RESEARCH ARTICLE

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# Anticancer Effect of Plectranthus Amboinicus and Glycyrrhiza Glabra on Oral Cancer Cell Line: An Invitro Experimental Study

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## Abstract

**Background and Objective:** Oral cancer is a commonly encountered type of cancer with multifactorial etiology. The number of oral cancer cases are increasing in the recent past. Advancements in cancer therapy are continuously evolving. In spite of that, the survival rate is very low along with adverse effects associated with each treatment modality. This poses a need for a constant search for newer or alternative treatment options. Herbal medicines or plant-based medicines are derived from plant sources. Evidence supports that incorporating herbal medicines in cancer therapy offers many advantages. Hence, exploring plant species for anticarcinogenic properties can potentially benefit cancer therapy. Studies show that the two medicinal herbs Plectranthus amboinicus (Indian borage, Karpooravalli) and Glycyrrhiza glabra (Liquorice, Athimathuram) have shown excellent anticancer activity on various cancers. The present study aimed to evaluate and assess the anticancer property of two medicinal plants Plectranthus amboinicus (Indian borage, Karpooravalli) and Glycyrrhiza glabra (Liquorice, Athimathuram) on oral cancer (KB) cell line. **Materials and Methods:** Ethanolic extracts of leaves of Plectranthus amboinicus and roots of Glycyrrhiza glabra were prepared. The oral cancer (KB) cell line was procured and cultured. Cell viability (MTT) assay was performed using various concentrations of both the plant extracts on oral cancer cells. The percentage of cell viability for each concentration was calculated and the IC50 value was derived for the two plant extracts. **Results:** The results revealed a decrease in the percentage of viable cells with increasing concentration of the extracts. The $IC_{50}$ values of Plectranthus amboinicus and Glycyrrhiza glabra were 53.0 µg/ml and 43.6 µg/ml respectively. On comparing the anticancer effect of the two extracts, Glycyrrhiza glabra was more cytotoxic than Plectranthus amboinicus on oral cancer (KB) cells. **Conclusion:** The two medicinal plants Plectranthus amboinicus and Glycyrrhiza glabra exhibited potent anticancer activity against oral cancer (KB) cells.

**Keywords:** Glycyrrhiza glabra- oral cancer- plectranthus amboinicus

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## Introduction

Nature is a powerful asset to mankind. It showers countless benefits for the health and wealth of living beings. Hippocrates once stated, "Nature itself is the best physician". It offers therapeutic benefits for many debilitating diseases. Cancer is a debilitating disease characterized by abnormal and uncontrolled tissue growth. In India, oral cancer is a frequently encountered type of cancer with increasing morbidity and mortality (Borse et al., 2020).

Until now, surgery, radiotherapy, and chemotherapy remain the mainstay treatment modalities for oral cancer. Despite that, the survival rate of oral cancer remains low. Moreover, each therapy is associated with a myriad of complications (Ganjibakhsh et al., 2017). Advances in cancer therapy include novel techniques like targeted biological therapy, immunotherapy, and gene therapy which are less cost-effective and are still in the evolving phase (Nandini et al., 2020). Hence, in the current scenario, the need to search for alternative treatment options remains essential.

Nature has provided us with abundant plant resources. Herbal medicines or phytomedicines are a group of drugs derived from plant sources. According to the World Health Organization (WHO), plant-derived medicines are used by 80% of the world's population (Djordjevic, 2017). Studies show that adding phytomedicines to conventional cancer treatment reduces mortality, decreases adverse drug reactions, and is cost-effective (Chaudhary et al., 2015). Several plant species have shown promising anticancer activity on various cancers like cervical cancer, breast cancer, and colon cancer (Yin et al., 2013).

The two plant species Plectranthus amboinicus and

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Glycyrrhiza glabra are well known for their medicinal value. Plectranthus amboinicus, commonly known as Indian borage (Karpooravalli), is a semi-succulent perennial plant. The leaves of the plant contain essential oil rich in bioactive compounds like Carvacrol, Thymol, β- Caryophyllene, α- Humulene, γ- Terpinene, p- Cymene, α- Terpineol, β- Selinene, and phytocompounds such as flavonoids, cinnamic derivatives, and terpenes, which contain anti-inflammatory, antioxidant and anticancer property (Arumugam et al., 2016; Gurgel et al., 2009). Many invivo and invitro studies focusing on the anticarcinogenic activity of the plant were documented in the literature (Gurgel et al., 2009; Rosidah (2014); Hasibuan and Sumaiyah, 2019).

