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# LC-MS Profiling and Cytotoxic Evaluation of Ashwagandha and Liquorice Aqueous Extracts in HT29 and MCF-7 Cell Lines with Relevance to Chemotherapy-Induced GI Toxicity

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## ABSTRACT

Chemotherapeutic agent 5FU reduces quality of life by causing many toxicity and severe GI toxicity. To evaluate this Glycyrrhiza Glabra (GG)and Withania Somnifera (WS) were examined for bioactivity in GI cell line HT 29, DPPH, and MCF 7 MTT assays. MTT studies revealed IC50 values of 36.38 ± 0.8 and 34.32 ± 0.7 µg/mL for Withania Somnifera (WS) and Glycyrrhiza Glabra (GG) for MCF-7 breast cancer cells. The invitro antioxidant experiment showed that WS had a radical scavenging activity of 134.4 ± 0.3 µg/mL, while GG had an EC50 of 99.75 ± 0.8 µg/ The proliferation assay determined GI cell line HT 29's bioactivity. WS' EC50 was 76.78 ± 0.3 µg/mL, while GG's was 77.90 ± 0.6. Studies show that WS and GG may reduce 5FU-induced GI toxicity and scavenge radicals. HPLC–ESI–MS/MS identified withanolides and Glycyrrihizic acid, demonstrating performance. This may improve patient quality of life.

***Keywords:*** *Ashwagandha, Liquorice, GI toxicity, Chemotherapy, adverse effects, Cell Line*

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## 1. INTRODUCTION

Cancer continues to be a leading cause of disease and death globally. Cancer ranks as the second leading cause of death among noncommunicable diseases, trailing only cardiovascular disease. Chemotherapy is frequently employed in the treatment of cancer. Cancer cells persist in their division even in the absence of numerous regulatory functions typically present in normal cells [1]. This trait makes cancer cells susceptible to chemotherapy treatments. Chemotherapeutic therapies, however, are not without their disadvantages. Chemotherapeutic treatments may result in a range of toxicities. For instance, 5-fluorouracil, a widely used chemotherapeutic agent, has been documented to induce myelotoxicity,

cardiotoxicity, and significant gastrointestinal toxicity. It has been shown to act as a vasospastic agent in documented instances. These complications result in treatment interruption and diminish the quality of life (QoL) [2]. At present, pharmaceutical antiemetic therapy has become an important aspect of supportive care; however, its effectiveness in reducing vomiting and related issues is still notably limited. Therefore, it is crucial to develop more effective therapeutic techniques or adjunct treatments to collaboratively enhance the efficacy and reduce the toxicity of chemotherapy and radiotherapy. Furthermore, herbal and plant-based medications have demonstrated the potential to mitigate and alleviate the side effects of chemotherapeutic agents, thereby contributing to the maintenance of quality of life [3].

To deal with the complexities of GI track different drugs have shown promising results in literature and quoted in ancient text for their efficient activity in reducing or preventing the GI mucositis, potent antioxidant and falling in safe limits along with showing prominent activities. To confirm the principal active constituents present study involves aqueous extract of *Withania Somnifera* (WS), *Glycyrrhiza glabra* (GG) with identification high-performance liquid chromatography (HPLC)–mass spectrometry (ESI–MS/MS) conveniently known as LCMS is described. HPLC was used in combination with a triple quadrupole mass spectrometer in multiple reaction monitoring (MRM) mode is used to confirm the presence of active

components in the given aqueous extracts [4]. Numerous authors have reported quantitative liquid chromatography/mass spectrometry (LC/MS) examinations of withanolides and glycyrrhizic acid; however, these analyses typically use the less specific single-ion monitoring (SIM) mode or full scan methods rather than the more sensitive and dependable MS/MS approach [5]. The interference of other compounds and specificity of HPLC UV is being overruled by using LCMS analysis.

