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Investigation of Anti-Oral Cancer Drugs and Their Modulation by Vitamin C and Natural Supplements in Macrophage Cell Lines

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Abstract: *Carcinoma in the HNS region is the 6th most common cancer globally, with a high mortality rate. In India, cancer incidence could exceed 1.7 million by 2035, as enrollment is not mandatory. Combined therapies—chemotherapy, radiation, and surgery—are enhancing survival rates. Macrophages are crucial for immune surveillance, and vitamin C can boost the immune system and reduce DNA damage. Citrus fruits, abundant in vitamin C and other nutrients, contribute to cancer defense and may help mitigate side effects in treatments involving cisplatin, a common chemotherapy drug. This Research explores citrus fruits' nutritional and medicinal benefits, particularly their antibacterial and antioxidant properties and macrophage protectivity. Cisplatin has a mortality rate of 50% at conc. between 0.188 and 0.944 ± 0.1678 mg/mL after 24 hours of incubation. In studies with different citrus fruit extracts on macrophage cells J774A1, a concentration of 0.09375 ± 0.189 mg/mL of cisplatin resulted in 14.73% cell viability. When macrophages were treated with citric acid, ascorbic acid, and D-limonene, the results showed improved cell viability: ascorbic acid combined with cisplatin enhanced viability by 40%, citric acid improved it by 30-40%, and D-limonene significantly increased it by 80-90%. Vitamin C is noted for enhancing the efficacy of macrophage cells when combined with several anticancer drugs, including cisplatin. It has protective effects against cisplatin-induced cytotoxicity. Limonene, found in citrus fruits, may reduce inflammation by inhibiting pro-inflammatory mediators in macrophages. A concentration of 0.0058 mg/ml shows over 80% cell viability, while 0.125-0.015 mg/ml results in over 97% viability. In contrast, cisplatin without vitamin C causes 80-90% cell toxicity, but adding it maintains 80-90% viability, highlighting its protective effect. This study explored the effects of commonly used anti-oral cancer drugs, both alone and in combination with Vitamin C and its natural supplements, on a macrophage cell line. My findings suggest that the presence of Vitamin C and its natural derivatives can modulate the efficacy of these drugs, potentially influencing macrophage activity and immune response. The results indicate a complex interaction between chemotherapy agents and antioxidants, which may have significant implications for cancer treatment strategies. While Vitamin C has been widely recognized for its antioxidant and immune-boosting properties, its role in combination with anti-oral cancer drugs requires further investigation to determine optimal therapeutic approaches. Future studies should focus on detailed molecular mechanisms and in vivo models to validate these findings and assess their clinical relevance in oral cancer treatment.*

Keywords: Oral Squamous Cell Carcinoma (OSCC), Vitamin C, Cisplatin, Macrophage Cell Viability, Antioxidant Therapy

I. INTRODUCTION

Nowadays a very common term ‘Cancer’ is nothing but Cytological atypia and archaeological changes that stimulate abnormal growth of malignant cells. If it is found in the Head and neck region, it becomes a complex disease with multifactorial etiologic traits (HNSCC), including genetic and epigenetic factors.(*Rastogi et al*, 2013)The prediction

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reports indicate that India's incidence of cancer will rise to more than 1.7 million in 2035, in 2012 it was around 1 million. (*Bray F et al.,*2013)The surprising thing is that enrolment cases of this deadly disease are not mandatory in India,which suggests that throughout the same period, the death rate from cancer will rise from 680000 to 1-2 million. Carcinoma in the Head and Neck Squamous region is an extremely fatal disease and is the 6th most frequent disease in the world, reported bythe World Agency for Research.(*Chen Y-F et al.,*2019; *Kao SY et al.,*2015; *Swaminathan R et al*., 2008) Some scientists first proposed an analogy between cigarette smoking and cancer in the 1920s, and by the 1950s, a tenuous link between the two had been firmly established. (Gandini S *et al.,*2008*)*The International Agency for Research declared worldwide It is the 6th most common disease and in India, researchers approve that Elderly people are most susceptible to oral cancer, according to epidemiologic research, smoking cigarettes and bidi may play a significant part in the etiological causes of OSCC and the risk of tumor development also depends on human papillomavirus (HPV), which can be of two subgroups: HPV positive and HPV negative (*Seiwert TY et al*., 2015).Additionally, factors such as DNA damage, heavy alcohol consumption, nonsmoking (chewing tobacco or areca nut), exposure to carcinogens like HPV, poor oral hygiene, and genetic predisposition can contribute to the risk of tumor development. (*KaratasOF et al.,*2017; *Ide R, Mizoue, et al.,*2008; *Subapriya R et al.,*2007; *Santos H-B et al.,*2016; *Solomon B. et al.*, 2018; *Ganci F et al.*,2016)Development and progression of cancer depend upon some oncogenes, also survival rate analysis can be done by determining cohorts, therefore comprehensive medication is implemented with success(*Irimie, Alexandra Iulia, et al. 2018*; *Ralhan, Ranju, et al*.,2007)specifically discusses the role of epigenetic modifications, HPV-positive HNSCC mostly occurs in Latino whitemen in the United States, and HPV-negative has been reported in sublingual sites of OSCC, especially in men. *Johnson. DE et al.*2020 & *Gillison ML et al.,*2015. Surprisingly, there is evidence connecting the oral microbiota to a higher risk of gastrointestinal and oral malignancies (*Ahn et al.,*2011; *Zhang et al.,*2019).