Glycyrrhiza glabra is commonly known as Liquorice (Athimathuram). The root of the plant contains oils, alkaloids, polysaccharides, polyamines, triterpenes, phenolic acid, flavones, flavans, chalcones flavonoids, and isoflavonoids which are known to exhibit antioxidant, anti-inflammatory, and anticarcinogenic property (Bode and Dong, 2015). Glycyrrhizin or glycyrrhizic acid is an important bioactive compound present in the root of the plant with apoptotic activity (Farooqui et al., 2018). A great deal of research has been reported on the anticarcinogenic activity of the plant against various cancers. (Pandian and Chidambaram, 2017; Goel et al., 2021).

The two plant species apart from having exceptional medicinal value, are easily available, cost-effective, and can be grown in households. Studies analyzing their anticarcinogenic activity on oral cancer are scarce. Hence, this study attempted to evaluate the anticarcinogenic effect of Plectranthus amboinicus and Glycyrrhiza glabra on the oral cancer cell line. This opens a new avenue in the formulation of novel plant-based drugs in oral cancer therapy.

## Materials and Methods

*Cell line*

This is an in vitro experimental study. The cell line used in this study was a commercially available human oral cancer KB (Keratin forming) cell line procured from National Centre for Cell Science (NCCS, Pune, India). The cell lines available in NCCS were authenticated using Short Tandem Repeat (STR) analysis and mycoplasma tested.

*Plant specimen*

Fresh plant specimens, (leaves of P. amboinicus and roots of G. glabra) were procured from the medicinal plant garden (Chettinad academy of research and education, Chennai, India) and were submitted for authentication. Both the samples (P. amboinicus and G. glabra) were identified and authenticated respectively by the Plant Anatomy Research Centre (PARC), Chennai.

*Authentication certificate number*

P. amboinicus – PARC/2021/4530

G. glabra – PARC/2021/4529

*Chemicals and reagents*

Dimethyl sulfoxide (RANKEM chemicals, Haryana, India), 10% Fetal Bovine Serum (Gibco, Grand Island, New York, USA), Ethanol (Changshu Hongsheng fine chemicals Co., Ltd, Jiangsu province, China), Minimal essential media (MEM) prepared from MEM powder, Streptomycin Penicillin G, L- glutamine, sodium hydrogen carbonate (HIMEDIA, Mumbai, India) and diluted HCl (RANKEM chemicals, Haryana, India). 1XPhosphate Buffer Saline (PBS) prepared from sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate (HIMEDIA, Mumbai, India), Trypsin PBS Versene (0.2% EDTA) Glucose (TPVG) solution (HIMEDIA, Mumbai, India), MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide] dye (HIMEDIA, Mumbai, India).

*Procedure*

*Preparation of plant extracts*

Plant extracts were prepared by cold maceration method. Fresh leaves of Plectranthus amboinicus (sample 1) and roots of Glycyrrhiza glabra (sample 2) were washed thoroughly in water. Samples were shade dried, powdered, and soaked in ethanol for 72 hours at room temperature. After 72 hours, both the solutions were filtered using sterile filter paper No. 1. The filtrate was further evaporated using a rotary mantle heater until a thick paste is obtained. The final yield of sample 1 and sample 2 was 0.59g and 0.54g respectively. The obtained paste was then diluted using the vehicle 0.1% Dimethyl sulfoxide (DMSO) in the following concentrations 15.6 µg/ml, 31.2 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, and 500 µg/ml.

*Cell culture*

KB cells were cultured in Minimal essential media (MEM) containing 0.1% streptomycin, 0.1% penicillin G and 0.1% amphotericin B, 10% Fetal bovine serum & L- glutamine (Figure 1). The cells were maintained at 37°C, 95% air, and 5% $CO_2$ atmosphere at a pH of 7.2 -7.4. The cells were subjected to cell viability assay once confluence was reached.

Passaging the cells: The medium in the culture flask was discarded and washed with fresh medium twice. 5 ml of TPVG solution (Prewarmed to 37°C) was added and kept for 1-2 minutes. The solution was then discarded and left for 5 – 10 minutes. 5 ml of 10% FBS was added to the culture flask and mixed thoroughly by pipetting back and forth to break the cell clusters into individual cells. The cells were then transferred to a 96-well plate for cell viability assay.