## Etiology

Mucositis resulting from radiation therapy, chemotherapy, or combined modalities is believed to include a complex process initiated by tissue injury, as proposed by Sonis in a five-phase model. The five sequential stages of oral mucositis generated by radiotherapy and chemotherapy include initiation, signalling, amplification, ulceration, and healing. Tissue injury is mostly induced by radiotherapy or chemotherapy, leading to the demise of basal epithelial cells and the generation of reactive oxygen species [6]. Subsequently, reactive oxygen species induce direct cellular apoptosis and enhance the inflammatory process, leading to more cellular demise. Additionally, other mechanisms, such as TNF alpha, are enhanced. Fourthly, mucosal ulcerations arise concomitantly with further inflammation. Finally, the epithelium experiences repair through epithelial proliferation [7].

Reactive oxygen species (ROS) are produced by cancer therapy methods such as radiation, cisplatin, and 5-fluorouracil (5-FU), which harm and kill the DNA of neoplastic cells. Oxidative stress is caused by an excess of reactive oxygen species. Numerous enzymes with antioxidant properties, such as glutathione peroxidase, catalase, and superoxide dismutase, are involved in the maintenance and control of reactive oxygen species (ROS) [8]. This cellular redox balance can be upset by oxidants generated during the generation of free radicals or by an issue with the antioxidant defense system. Increased DNA damage, structural protein impairment, and elevated levels of malondialdehyde and 4-hydroxynonenal are often associated with elevated oxidative stress. Pro-inflammatory cytokine production is increased when nuclear factor kappa B (NFκB) is activated during chemoradiation [9]. Treatment-induced oral mucositis is the result of mucosal ulceration caused by this sequence of inflammatory events.

2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, often known as the MTT reagent, is a mono-tetrazolium salt that is composed of a positively charged quaternary tetrazole ring core that has four nitrogen atoms. This core is surrounded by three aromatic rings, which include two phenyl moieties and one thiazolyl ring. Formazan is a violet-blue molecule that is insoluble in water and is formed when MTT is reduced [10]. This disruption of the core tetrazole ring is the outcome of the reduction of MTT. Given that the MTT reagent has a positive charge [11] and a lipophilic structure, it is able to traverse both the cell membrane and the mitochondrial inner membrane of live cells. Furthermore, metabolically active cells are able to convert the MTT reagent into formazan. A colorimetric-based measurement of intracellular formazan synthesis is provided by the chromogenic character of this redox chemical reaction. This measurement served as the foundation for the development of the MTT test by Mosmann et al. in 1983 [12]. Considering this, the assay is extremely useful as a method for determining the metabolic activity of single cells.

### *Withania Sominfera* as Potential herb

The Solanaceae family is made up of 84 different genera, which together contain roughly 3000 different species. Traditional medicine in Southeast Asia has made extensive use of the genera Withania and Physalis, particularly within the framework of the Unani and Ayurvedic medical systems. *Withania Somnifera* L. Dunal, also known as "Ashwagandha," is a small green shrub belonging to the family Solanum. It is a primary component in Ayurvedic formulations [13]. It is recognized for its numerous properties, such as its anti-inflammatory, antitumor, and antioxidant effects. Additionally, it has been utilized in the treatment of ulcers, bacterial infections[14].

Withanolides are a type of naturally occurring C28-steroidal lactones that are distinguished by an intact or rearranged ergostane framework. This framework is composed of C-22 and C-26, which are oxidized in the appropriate manner to produce a lactone ring including six members. A medicinal plant known as WS (also known as Indian winter cherry, Indian ginseng, and Ashwagandha) has been utilized for thousands of years in the traditional medical practices of South Asian countries [15]. For flowering plants, it is a member of the Solanaceae family, which is widely distributed. The WS species have a range that is unusually widespread in the dry parts of the world. Only *W. somnifera and W. coagulans*, out of the 23 species of Withania that have been identified, are thought to have the potential to be used in medicinal applications [16].

### Liquorice as a choice of drug

Liquorice belongs to the genus Glycyrrhiza, and its desiccated roots and rhizomes are referred to as radix glycyrrhiza (RG). Liquorice is frequently utilized as a natural sweetener, with its triterpenoid saponins—especially glycyrrhizic acid, a principal component and bioactive ingredient—being applied in herbal medicine [17].