Currently, post-transcriptional or translational levels of genes are understood to negatively regulate protein expression and are involved in various normal physiological processes and cellular behaviors, such as apoptosis, migration, invasion, stress response, inflammation, differentiation, and cell proliferation.

Ascorbic acid, also known as vitamin C, is a fundamental nutrient that is essential to many bodily processes. It has several vital roles, including collagen hydroxylation, tyrosine metabolism, ferric metabolism, and support for the immune system and collagen synthesis. It also operates as an antioxidant. Some ascorbic acid derivatives, including certain mineral ascorbates or esterified forms, may have a lower bioavailability than ascorbic acid, which is highly accessible and readily absorbed by the body.(*Sengupta, S. et al.,*2023)

Eating fruits that are high in fiber and low in fat, such as lemons, pummelos, and Gandharaj, can help us fight various chronic diseases and boost our immunity. Oranges contain the highest levels of Vitamin C (10.13 ± 0.10 mg/100 mL) compared to other fruits such as apples. Fruits are rich in antioxidants that are beneficial for preventing degenerative diseases. They also contain phenolic compounds, important minerals, vitamin A, vitamin B, and vitamin C. The fruit's pericarp contains a number of phenolic chemicals, including as ferulic acid and p-coumaric acid. epicatechin, catechin, caffeic acid, and their esters. Estimation of the protein content of fruits through reliable analytical methods because ascorbic acid is known to affect the immune system, and researchers must still be exploring its effects. Immune cells such as leukocytes and neutrophils can store high concentrations of vitamins in intracellular regions, which enhance phagocytosis, chemotactic activities, and ROS production, leading to better antimicrobial activities. Vitamin C is essential for triggering apoptosis and facilitating the removal of spent neutrophil cells from infected areas by macrophages. This process reduces cell damage and necrosis.

II. LITERATURE REVIEW

Chen et al. (2014) In a mouse xenograft model, ascorbic acid (vitamin C) reduced cisplatin-induced nephrotoxicity without impairing cisplatin's antitumor efficacy, supporting a protective role for vitamin C against cisplatin toxicity in vivo.

Elballat et al. (2016) Animal and in-vitro studies reported that antioxidant agents (including vitamin C and curcumin) can ameliorate cisplatin-induced tissue damage, indicating potential benefits of antioxidant co-treatment to reduce chemotherapy side effects.

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García et al. (2017) Work on macrophage cell lines (RAW 264.7 / J774) established protocols for assessing cytotoxicity of botanical extracts and confirmed that macrophage viability assays are robust models for testing natural product safety and immunomodulatory effects.

Vissers & Das (2018) Mechanistic review summarized how vitamin C exerts context-dependent actions in cancer (antioxidant at low doses, pro-oxidant at high/intravenous doses), and can interact synergistically with chemotherapeutics; route and concentration critically determine outcomes.

Ghavami et al. (2019) Experimental studies showed **synergistic** effects of vitamin C with cisplatin in some cancer models, allowing reduced cisplatin doses while maintaining cytotoxicity—supporting combination strategies to lower toxicity.

Huang et al. (2019) Although focused on vitamin D, this study illustrated that micronutrients can modulate cisplatin sensitivity in oral cancer cells, reinforcing the concept that dietary/phytonutrient supplements affect chemotherapy response.