*Cell viability assay*

The MTT assay was performed according to Mosmann et al., (1983). KB cells were seeded $1X10^5$ in 0.2 ml of medium /well in 96 well plates. The cells were then incubated at 37°C in a 5% $CO_2$ incubator for 72 hours. The test samples of P. amboinicus and G. glabra were added at various concentrations (15.6 µg/ml, 31.2 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, and 500 µg/ml) and kept in 5% $CO_2$ incubator for 24 hours. KB cells treated with DMSO and KB cells in culture media were used as controls

(Figure 4). After 24 hours, cells were observed under an inverted microscope at 40X for any morphological changes. Upon removal of the residual sample solution, 20µl/well MTT reagent was added and incubated for 4 to 6 hours at 37°C. 200 µl DMSO was added to make the formazan crystal soluble. Absorbance (OD) was measured at 540 nm. The $IC_{50}$ value (half maximal inhibitory concentration) was determined graphically. The effect of the test samples on the proliferation of the treated KB cells was calculated and expressed as the percentage cell viability using the formula:

*Calculation*

**% Cell viability = A 540 of treated cells / A 540 of control cells × 100%**

*Statistical Analysis*

The study obtained data for cell viability. The values were tabulated to analyze the anticarcinogenic activity of Plectranthus amboinicus (sample 1) and Glycyrrhiza glabra (sample 2) on the oral cancer cell line. Statistical analysis was done using SPSS software version 20.0. Pearson's correlation test was performed to measure the correlation between the variables extract concentration and percentage cell viability for both samples.

## Results

The study analysis expressed a reduction in the viable cell count in those treated at different concentrations of Plectranthus amboinicus (sample 1) extract and Glycyrrhiza glabra (sample 2) extract. The anti-carcinogenic activity of both extracts was directly proportionate to the extract concentration and expressed by the percentage of the number of viable cells. Meanwhile, KB cells treated with vehicle DMSO and KB control cells remained 100% viable thus eliminating the risk of vehicle cytotoxicity. (Tables 1 and 3) (Figure 4).

*Cell viability after sample 1 (Plectranthus amboinicus) treatment*

Sample 1 Plectranthus amboinicus revealed a minimum number of viable cells of 2.5% at the highest extract concentration of 500 µg/ml and a maximum of 80.8% of viable cells at the minimum extract concentration of 15.6 µg/ml (Table 1). The half maximal inhibitory concentration ($IC_{50}$) value of 53.0 µg/ml was derived for sample 1. Pearson's correlation revealed a statistically significant strong negative correlation of -0.818 (P value 0.047) (Table 2). This shows that as the concentration of the extract increases, a decrease in the percentage of viable cells was seen (Graph 1).

Table 1. Plectranthus Amboinicus Extract Concentrations and Percentage of Viable Cells

| S. No | Concentration µg/ml | Absorbance 540nm | % Cell Viability |
|---|---|---|---|
| 1 | 500 | 0.03 | 2.5 |
| 2 | 250 | 0.07 | 5.8 |
| 3 | 125 | 0.23 | 19.1 |
| 4 | 62.5 | 0.53 | 44.1 |
| 5 | 31.2 | 0.78 | 65 |
| 6 | 15.6 | 0.97 | 80.8 |
| 7 | DMSO | 1.2 | 100 |
| 8 | Control Cells | 1.2 | 100 |

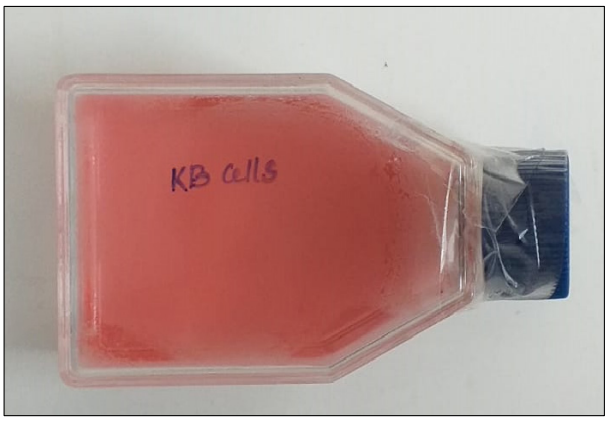



Figure 1. KB Cells in Culture Flask

The microscopic images of the Plectranthus amboinicus (Sample 1) treated cells showed alterations in the cellular morphology. Changes in the epithelial property of KB cells such as loss of adhesiveness between the cells as evident by rounded cellular outline were seen due to the cytotoxic effect of Plectranthus amboinicus. These changes appeared to be pronounced with increasing concentrations of the extract (Figure 2).

