacter pylori* (Group 1) carcinogen and was first documented by the International Agency for Research on Cancer in 1994. Later development stages of Gastric Cancer (GC) also involve bacterial factors besides *H. pylori*. Recent studies indicated that patients with GC exhibit alterations in gastric microbiota composition, significantly showing reduced microbial diversity and an increase in other bacterial genera, particularly oral and intestinal dependent (Petryszyn *et al.,* 2020).

The global survival statistics of GC show poor outcomes because of its dangerous nature. New GC cases continue to rise during each year mainly in Asian and South American regions. A total of 27,000 patients received GC diagnosis throughout the United States in 2020 (Sexton *et al.,* 2020). In 2018 GC resulted in 8.2% of all cancer deaths and was noted as the fifth most diagnosed cancer type during that year. The third place among deadly cancers is gastric cancer, that reports about one percent of annual cancer deaths above lung cancer (18.4%) and colorectal cancer (9.2%) (Thrift & El-Serag, 2020).

In India, digestive system cancers constitute a significant portion of the overall cancer burden, with an estimated 288,054 cases reported in 2022, making it one of the leading sites of cancer incidence in the country. This category includes cancers of organs such as the stomach, liver, gallbladder and others within the digestive tract. The high prevalence underscores the substantial impact of digestive cancers on public health, emphasizing the importance of targeted prevention, early detection and intervention strategies to manage this growing health concern in India (Sathishkumar *et al.,* 2022).

Gastric cancer incidence tends to be higher in nations with a very high Human Development Index (HDI). Despite this higher incidence, GC-related mortality is lower in high HDI nations compared to those with medium or low HDI nations. This discrepancy highlights the complex interplay between healthcare access, early detection, treatment options and overall socio-economic development in influencing cancer outcomes (Morgan *et al.,*2022).

In 2020, Eastern and South Eastern Asia with high HDI and prevalent virulent *H. pylori* strains, accounted for nearly two-thirds of global gastric cancer cases (6,96,112). Eastern Asia had the highest incidence rate (22.4 per 1,00,000) followed by Central / Eastern Europe (11.3) and South America (8.7). Conversely, North America (4.2) and Africa (3.4) had the lowest rates. Since the mid-20th century, GC rates have declined in high HDI countries but with varying speeds and onset times (Thrift *et al.,* 2023).

Advanced gastric cancer is classified according to the Borrmann classification system (1926), which categorizes tumours based on their gross appearance. There are four types of tumours classified using the Borrmann classification system (Figure. 1).

1. Patients with Type I tumours present with well-defined polypoid or fungating masses inside the stomach lumen.
2. Tumours belonging to Type II (Fungating with ulceration) show elevation from the stomach wall but reveal ulcerated regions on their surface.
3. The Type III tumours exhibit ulcerative lesions that show elevated borders and develop craters on their surfaces.
4. The fourth classification (Diffuse infiltrative) includes stomach tumours which disperse diffusely into the wall while remaining mass-less and become stiff and thick.

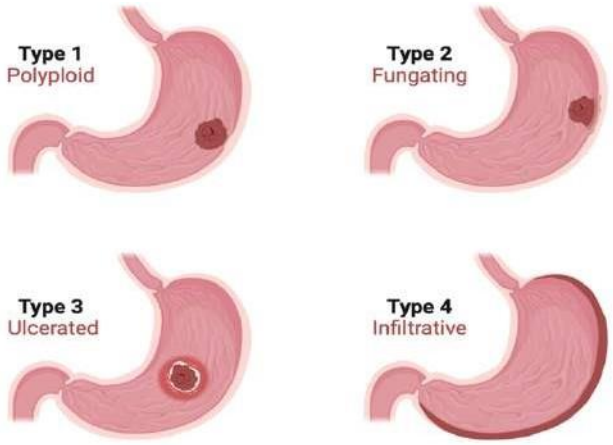

Figure 1. Borrmann Classification of Gastric Cancer (created with BioRender.com)

The visual classification system of Borrmann types helps medical professionals understand both the structure and growth patterns of advanced gastric cancer thus influencing treatment choices and predicts patient outcomes (Figure. 2) (Hong *et al.,* 2023).