Radiation and chemotherapy induce mucosal irritation during cancer treatment and rapidly eliminate highly proliferative cells, including both cancerous and dividing epithelial cells [18]. Mucositis is the most common adverse event to cancer

treatment in individuals with head and neck cancer. Glycyrrhiza aqueous extract was administered to head and neck cancer patients on the initial day of radiation therapy in a double-blind clinical trial. This was found to diminish the severity of mucositis and mucosal irritation post-intervention [19]. The anti-inflammatory effects of Glycyrrhiza may inhibit macrophage activation, decrease prostaglandin E2 levels, and diminish the generation of free radicals by macrophages [20]. Moreover, it can diminish reactive oxygen species and neutralize free radicals owing to its antioxidant properties. During chemotherapy, gastrointestinal adverse reactions result in side symptoms such as nausea, vomiting, and diarrhoea [21]. A patient's discomfort level may fluctuate based on the type of chemotherapy, its duration, and their tolerance to it. Emetogenic agents include capecitabine, cisplatin, doxorubicin, and carboplatin. PHY906 can obstruct the mechanisms that initiate intestinal inflammation induced by CPT-11, including NFκB, cyclooxygenase-2 (COX2), and inducible nitric oxide synthase (iNOS) [22].

This study aims to assess the potential of WS and GG regarding toxicity, bioactivity, and antioxidant effects utilizing various cell lines.

## 1. MATERIAL AND METHODS

2. **Drug preparation:** The raw material was purchased form local vendor, identified and analysed for authenticity. The aqueous extract was prepared and evaluated for active constituents and its content, used for further study.

**Antioxidant assay:**
The antioxidant capabilities of the WS and GG extracts were assessed by examining their free radical scavenging activities on the DPPH radical, using the methodology established by Ferreira et al. [23]. In summary, 1 ml of each extract (1 mg/ml) was combined with 2.7 ml of a methanol solution containing DPPH radicals (0.024 mg/ml). The mixture was agitated vigorously and incubated at ambient temperature in the absence of light for 60 minutes, during which their absorbances remained constant. The reduction of the DPPH radical was assessed by measuring the absorbance at 517 nm. Radical scavenging activity (RSA) was determined as the percentage of DPPH discoloration using the equation: % RSA = [(aDPPH − AS)/aDPPH] × 100, where AS represents the absorbance of the solution upon the addition of the sample extract at a specified concentration, and aDPPH denotes the absorbance of the DPPH solution [24]. The 50% maximal inhibitory concentration (IC50) was defined as the concentration of the evaluated WS and GG sample that achieved a 50% decrease in the initial DPPH concentration, as derived from the linear regression concentration curve of the test extract (µg/ml) plotted against the percentage of radical scavenging inhibition.

**MTT Assay:** For biological studies, 20 mg of methanolic extract was dissolved in 1%, 10%, and 50% DMSO and sterile water, respectively, at a concentration of 20 mg/ml. The extracts were passed through 0.22-µm sterile Millipore syringe filter units (Fisher Scientific) prior to being used in the cell culture studies. Modified Eagle's Minimal Essential Medium (DMEM), Phosphate buffer (pH 7.4), trypsin-EDTA, penicillin, streptomycin, Glutamine, foetal bovine serum (FBS), Dimethyl sulfoxide, Trypan blue, Thiazolyl Blue Tetrazolium Bromide were obtained from sigma aldrich (UK) [25]. Human Dermal Fibroblasts (2DD) and breast cancer cells (MDA- MB-231) were purchased from Health protection agency culture collection. Disodium phosphate (Na2 HPO4), Monopotassium phosphate (KH2 PO4), Ethylenediaminetetraacetic acid (EDTA) were purchased from sigma Aldrich (UK). Deoxyribose, Ethylenediaminetetraacetic acid (EDTA), L-ascorbic acid, Iron(III) chloride hexahydrate, Thiobarbituric acid, Trichloroacetic acid Hydrogen peroxide, sodium hydroxide(NaOH), potassium nitrite, Manganese dioxide Diethylenetriamine pentaacetate (DTPA), sodium chloride (NaCl), potassium chloride (KCl), Evans Blue, Nicotinamide adenine dinucleotide, nitro blue tetrazolium, phenazine methosulphate Sodium nitroprusside, sulphanilamide, glacial acetic acid, naphthylethylenediamine dihydrochloride (NED) and Griess agent were purchase from sigma Aldrich (UK). All were analytical grades [26].