*Cell viability after sample 2 (Glycyrrhiza glabra) treatment*

Sample 2 Glycyrrhiza glabra revealed a reduction in the viable cells at a concentration as low as 15.6 µg/ml, expressing 87.5% of viable cells (Table 3). The half maximal inhibitory concentration ($IC_{50}$) value of 43.6 µg/ml was obtained for sample 2. Pearson's correlation revealed a strong negative correlation of -0.731, but was statistically insignificant (P value 0.099) (Table 4). This shows that as the concentration of the extract increases, the percentage of viable cells decreases (Graph 2).

The microscopic images of the Glycyrrhiza glabra (Sample 2) also showed changes in the cellular morphology such as loss of adhesiveness and rounded cellular outline which increased with increasing concentrations of the extract (Figure 3).

Table 2. Pearson's Correlation for Plectranthus Amboinicus (Sample1)

| | | Sample 1 Concentration | Sample 1 Cell Viability |
|---|---|---|---|
| Sample 1 Concentration | Pearson Correlation | 1 | -0.818* |
| | Sig. (2-tailed ) | | 0.047 |
| | N | 6 | 6 |
| Sample1 Cell Viability | Pearson Correlation | -0.818* | 1 |
| | Sig. (2-tailed) | 0.047 | |
| | N | 6 | 6 |

*Correlation is significant at the 0.05 level (2-tailed).

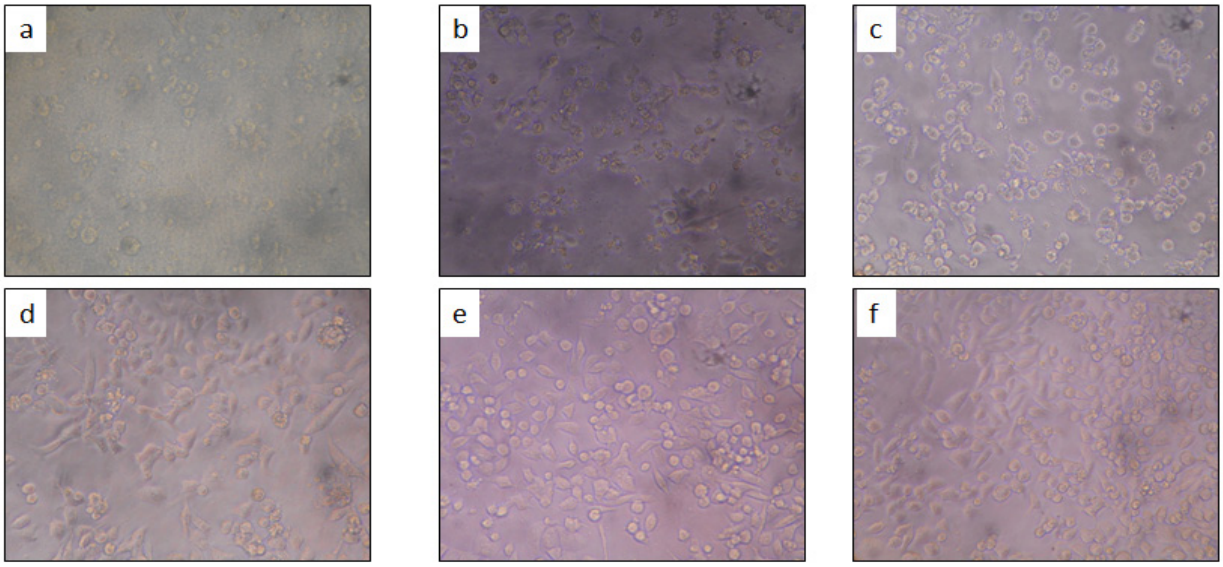



Figure 2. Microscopic Images of Cells at Various Concentrations of P. amboinicus a) 500 µg/ml, b) 250µg/ml, c) 125 µg/ml, d) 62.5 µg/ml, e) 31.2 µg/ml, f) 15.6 µg/ml.