Zhou et al. (2020) In vitro data confirmed vitamin C can induce apoptosis and cell-cycle arrest in certain cancer cell lines, and when combined with chemotherapeutics may enhance anti-tumor responses—again dependent on concentration and delivery method.

Schoeberl et al. (2021) Single-cell/analytical studies revealed differential cisplatin uptake among macrophage subtypes (M0/M1/M2), with M2 TAM-like macrophages incorporating more platinum—this finding links macrophage phenotype to cisplatin handling and potential toxicity/efficacy modulation.

Böttger et al. (2021) A review of high-dose intravenous vitamin C highlighted emerging clinical phase-I/II data suggesting safety and possible efficacy as an adjunct to chemotherapy in multiple cancers, while emphasizing the need for controlled trials specific to tumor types.

Sears et al. (2022) Resident macrophages (F4/80^hi^) were implicated in mediating cisplatin-induced organ damage (kidney), demonstrating that macrophages are active participants in cisplatin toxicity and a target for protective interventions.

Sung et al. (2023/2024) & Elmorsy (2024) Ablation or modulation of tissue macrophages was shown to reduce cisplatin-induced ototoxicity/nephrotoxicity, and recent reviews summarized evolving strategies to mitigate cisplatin toxicity (including antioxidant co-treatments and immune modulation). These works underscore macrophages' central role in both toxicity and therapy response.

El Hachlafi et al. (2024) Studies on citrus essential oils and limonene reported strong antioxidant and anti-inflammatory activity and favorable macrophage biocompatibility/cytoprotective effects in vitro, supporting citrus constituents (ascorbic acid, citric acid, D-limonene) as candidates to protect immune cells during chemotherapy.

III. RESEARCH METHODOLOGY

The study begins with the formulation of a clear clinical objective, focusing on evaluating how commonly used chemotherapeutic agents such as Cisplatin and 5-Fluorouracil (5-FU) influence macrophage function, both individually and in combination with antioxidants like Vitamin C and bioactive natural compounds such as citric acid, D-limonene, and other plant-derived extracts. Following a comprehensive literature review and protocol design, suitable macrophage cell lines—J774A.1 (murine macrophages) and RAW 264.7—are selected as experimental models due to their relevance in tumor immunology and inflammation studies.

Next, test agents are prepared, ensuring accurate concentrations and proper solubility for cell culture use. The treatment groups are carefully structured into multiple categories, including a vehicle control, drug-only treatments at varying concentrations, Vitamin C alone, natural supplement alone, and combined treatments of drugs with Vitamin C or natural supplements. This structured approach enables comparative analysis of cytotoxicity and protective mechanisms.

The macrophage culture and treatment phase involves seeding cells into 96- or 24-well plates, followed by incubation for 24, 48, and 72 hours, ensuring biological replicates ($n \geq 3$) for statistical reliability.

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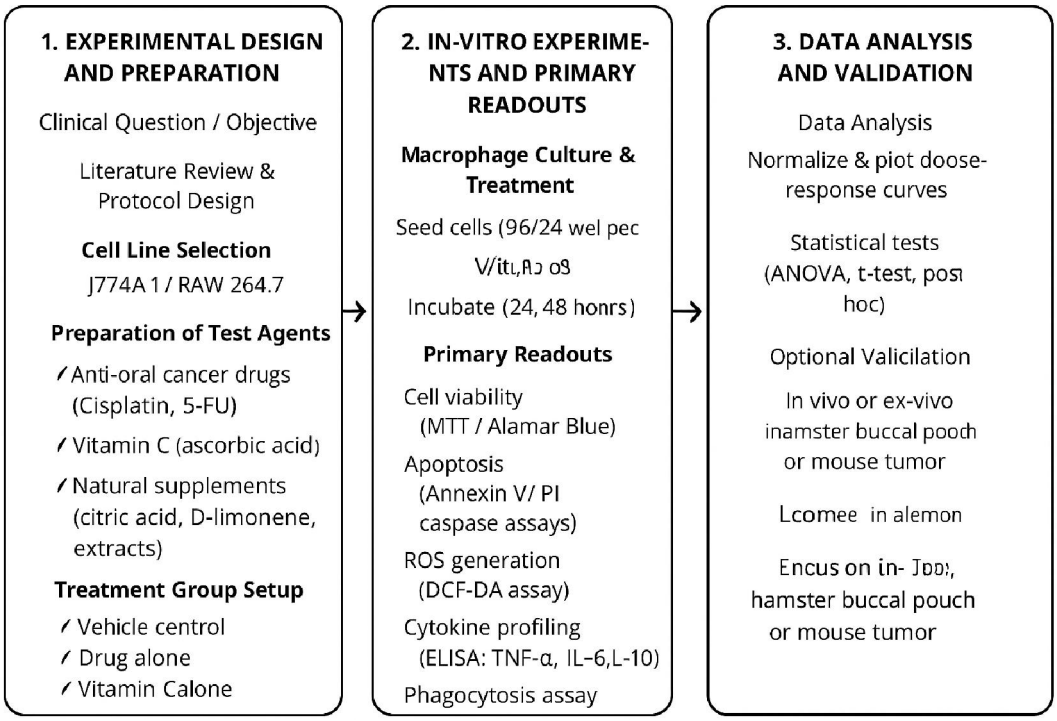