| Sr No | Name of Ingredient | Quantity |
|---|---|---|
| 1 | Dulbecco's Modified Eagle's Medium with a high glucose content (4.5 g l−1) | 500 mL |
| 2 | 10% v/v Foetal Bovine serum | 56.5 mL |
| 3 | 100 U ml−1 Penicillin and 10µg ml−1 streptomycin | 6 mL |
| 4 | 1% Glutamine | 6 mL |
| 5 | 1% non-essential amino acid | 6mL |

**Table 1: List of ingredients required for the preparation of media for MTT assay.**

### Cell culture

Human breast cancer cell line (MCF 7) was grown with the help of culture media. All ingredients listed above were pre-warmed at $37^0$C before starting to prepare the medium. All the materials placed in the cabinet were sprayed with bio guard. Hand gloves were used while working in the cabinet to maintain the aseptic conditions. Ingredients 2 - 5 from table 1 were measured and added to Dulbecco's Modified Eagle's bottle. Medium was filtered using a 0.2µ filter. The cell vials were

removed from the nitrogen freezer and placed in a $37^0$C water bath to rapidly defrost the suspension. Cells were plated in 90 mm petri dishes and placed into a humidified incubator at $37^0$C with 5% carbon dioxide. Medium was changed on Tuesday and Friday. The cells were passaged twice every week. The medium was removed and the plate with culture was washed using versene (KCl 0.02% (w/v), NaCl 0.8% (w/v), KH2 PO4 0.02% (w/v), Na2 HPO4 0.0115% (w/v), and 0.2% EDTA (w/v)). The cultures were then treated with a solution of 0.25% trypsin: versene (1:10, v: v) to detach the cells from the tissue culture flasks (approximately 3 - 5 minutes). The effect of trypsin was neutralized by addition of an equal volume of DMEM medium. This cell suspension was centrifuged at 1000 rpm for 5 minutes. The supernatant was removed and the cells were re-suspended in a known volume of fresh medium [25,27].

**MTT assay procedure:** MTT Assay was performed on the MCF 7 cells to find the cytotoxicity of the molecules on the cells. The molecules were applied in serial dilution. Cytotoxicity is determined by plotting the graph of cell viability vs concentration: Cell viability formula = (Absorbance of sample/Absorbance of positive) x 100. Cells were seeded on 96 well plates at a final concentration of approximately 1.5 x 104 cells per 200 µl medium per well 24 hours before the assay. 96 well plates with cell suspensions were then incubated at 370C for 24 hours. After 24 hours the cell media was removed and the cells were treated with different concentrations of molecules and incubated at 370C for 24 hours. After 24 hr 20 µL MTT (5 mg/mL) dye solution in PBS was added to 96 well plates and incubated with cells for 3hrs at 370C. After 3hrs the media containing MTT was removed and the plates were washed with 100 µl of PBS. After washing with PBS the solution of DMSO (200 µl) was added to the wells and kept on shaker for 5 to 10 mins. The absorbance was measured at 580 nm using a microplate reader [27, 28].

**3. Proliferation assay:**

To determine the effect of the fractions on the cells before and after exposure to hydrogen peroxide and UVC, an assay was conducted on the HT 29 cells. Serial dilution is used while applying the plant extracts [29]. A graph showing cell viability against concentration can be used to determine activity (Sample absorbance divided by positive absorbance) x 100 is the cell viability formula. Same procedure was applied as used in MTT assay. The absorbance was measured at 580nm using microplate reader [30].