Table 3. Glycyrrhiza Glabra Extract Concentrations and Percentage of Viable Cells

| S. No | Concentration µg/ml | Absorbance 540nm | % Cell Viability |
|---|---|---|---|
| 1 | 500 | 0.02 | 1.6 |
| 2 | 250 | 0.03 | 2.5 |
| 3 | 125 | 0.09 | 7.5 |
| 4 | 62.5 | 0.41 | 34.1 |
| 5 | 31.2 | 0.72 | 60.0 |
| 6 | 15.6 | 1.05 | 87.5 |
| 7 | DMSO | 1.20 | 100.0 |
| 8 | Control Cells | 1.20 | 100.0 |

*Comparison of the anticancer potential of Plectranthus amboinicus and Glycyrrhiza glabra*

The values of both samples were compared to arrive at the most potent herbal extract. The half maximal inhibitory concentration $IC_{50}$, which determines the efficacy of the extract was 53.0 µg/ml for sample 1 and 43.6 µg/ml for sample 2 which infers that sample 2 Glycyrrhiza glabra is more cytotoxic than sample 1 Plectranthus amboinicus on oral cancer (KB) cell line (Table 5) (Graph 3).

Table 4. Pearson's Correlation for Glycyrrhiza Glabra Extract (Sample 2)

| | | Sample 2 Concentration | Sample 2 Cell viability |
|---|---|---|---|
| Sample 2 Concentration | Pearson Correlation | 1 | -0.731 |
| | Sig. (2-tailed) | | 0.099 |
| | N | 6 | 6 |
| Sample 2 Cell viability | Pearson Correlation | -0.731 | 1 |
| | Sig. (2-tailed) | 0.099 | |
| | N | 6 | 6 |

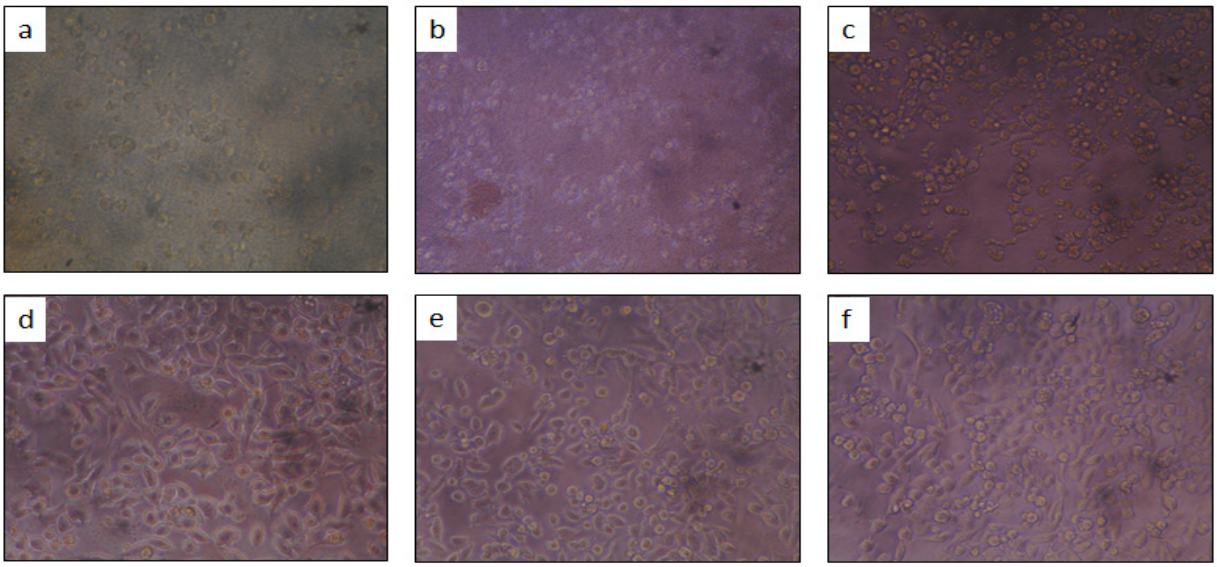



Figure 3. Microscopic Images of Cells at Various Concentrations of G. glabra Extract a) 500 µg/ml, b) 250µg/ml, c) 125 µg/ml, d) 62.5 µg/ml, e) 31.2 µg/ml, f) 15.6 µg/ml.