Fig 1 Research Methodology

The primary assays focus on cell viability using MTT or Alamar Blue assays, apoptosis determination via Annexin V/PI staining and caspase activity, measurement of reactive oxygen species (ROS) using DCF-DA fluorescence, cytokine profiling (ELISA for TNF-α, IL-6, IL-10), phagocytosis assays employing fluorescent beads, and surface marker analysis through flow cytometry to distinguish M1/M2 macrophage polarization states. The secondary and supportive analyses include Western blotting for signaling proteins like AKT, NF-κB, and caspase-3, qPCR for gene expression related to inflammation and antioxidant defense, and fluorescence microscopy for morphological and localization studies.

Data from all experiments are subjected to statistical analysis, involving normalization, dose–response curve plotting, and comparison using ANOVA and t-tests with post hoc validation to determine significance. The findings are interpreted to assess whether the combinations provide protective or synergistic effects against drug-induced oxidative or apoptotic damage. Optionally, the study may extend to in vivo or ex vivo validation using animal models such as hamster buccal pouch or mouse tumor systems to examine macrophage infiltration, toxicity profiles, and therapeutic efficacy, ensuring translational relevance of the results.

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IV. RESULTS AND DISCUSSION

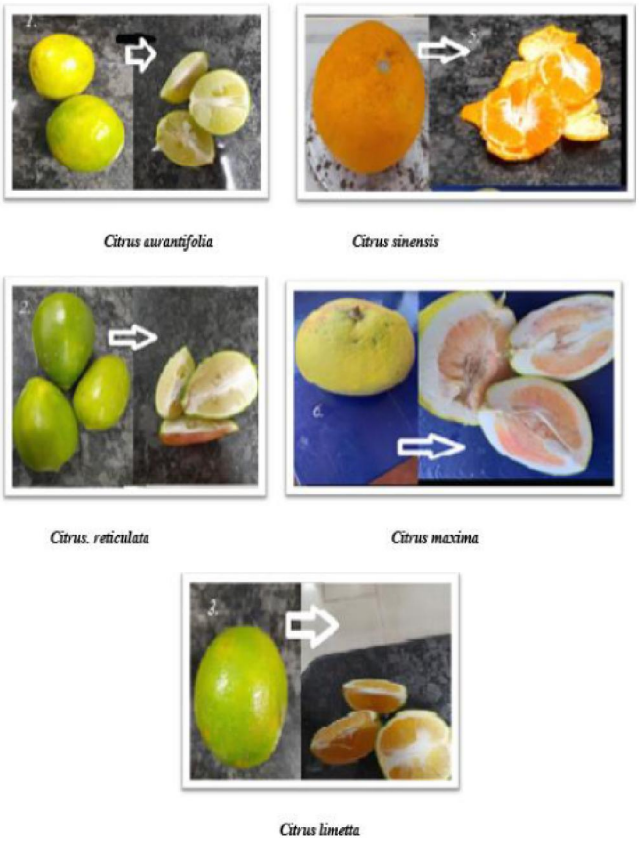

Fig. 2: Pictures of the citrus lemons, used for the experiments.