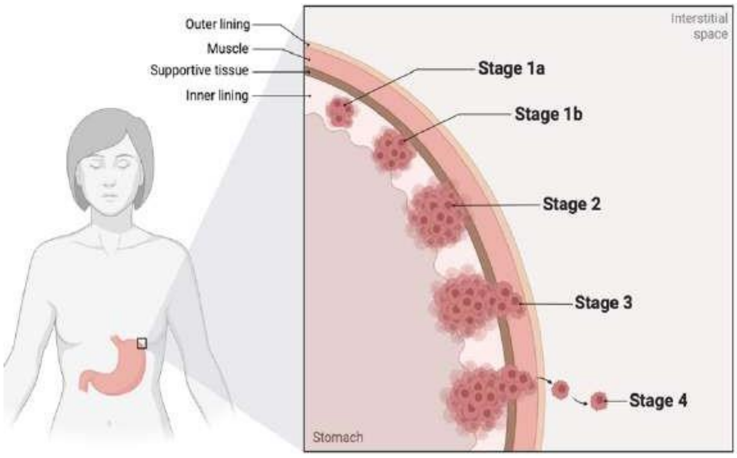

Figure 2. Gastric Cancer Staging (created with BioRender.com)

1.2. Role of Antioxidants in Preventing Oxidative Damage

During metabolism, our body cells give rise to free radicals and reactive oxygen species (ROS) due to non-equivalent cell redox reactions. Superoxide ($O^{-\bullet}$), peroxide ($^{\bullet}OOH$), singlet oxygen (O2), hydroxyl radical ($^{\bullet}OH$) and nitric oxide ($NO^{\bullet}$) are some of them. An excessive or inappropriate generation of reactive oxygen species (ROS) (Jomova *et al.*, 2023) is the result of dietary patterns, lifestyle and environmental factors leading to oxidative stress which is implicated in damage and dysfunction of essential cellular components (Figure. 3) (Azarova *et al.,* 2023). For instance, they oxidize biomolecules like proteins, lipids, carbohydrates and nucleic acids in the human body (Snezhkina *et al.,* 2019) thereby contributing to diseases, such as cancer, cardiovascular and neurodegenerative disorder.

A free radical is a chemical species able to exist independently, possessing one / more unpaired electrons; these molecules are highly unstable. They produce internally a natural portion of metabolism within mitochondria facilitated by enzymes such as xanthine oxidase (XO) in peroxisomes (Chaudhary *et al.,* 2023) during physiological processes like phagocytosis, inflammation, ischemia and arachidonate pathways as well as through physical exercise (Ghimire, 2013). Additionally, external factors that contribute to the production of free radicals include environmental pollutants, smoking, radiation, certain drugs, ozone, pesticides and industrial solvents.

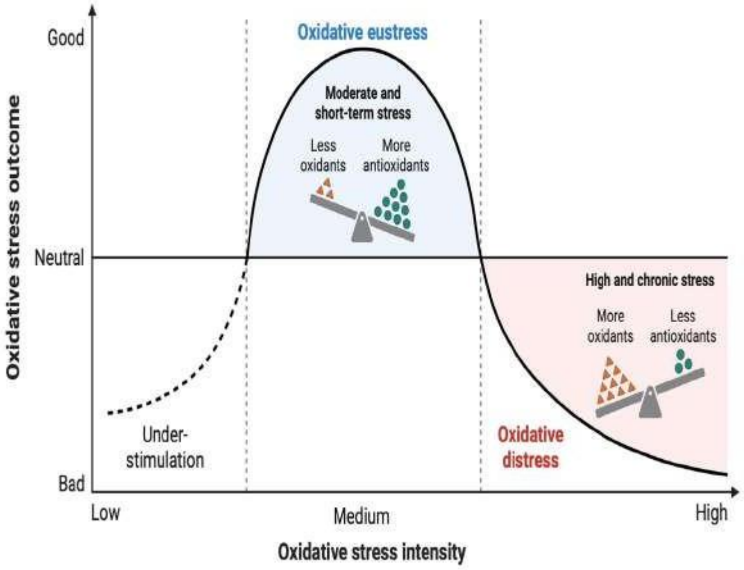

Figure 3. Oxidative Stress Intensity (created with BioRender.com)

Antioxidants play a crucial role in maintaining balanced metabolic processes by inhibiting the de-toxification chains of reactive oxygen species (Rammohan *et al.*, 2020). Consuming foods rich in natural antioxidants, such as green leafy vegetables, nuts, fruits and beverages like tea/coffee and traditional medicinal herbs, helps in preventing diseases related to oxidative damage.