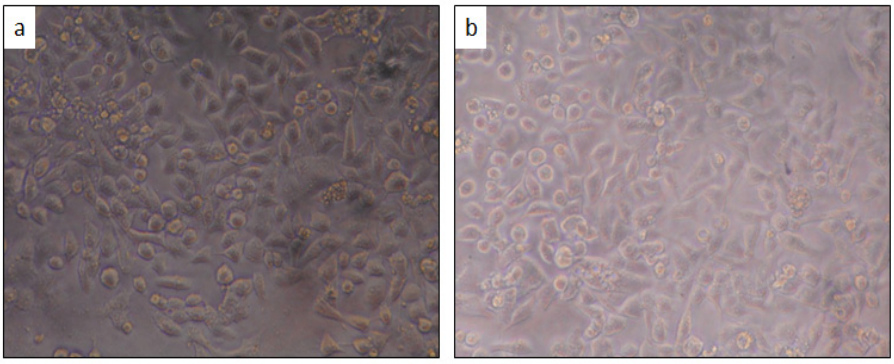



Figure 4. a) Cells in DMSO and b) KB control cells

Table 5. Comparison of Percentage of Viable Cells in Sample 1 and Sample 2

| S. No | Concentration µg/ml | % Cell Viability Sample 1 | % Cell Viability Sample 2 |
|---|---|---|---|
| 1 | 500 | 2.5 | 1.6 |
| 2 | 250 | 5.8 | 2.5 |
| 3 | 125 | 19.1 | 7.5 |
| 4 | 62.5 | 44.1 | 34.1 |
| 5 | 31.2 | 65 | 60 |
| 6 | 15.6 | 80.8 | 87.5 |
| 7 | DMSO | 100 | 100 |
| 8 | Control Cells | 100 | 100 |

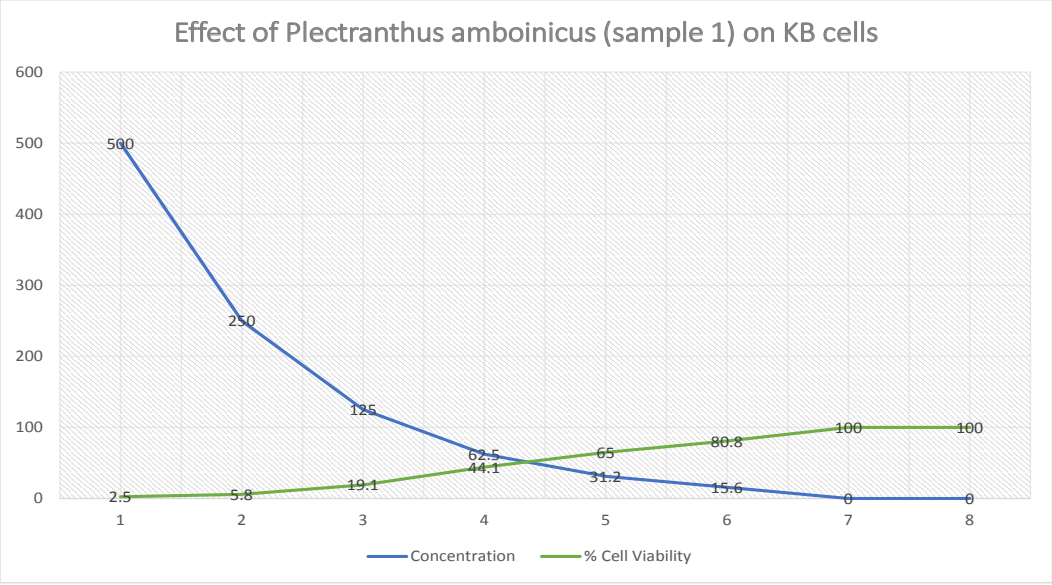
## Discussion

Cancer has become a major health problem worldwide. In India, oral cancer contributes to 30 % of all cancers (Sankaranarayanan et al., 2005). About 70 % of oral cancer cases are diagnosed at the advanced stage leading to a very low 5-year survival rate of 20% (Borse et al., 2020).