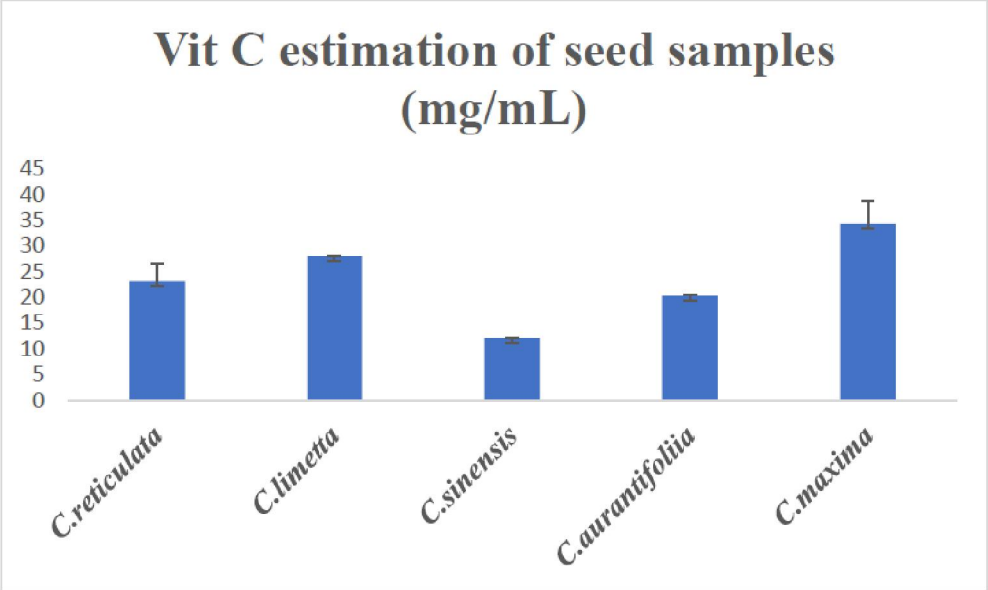

Fig. 3: Vitamin C estimation ofdifferent SEED samples

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The results indicate that C. maxima (34.34±2.6 mg/mL) have a significantly higher amount of vitamin C, than the other samples. The standard deviation was measured three times for each sample (n=3).

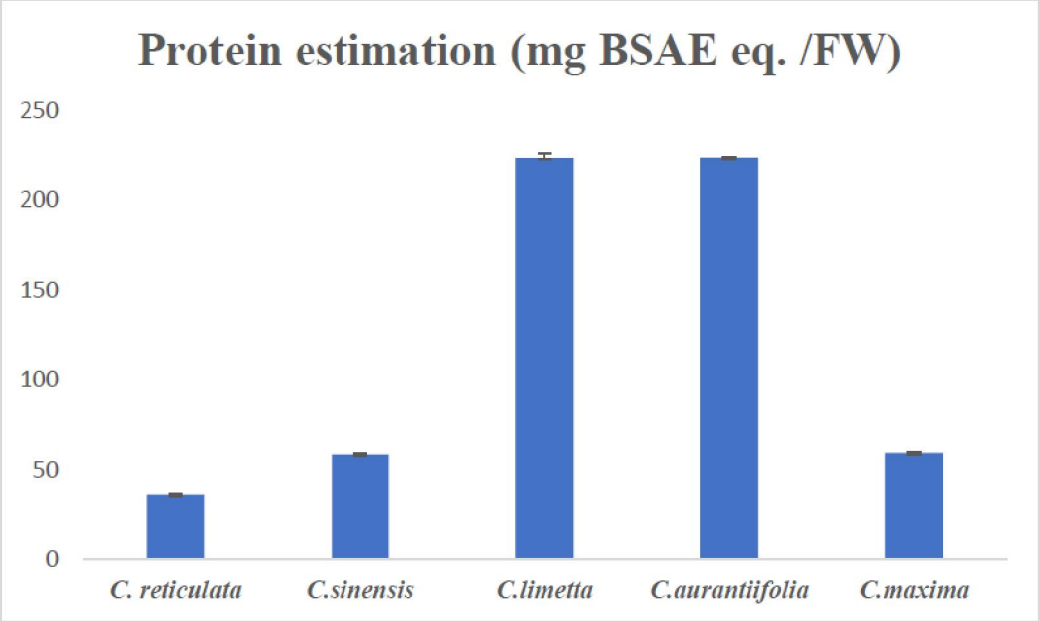

Fig. 4: Protein estimation of different seed samples

The results indicate that *C. aurantiifolia* and *C. limetta* contain significantly higher amounts of vitamin C, than the other samples following closely behind. The standard deviation was measured three times for each of the samples. [fig 4]