**4. LCMS Study:**

**Sample preparation:** The aqueous extract of WS and GG was prepared and dissolved in 500 µL of HPLC grade methanol separately. The solutions were filtered through Milipore filter (0.45 µm) before injecting. The stock solution of withaferin-A and glycyrrhizic acid was prepared by dissolving the appropriate amount of standard, accurately weighed, in methanol to yield a final concentration of 330 µg/mL [31].

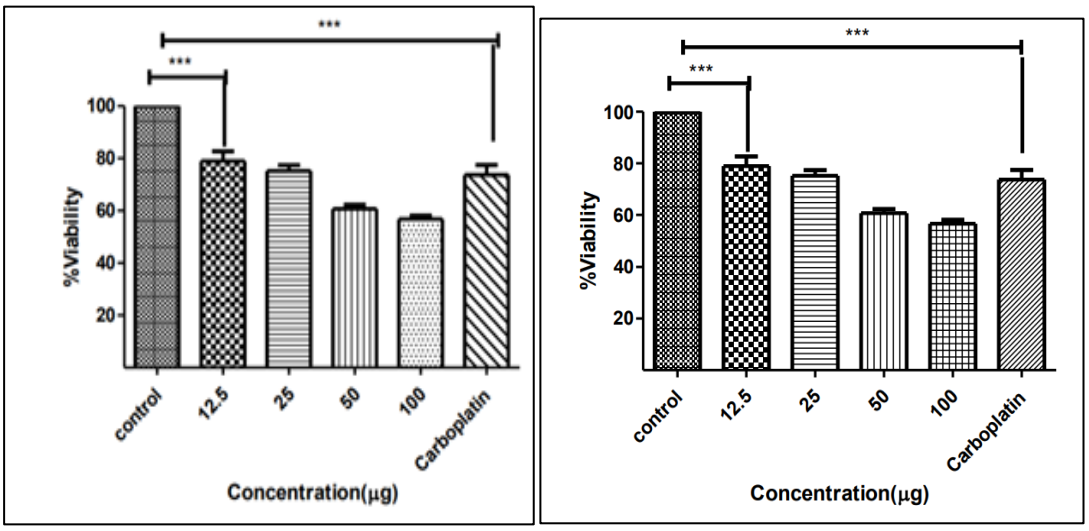
**Instrumentation:** HPLC analysis was performed on an Agilent 1100 equipped with a thermostated autosampler system (Waldbronn, Germany). Separations were carried out using a Waters XBridge Shield RP18 (2.1 × 100 mm; 3.5 µm) column.

The mobile phase consisted of water (A) and ACN (B) using gradient elution: (time, %B) = (0, 20), (5, 55), (5.5, 80), (5.8, 80), (6, 20), (11, 20) at a flow rate of 0.4 mL/min. qudrapole mass spectrometer API 3000 (ABSciex, Ontario, Canada) equipped MS/MS analysis was performed on a triple quadrapole with a Turbo Ionspray source operating in positive ion mode with the following settings: nebulizer gas (N2) at 10 (arbitrary units), curtain gas (N2) at 12 (arbitrary units), auxiliary gas (N2) at 8000 cc/min and CAD gas (N2) at 6 (arbitrary units). Ionspray voltage 5000 V, declustering potential (DP) at 80 V, focusing potential (FP) at 200 V, entrance potential (EP) at 10 V, collision energy at 30 and collision cell exit potential at 15. Mass exact information was obtained on a QSTAR Elite hybrid Q-TOF mass spectrometer (ABSciex, Ontario, Canada) coupled to the Agilent 1200 Rapid Resolution liquid chromatograph. The instrument provided a typical resolution of 10,000 (*m/z* 609.2812). TOF MS data were recorded from *m/z* 70 to 700 with an accumulation time of 1 sec and a pause between mass ranges of 0.5 ms, operating in the positive mode. Reserpine (1 pmol/µL) in product ion scan mode of *m/z* 609 was used to calibrate the mass spectrometer (ions *m/z* 195.0651 and *m/z* 609.2812). The instrument parameter settings were the following: ion spray voltage +5200 V, nebulizer gas (N2) 40 (arbitrary units), curtain gas (N2) 40 (arbitrary units), auxiliary gas (N2) 50 (arbitrary units), heated to 400°C, declustering potential 60, focusing potential 190, declustering potential 2 (DP2) 15 [31, 32].