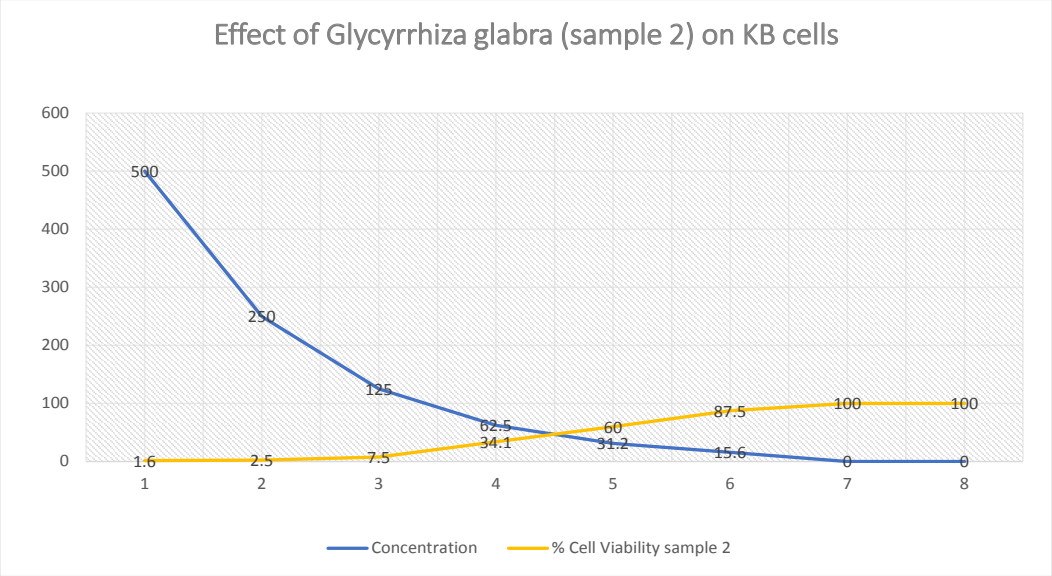
The search for newer treatment options and the discovery of a novel drug becomes a never-ending pursuit. Plant-based medicines or herbal medicines provide an excellent area of scope to explore more plant species with anticarcinogenic properties. The present study aimed to evaluate the anticancer property of two important medicinal plants Plectranthus amboinicus (Indian borage, Karpooravalli) and Glycyrrhiza glabra (Liquorice, Athimathuram) on oral cancer.

The morphological alterations of cells in the two study samples were observed through an inverted microscope and the anticarcinogenic activity of each was analyzed and compared by assessing the cell viability using MTT assay. MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide] is a yellow dye, which in reaction with mitochondrial succinate dehydrogenase enzyme present in viable cells, gets reduced to insoluble purple color formazan. The optical density (OD value) of the color change in the viable cells was measured using a colorimeter at 540 nm. The efficacy of the study samples was inferred based on the $IC_{50}$ value. A decrease in the percentage of viable cells on treatment with test compounds was the major finding in the present study proving their anticarcinogenic potential.

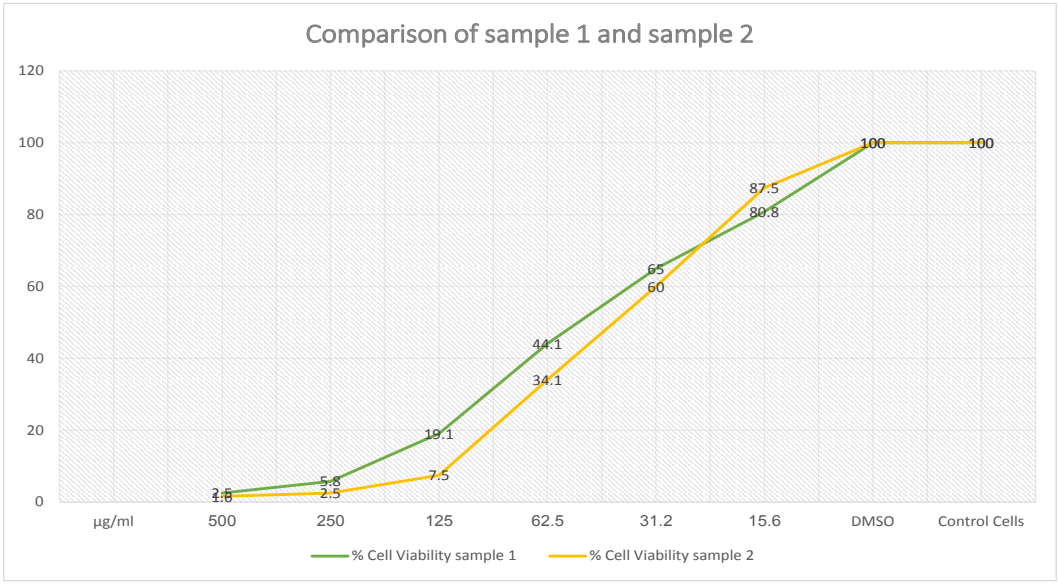
Rosidah (2014) study on Hela cell line using ethanolic extract of Plectranthus amboinicus showed anticancer effect with an $IC_{50}$ value of 88.99 µg/ml. Hasibuan and




