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**Jainul Basher, Radheshyam**

Faculty of Pharmacy, Bhagwant  
University, Ajmer, Rajasthan, India

**Prabha Shankar Maurya**

SCOP, Balrampur, Uttar Pradesh,  
India

## Correspondence

**Jainul Basher**

Faculty of Pharmacy, Bhagwant  
University, Ajmer, Rajasthan, India

Email: [basherjainul29@gmail.com](mailto:basherjainul29@gmail.com)

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## *In Vitro* Anticancer Activity of Gamma Oryzanol in Skin Cancer

**Jainul Basher, Prabha Shankar Maurya, Radheshyam**

### ABSTRACT

Plants have had an essential role in the folklore of ancient culture. In addition to the use as food and spices, plants have also been utilized as medicines for over 5000 years. Herbal products such as plant extracts, dry powder and parts of plants, fungi, and algae have been used as complementary treatments alongside conventional drugs. Cancer is a severe metabolic syndrome and is one of the leading causes of death regardless of developments in the tools of disease diagnosis, treatment and prevention measures. Cancer is one of the principal causes of mortality and morbidity around the globe and the number of cases are constantly increasing estimated to be 21 million by 2030. Several medicinal plant species and their phytochemicals inhibit the progression and development of cancer. In the present work *In vitro* anti cancer activity of gamma oryzanol was performed using SRB assay. Result shown that gamma oryzanol has anti cancer activity for SKMEL-2 cancerous cell line. On the basis of this result further study plan can be designed.

**Key words:** Cancer, Gamma oryzanol, Phytochemicals.

### 1. INTRODUCTION

Plants have had an essential role in the folklore of ancient culture. In addition to the use as food and spices, plants have also been utilized as medicines for over 5000 years. It is estimated that 70-95% of the population in developing countries continues to use. Today medicinal herbs are defined as plants that contain valuable substances with therapeutic or beneficial effect in healing and prevention of various ailments in man and animals. Herbal products such as plant extracts, dry powder and parts of plants, fungi, and algae have been used as complementary treatments alongside conventional drugs.<sup>1</sup>

#### 1.1. Cancer

Cancer is a severe metabolic syndrome and is one of the leading causes of death regardless of developments in the tools of disease diagnosis, treatment and prevention measures.<sup>1-3</sup> Cancer is one of the principal causes of mortality and morbidity around the globe and the number of cases are constantly increasing estimated to be 21 million by 2030.<sup>4-5</sup> It is estimated that in 2017, the United States alone will have approximately 1 688 780 new cancer diagnoses cases and 600 920 cancer deaths.<sup>6</sup> This uncontrolled proliferation of a normal cell which produces genetic instabilities and alterations accumulates within cells and tissues which transforms normal cell into a malignant cell. These genetic instabilities include mutations in DNA repair genes (p21, p22, p27, p51, p53 and tool box for DNA), tumour suppressor genes (p53, NF1, NF2, RB and biological breaks), oncogenes [MYC, RAF, Bcl-2, RAS (biological accelerators)] and genes involve in cell growth metabolism.

Both external factors (radiations, smoking, tobacco, and pollutants in drinking water, food, air, chemicals, certain metals and infectious agents) and internal factors (genetic mutations, body immune system and hormonal disorders) can cause cancer.<sup>7</sup> There are several types of cancer in human being; among these the lung cancer is reported the top listed in male followed by breast cancer in female.<sup>8-9</sup>

## 1.2 Current cancer therapy via Phytochemicals: a novel approach

Medicinal Plants serve as nature's gift to humans to help them pursue better health. Plants and their bioactive compounds are in medicinal practices since ancient times. Several medicinal plant species and their phytochemicals inhibit the progression and development of cancer.<sup>22</sup> It has been researched that plant kingdom comprised of approximately 250 000 plant species and only around 10% have been studied for treatment of different diseases. Phytochemicals and their derived analogues are present in different parts of the plant, *e.g.*, flower, flower stigmas, pericarp, sprouts, fruits, seeds, roots, rhizomes, stem, leaf, embryo, bark and perform several pharmacological functions. Several plant products such as alkaloids, flavonoids, lignans, saponins, terpenes, taxanes, vitamins, minerals, glycosides, gums, oils, biomolecules and other primary and secondary metabolites play significant roles in either inhibiting cancer cell activating proteins, enzymes and signalling pathways [Cdc2, CDK2 and CDK4 kinases, topoisomerase enzyme, cyclooxygenase and COX-2 (Cyclooxygenase), Bcl-2, cytokines, PI3K, Akt, MAPK/ERK, MMP, TNF, mechanistic target of rapamycin (mTOR) or by activating DNA repair mechanism (*p21*, *p27*, *p51*, *p53* genes and their protein products), Bax, Bid, Bak proteins, stimulating the formation of protective enzymes (Caspase-3, 7, 8, 9, 10, 12), inducing antioxidant action (antioxidant enzymes *e.g.* GSH, GST and GPxn), thus showing strong anticancer effects in terms of their efficacy on the above mentioned proteins, enzymes and signaling pathways.<sup>15</sup>

## 2. MATERIALS

Gamma-Oryzanol was procured as gift sample from A. P. organic Pvt Ltd. Punjab. Other analytical grade chemicals used in this study were purchased from Marke Pvt. Ltd., Delhi.

## 3. METHODS

### 3.1 SRB Assay

According to SRB assay,<sup>23-24</sup> *in vitro* testing was done by using SRB assay protocols. Cell lines were counted, cultured and inoculated in 96 well plates. In these drugs should be tested at 4 dose levels at 10, 20, 40, 80 µg/ml. Each experiment should be

repeated three times. After incubation with different concentrations of test compounds, the cell cultures were stained with SRB dye. Washing with 1% acetic acid (CH<sub>3</sub>COOH) removed the unbound dye and the protein bound dye gets extracted using Tris-HCl bufferbase (100 µl, 0.01 M, pH 10.4). The optical density was determined by 96-well plate ELISA reader at 540 nm. Table 1 summarized the GI<sub>50</sub> values of various formulations. Different concentrations of drugs showed improved anticancer effect and reduction in cell growth compared to sample drug solution after 48 h incubation period in SKMEL-2 cancerous cell line.

**Table 1: Different GI<sub>50</sub>, TGI, and LC 50 values for different doses of gamma oryzanol**

S.No.	Dose (µg/ml)	GI 50	TGI	LC 50
1	10	>80	>80	>80
2	20	>80	>80	>80
3	40	>80	>80	>80
4	80	21.3	51.8	>80

## 4. RESULT & DISCUSSION

From different studies it was found that several plant materials have anti cancer activities. OZ is a mixture of ferulate esters of different sterols. Important components of OZ are cycloartenyl ferulate, 24-methylene cycloartenyl ferulate and campesterol ferulate. In the present work *In vitro* anti cancer activity of gamma oryzanol was performed using SRB assay. On the basis of results it can be concluded that with higher doses gamma oryzanol is showing anti cancer activity. Standard drug doxorubicin has more activity compared to gamma oryzanol.

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