1.3. Animal Models in Cancer Therapeutics

Cancer research commonly utilizes four types of animal models: syngeneic models, Genetically Engineered Mouse Models (GEMMs), chemically-induced models and xenograft models. Xenografts, in particular are categorized based on the origin of the tumour: those derived from standard cell lines (known as cell line-derived xenografts, CDX) or from patient specimens with renal cell carcinoma (referred to as Patient-Derived Xenografts, PDX) (Sobczuk *et al.,* 2020).

Animal immunochemical assays are well-established in identifying the carcinogenic potential of natural influences such as physical, chemical and biological agents. They are reflected as an authentic approach for threat identification of possible human carcinogens, as emphasized by the International Agency for Research on Cancer (IARC, 2019a). These immunochemical assays are also universally used for cancer probability assessment, particularly in cases where human data do not allow for quantitative dose-response analysis (Mevissen *et al.,* 2022).

The use of animals in scientific investigation presents both scientific and ethical challenges. In 1959, Russell and Burch published "The Principles of Humane Experimental Technique," advocating for the integration of the 3Rs wherever feasible: replacement, reduction and refinement (Workman *et al.,* 2010). These principles aim to promote ethical practices and improve the welfare of animals used in experimentation, reflecting ongoing efforts in research ethics and animal welfare considerations. A rodent model used for studying human disease should be affordable, easy to work with and able to accurately replicate the disease in terms of its morphological, biomolecular and behavioural features (Nascimento *et al.,* 2021).

In cancer research, experimental animal are important for the development of vaccine-mediated immunity. Insights from the study show that T-cells mediated cellular responses perform an important role in therapy of cancer (Mak *et al.,* 2014). These models help evaluate the effectiveness of vaccines in triggering T-cells that target cancer related antigens. They facilitate the design of efficient cancer vaccines and treatment methods.

The C57BL/6 mice model for gastric cancer research is crucial due to their genetic uniformity, which minimizes experimental variability and ensures that observed effects are attributable to the treatments rather than genetic differences (Benavides *et al.,* 2020). This particular strain is essential because these models closely resemble human biology and thus allow us to study tumour development and progression of gastric cancer (Zhou *et al.,* 2021). Also it has super-characterised immune systems and that makes them perfect for working out how cancer interacts with the immune system and testing out different immunotherapies (Hau and Schapiro, 2021; Connolly *et al.,* 2022).

Moreover, these mice are commonly utilized in GEMMs to study certain gene functions connected to gastric cancer which aids in understanding the molecular basis tumour initiation, progression and metastasis (Won & Choi, 2022). The use of male C57BL/6 mice is their strength relative to female and other strains which is accompanied by lower health problems, more suitable for long-term experiments that are often needed in cancer research to follow the growth of tumours, metastases and the effects of treatment (Ericsson & Franklin, 2021). This combination of genetic stability is important to immunology, susceptibility to the induction of gastric cancer and other practical factors such as efficiency of breeding and ease of handling male C57BL/6 makes these mice a primary model for understanding adenocarcinoma of the stomach and developing novel therapeutic approaches.

1.4. Network Pharmacology

Network pharmacology stems from a modern approach that transcends beyond traditional drug research phenomena by focusing on how drugs interact with a whole biological system or organism instead of specific biological components (Noor *et al.,* 2022). It is a multi-disciplinary approach encompassing systems biology, bioinformatics and pharmacology to meet the challenges of the intricacy of drug and target interactions (Li *et al.,* 2021). In classical pharmacology, the investigations focus on the actions of medicines on certain constituents such as molecules, proteins and enzymes. Network pharmacology, on the other hand, is holistic; it considers how a drug affects a network of interactions within the body consisting of proteins, genes and numerous cellular pathways (Zhao *et al.,* 2021). It treats the body as a network of components that correlate with each other (Li *et al.,* 2023). When a drug is introduced, it does not seem to impact a particular part of the network but on a network level, it can affect many regions (Silverman *et al.,* 2020). This greatly influences the impact of disease development and progression as well as the curative efficacy and adverse impact of the drugs (Alshabrmi *et al.,* 2024).