Graph 1. Percentage of Viable Cells Decreases with Increase in the Concentration of Sample 1




Graph 2. Percentage of Viable Cells Decreases with Increase in the Concentration of Sample 2




Graph 3. Comparison of Percentage of Viable Cells in Sample 1 and Sample 2

Sumaiyah (2019) evaluated the anti-cancer effect of ethanolic extract of leaves of Plectranthus amboinicus on T47D breast cancer cell line and obtained an $IC_{50}$ value of 89.166 µg/mL. Similarly, Goel et al., (2021) evaluated the anticancer effect of hydroalcoholic extract of Glycyrrhiza glabra on C6 glioma cell line which revealed an $IC_{50}$ value of 32 µg/ml. Pandian and Chidambaram (2017) studied the anticarcinogenic activity of aqueous extract and silver nanoparticles of Glycyrrhiza glabra on HeLa cell line which revealed an $IC_{50}$ value of 125 µg/ml and 62.5 µg/ml respectively leading to the anticancer effect of the extract. These findings were in accordance with the present study where both the extracts gave an $IC_{50}$ value of 53.0 µg/ml (sample 1) and 43.6 µg/ml (sample 2) which are within the range of the above investigations justifying their anticarcinogenic potential. The low $IC_{50}$ values of both test samples indicate that they have excellent anticarcinogenic potential, thereby advocating their use in subsequent research aiming toward the development of anticancer drugs. Previous studies show that induction of apoptosis and cell cycle arrest are the possible mechanisms of action of the extracts on other cancers (Farooqui et al., 2018; Hasibuan and Sumaiyah, 2019). However, the exact mechanism of anticarcinogenic activity of the extracts and the bioactive compound responsible for the same remains unexplored in this study, which is considered a limitation of the study.

Literature reveals that different plant extracts were tested on oral cancer (KB) cell line providing different $IC_{50}$ values. Sireesha et al., (2019) investigated the anticancer effect of amygdalin obtained from almonds and apricots on KB cells using MTT assay which gave an $IC_{50}$ value of 32 µg/ml and 61 µg/ml respectively. The study done by Ankola et al., (2020) evaluating the anticancer effect of Vaccinium macrocarpon (cranberry) on KB cells gave a very low $IC_{50}$ value of 3.564 µg/ml.

In conclusion, Herbal medicines or Phytomedicines remain an excellent area to inquire as an alternative and/or in addition to conventional cancer chemotherapeutic drugs. India, being a country with a medicinal heritage, owns plenty of greenery with inherent medicinal value. Many of these plants besides being a part of day-to-day food, provide exceptional preventive and therapeutic benefits. The present study was an attempt to explore the anticancer effect of the two Indian medicinal herbs Plectranthus amboinicus (Indian Borage) and Glycyrrhiza glabra (Liquorice) on oral cancer (KB) cell line. The results of the study established their role in cancer therapeutics by expressing good anticarcinogenic activity on oral cancer cell line. Studies to identify the exact bioactive compound and its mechanism of action will aid in the development of novel drugs in oral cancer chemotherapy. Such herbal formulation will pave way for easy availability and affordability to help, to combat the financial burden associated with cancer therapy.

## Author Contribution Statement

Study design: Dr. Leo Caroline.M and Dr. Harini Priya A.H, data collection: Dr. Leo Caroline.M and Dr. Nachiammai.N, analysis and interpretation of results: Dr. Harini Priya A.H and Dr. R. Sathish Muthukumar, manuscript preparation: Dr. Leo Caroline.M and Dr. R. Sathish Muthukumar. All the authors read and approved the manuscript.

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*Availability of data*

All the data used in this study are available from the corresponding author

*Conflict of interest*

The authors declare no conflict of interest

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