## 5. RESULT:

1. ***Cytotoxicity Assay:*** The cytotoxicity of the fractions was assessed, and the concentration of non-cytotoxic extracts was identified using the MTT assays. Fractions were screened within the range of 1.25 µg/ml to 0.156 µg/ml. Each experiment was conducted in triplicate. Data were expressed as means ± standard deviation. Statistical analysis was conducted using ANOVA, followed by a post-hoc Tukey test. The Tukey test compares standard deviations across varying concentrations, with asterisks denoting significant differences (***P<0.0005, **P<0.005, and *P<0.05) among cells treated with different quantities of extracts Where P= cells without samples. Extract of *Withania Somnifera (*WS), *Glycyrrhiza Glabra* (GG), were screened with MTT assay in MCF 7 cell line (Figure 1). Percentage of cell viability in petroleum ether

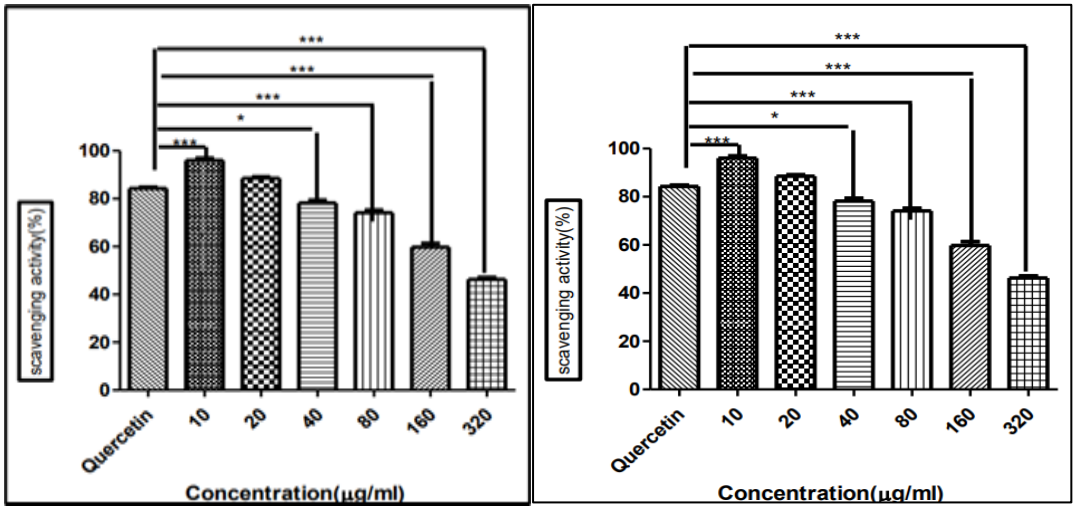
and acetone was observed to be in the range of 75-95 % at applied concentration. While in hexane and dichloromethane the observed percentage of cell viability was in the range of 60-95%. Significant cell viability was observed in all fractions at applied concentrations. The extract fractions were also screened with the via count to confirm the MTT results. It helps to confirm that as concentration was increased the cell viability decreased. P value was also observed less than 0.05. The IC50 value of WS was found to be36.38 ± 0.8 µg/mL whereas The IC50 value of GG was found to be 34.32 ± 0.7 µg/mL [25, 33].




**A** **B**

**Figure 1: Screening of *Withania Somnifera (*A) and *Glycyrrhiza Glabra* (B) on MCF 7 cell line with MTT for toxicity**