To explain these complex relations, network pharmacology relies on novel advanced computation softwares and techniques (Jiao *et al.,* 2021) which aids in identifying the whole network of interactions in the cells, tissues and the impact of the drug on a system rather than a single target (Yue & Dutta, 2022). This helps in the finding of new drug targets, refinement of existing treatments and anticipation of undesired outcomes by appreciating the ways medicines interact within a biological system (You *et al.,* 2022).

In network pharmacology, the STRING database is one of the most important tools and Cytoscape helps in finding druggable targets in PPI networks and evaluating the impact of drugs on networks rather than just proteins (Huang *et al.,* 2022). Cytoscape visualizes molecular network changes in response to drugs or diseases by integrating data like gene alterations, protein levels and other molecular changes.

The investigations of interactions and relationships of various parameters within network pharmacology involve functional enrichment analyses, GO enrichment or KEGG enrichment, all with equal significance (Huang *et al.,* 2022). Combining STRING, Cytoscape, GO enrichment, KEGG pathway enrichment analyses, it is a mechanistic framework for understanding the complex molecular interactions underlying disease and drug action (Zhao *et al.,* 2023). This strategy can target multiple mechanisms that can lead to similar clinical outcomes. Therefore, the multi-target approach is useful for cancer. Being able to identify new drug combinations or repurposed drugs that can act on them could enhance their therapeutic potential and simultaneously reduce major risks (Azer & Leaf, 2023). It also significantly impacts personalized medicine, where therapeutic strategies are customized to the specific molecular features of individual patients (Ceylan *et al.,* 2024), thereby potentially improving treatment outcomes while limiting side effects (Li *et al.,* 2023; Santamaría *et al.,* 2021).

1.5. Signaling Pathways in Cancer Biology

In gastric cancer, the EGFR tyrosine kinase pathway plays a key role in tumor development and progression of tumours (Palle *et al.,* 2020). EGFR is an important receptor which upon activation by its ligands such as Epidermal Growth Factor (EGF), triggers an intracellular cell signaling process that ultimately influences cell propagation, cell survival as well as cell migration (Uribe *et al.,* 2021; Rosenkranz & Slastnikova, 2020). Signaling abnormalities in this pathway can drive cancer growth and affect treatment outcomes (Figure. 4).

In a normal cell, the activation of EGFR initiates a pathway of downstream signals namely Ras/MAPK (Mitogen-Activated Protein Kinase) pathway and PI3K/Akt pathway (Uribe *et al.,* 2021; Cuesta *et al.,* 2021). These pathways help regulate cell cycle progression, anti-apoptotic mechanisms and cellular metabolism (Cuesta *et al.,* 2021). Targeted therapies that inhibit EGFR or its signaling pathways have been explored as treatment options for gastric cancer (Lei *et al.,* 2022). However, in gastric cancer- EGFR signaling gets dysregulated due to various factors such as gene mutations, overexpression of EGFR or alterations in downstream signaling components (Lei *et al.,* 2022; Bou & Chioni, 2023).

Dysregulation of the EGFR pathway in gastric cancer can result in uncontrolled cell proliferation and evasion apoptosis, leading to tumour formation and metastasis (Jiang *et al.,* 2024). EGFR overexpression or mutations in the stomach adenocarcinoma cells are linked to poor prognosis and increased disease aggressiveness (Sun *et al.,* 2020). As an example, some gastric tumours express high levels of EGFR, increasing the tumorigenic properties and developing resistance against conventional chemotherapies (Uribe *et al.,* 2021).

However, the effectiveness of these therapies differs and resistance mechanisms frequently develop, e.g., mutations in the EGFR gene or the activation of alternative molecular pathways (Uribe *et al.,* 2021; Dong *et al.,* 2021). This makes it imperative to comprehend the nature of the EGFR pathway in gastric cancer to formulate suitable targeted therapies that are likely to have a significant impact on the patients (Lei *et al.,* 2022).