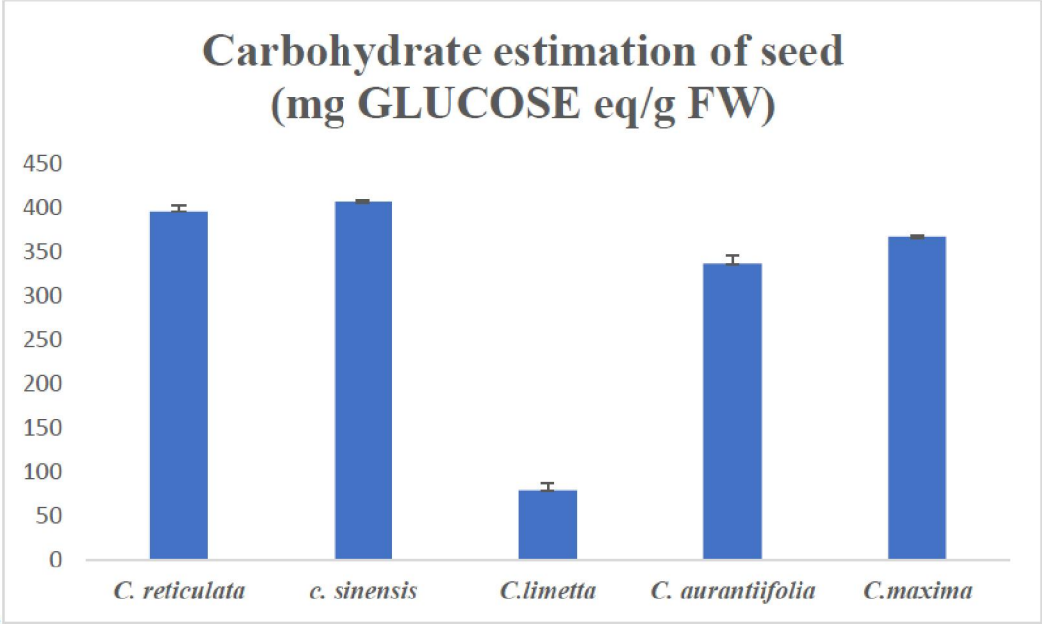

Fig. 5: Carbohydrate estimation of different seed samples.

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Each sample has a significantly high amount of carbohydrates. *C. sinensis* and *C.reticulata* have a higher amount of carbohydrate [fig 5], equivalent to glucose Sd measured (n=3) for each of the samples.

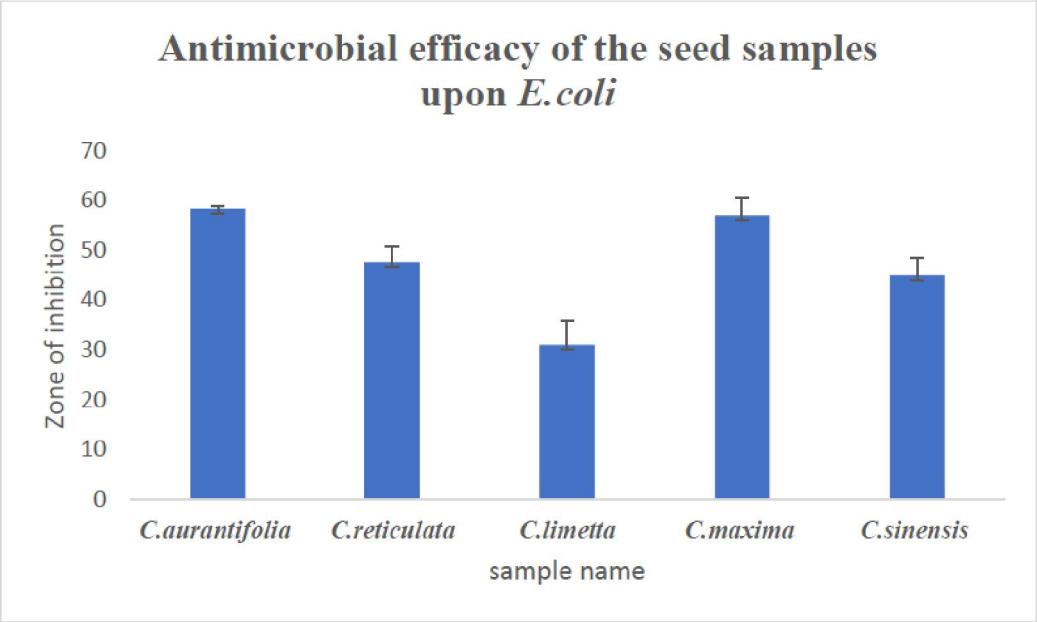

Fig. 6 a: Diagrammatic representation of the antimicrobial activity of the seed samples on *E. coli* bacteria.

The result shows C. aurantiifolia and C. maxima have the highest antimicrobial activity among other samples. Sd measured (n=3) for each of the samples [fig 6a]

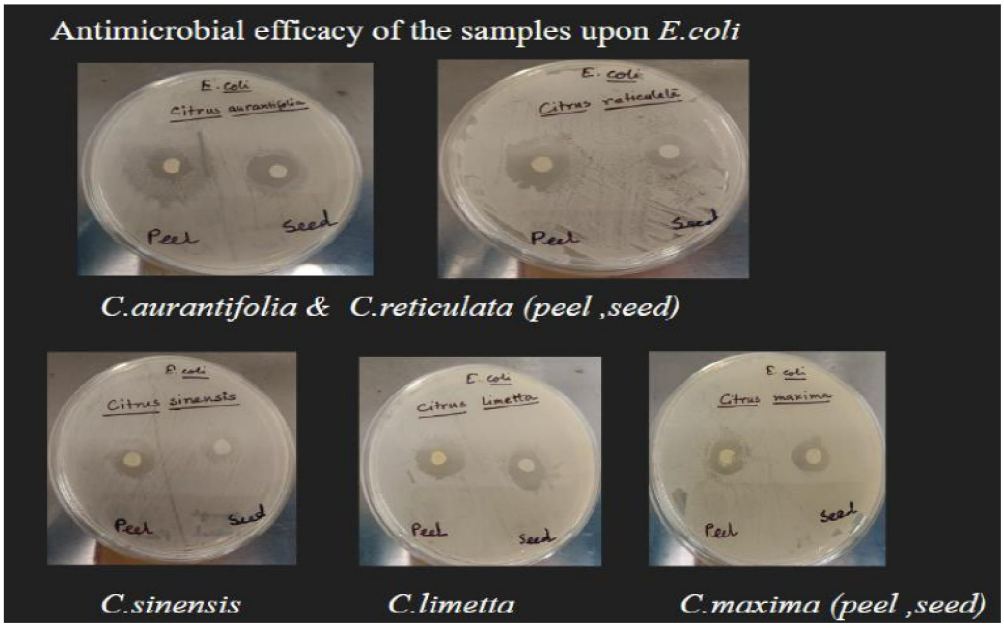

Fig. 6b: Pictorial representation of the antimicrobial activity of the seed samples on *E. coli* bacteria.

Pictures show the zones in which the samples have inhibited bacterial growth. Seed extracts of *C. arenicolid* (58.33 ±0.4 mm) and *C. maxima* (57 ±3.5 mm) showed good antibacterial efficacy [fig 6b]

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V. CONCLUSION

This study explored the effects of commonly used anti-oral cancer drugs, both alone and in combination with Vitamin C and its natural supplements, on a macrophage cell line. My findings suggest that the presence of Vitamin C and its natural derivatives can modulate the efficacy of these drugs, potentially influencing macrophage activity and immune response. Phytonutrients, abundant in various fruits, vegetables, herbs, and spices, are readily available and can be easily incorporated into diverse diets and research across different populations. Moving forward, medical practitioners recommend a diet rich in pigment-dense fruits and foods that enhance the body's defense system. However, when considering cancer medications and preventing the recurrence of malignant cells, some citrus fruits may prove to be more beneficial than others.The results indicate a complex interaction between chemotherapy agents and antioxidants, which may have significant implications for cancer treatment strategies. While Vitamin C has been widely recognized for its antioxidant and immune-boosting properties, its role in combination with anti-oral cancer drugs requires further investigation to determine optimal therapeutic approaches. ENTPD1-CD39 as a focus of the study. Specifically, it discusses its known role in regulating purinergic signalling, which influences immune cell activation and function. ENTPD1 (also known as CD39) is a key enzyme involved in ATP hydrolysis, leading to the production of adenosine, a potent immune regulator that can modulate macrophage and T cell activity, which is relevant in the context of cancer immunology.

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