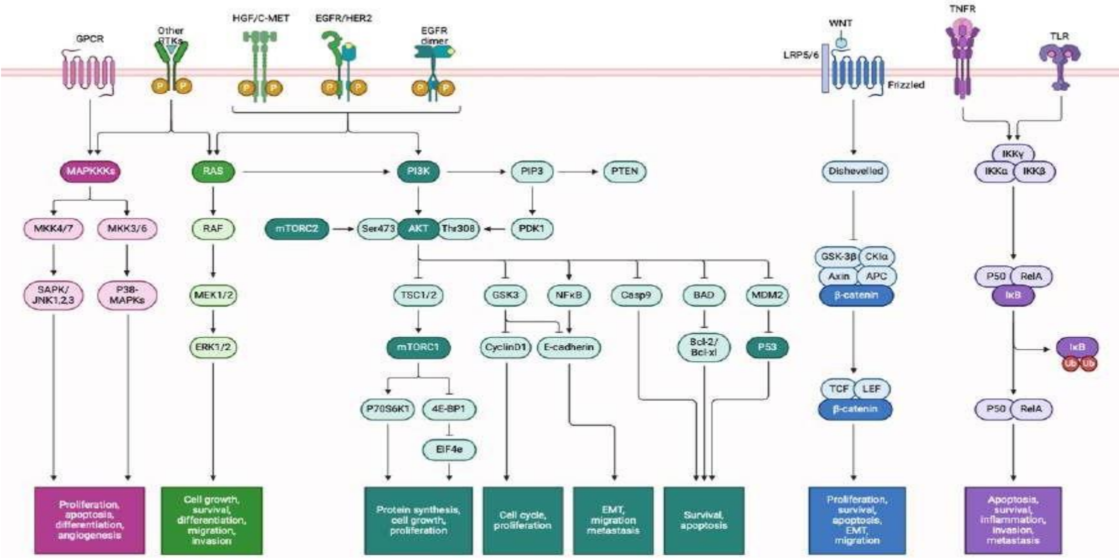

Figure 4. EGFR pathway in Gastric Cancer (created with BioRender.com).

1.6. *In silico* studies

Computational studies facilitate a more well-organized drug development process by significantly reducing time, resources and costs. Early identification of promising biomolecules through these studies helps mitigate potential failures during wet lab experiments, thereby paving the way for practical applications in designing and developing effective therapeutic options for gastric cancer treatment (Akash *et al.,* 2023). Computational methods are crucial in drug discovery for virtual screening and *de novo* compound design, predicting ADME properties and analyzing protein-ligand interactions to optimize drug efficacy and specificity. These tools help accelerate the development of new medications (Rao & Srinivas, 2011).

The effectiveness and recent advancements *in silico* analysis tools are crucial in drug discovery. Molecular docking is a widely utilized approach that visualizes interactions between proteins and small molecule ligands at the atomic level. Similarly, Molecular Dynamics (MDs) simulations employ computational methods to assess free energy, providing insights into drug-protein binding pockets in simulated environments where molecules move freely. Consequently, virtual drug screening using molecular docking and simulation models has emerged as a widely accepted strategy for enhancing the effectiveness of drug development (Vetrivel *et al.,* 2020).

1.7. Statement of the Problem

Gastric cancer is a major health concern globally, with high incidence and mortality rates as per recent Globocan and ICMR data. Existing treatments for gastric cancer often have limitations, including toxicity, drug resistance and high cost. There is a growing need for safer, effective and affordable alternatives derived from natural sources. *R*. *arboreum* is traditionally used in folk medicine but lacks scientific validation for its anticancer potential. It's phytochemical composition and bioactivities remain underexplored, especially in the context of gastric cancer. Based on the above information. The proposed study aims to evaluate the anti-cancer activity of *Rhododendron arboreum* Sm. leaf and flower extracts.

1.8. Hypothesis of the Study

The proposed study was framed to analyse the following hypothesis

- *Null Hypothesis*: The *R. arboreum* methanol leaf and flower extracts do not possess strong anti-gastric cancer activity.
- *Alternative Hypothesis*: The *R. arboreum* methanol leaf and flower extracts possess strong anti-gastric cancer activity.

Thus, the proposed study was framed with the following objectives to test the above mentioned null and alternative hypothesis.

- ➢ To evaluate the qualitative and quantitative phytochemical analysis of *R. arboreum* leaf and flower extracts.
- ➢ To determine the antioxidant potentials and anti-proliferative effects of *R. arboreum* extracts on gastric cancer cells.
- ➢ To validate the efficacy of *R. arboreum* leaf and flower extracts using gastric cancer induced mice model (C57BL/6).
- ➢ To decipher the prominent targets associated with gastric cancer through network pharmacology and elucidate the molecular interaction of bioactive compounds with the identified targets.