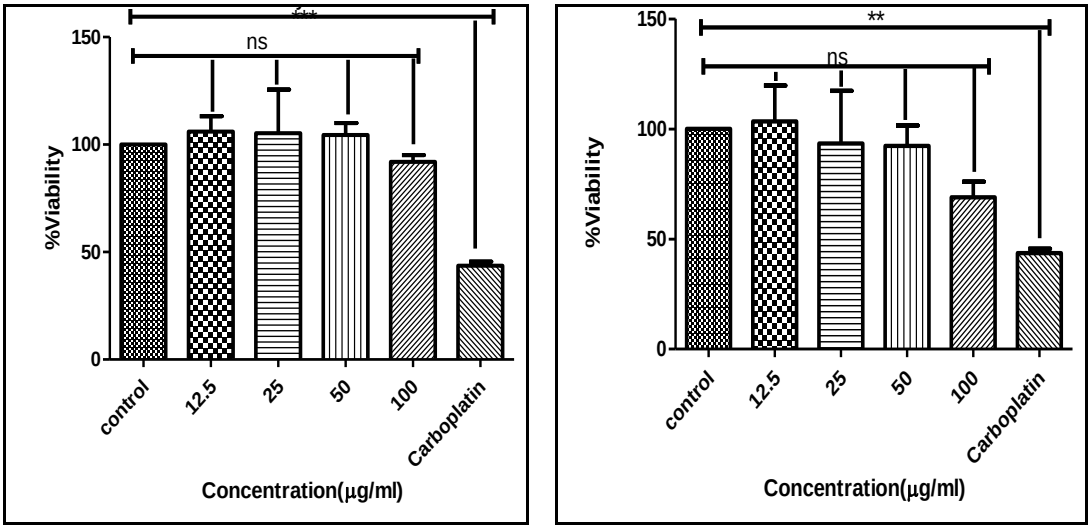
2. ***Antioxidants Assay:*** Dr. Blois devised the DPPH test in 1958 to measure antioxidant activity utilizing stable free radical α, α-diphenyl-β-picrylhydrazyl (C18H12N5O6, M = 394.33). The mechanism for measuring antioxidant scavenging capability is simple. Antioxidant hydrogen converts DPPH's odd nitrogen electron to hydrazine. Each experiment was tripled. Data format: means ± Standard Derivation. Anova and post-hoc tukey test were used for statistical analysis. Tukey test compares std of different concentrations and stars indicate a significant difference (***P<0.0005, **P< 0.005 and *P<0.05) to free radicals treated with different concentration of extracts. Where Neg Std= free radical solution without samples. The EC50 of WS was found to be 134.4 ± 0.3 µg/mL and for GG, EC50 was found to be 99.75 ± 0.8 µg/mL (Figure2) [27, 34].




**A** **B**

**Figure 2: DPPH assay of *Withania Somnifera (*A) and *Glycyrrhiza Glabra* (B) screened for its free radical scavenging property.**

3. **Proliferation Assay:** The in vitro proliferation assay was performed to determine whether cells are triggered to divide after treating with extract and Hydrogen peroxide/UVC and assess differences between cell populations. Each experiment was done in triplicate. Data were represented as means ± Standard Derivation. Statistical analysis was performed by Anova followed by post-hoc tukey test. Tukey test compare std with different concentrations and the stars indicate a significant difference (**P< 0.005 and *P<0.05) to cells treated with different concentration of fractions. Where Std= cells without samples. The extracts were tested on HT29. Similar concentrations of fractions were employed in prior tests. At all concentrations, viability was maintained. Low concentrations have high meaningful activity, which decreases with concentration. Percentage viability was counted and analysed I with different concentrations. Later its confirms that as concentration was increased the cell viability decreased. P value was also observed less than 0.05. [28,35 ] The EC50 value of WS was found to be 76.78 ± 0.3 µg/mL, EC50 value for GG was observed as to be 77.90 ± 0.6 µg/mL (Figure 3).




**A** **B**

**Figure 3: Proliferation assay of *Withania Somnifera (*A) and *Glycyrrhiza Glabra* (B) to identify the bioactivity of the compound.**

**4. LCMS Study:** In order to identify the Withaferin A (Figure 4) and Glycyrrhizic acid (Figure 5) the extract samples were scanned on above said mod e. The MS MS data was collected simultaneously with liquid chromatography. The peaks of Withaferin A showed as *m/z* 476.4 is being compared with samples where the *m/z 485*. For Glycyrrhizic acid *was* found to be *m/z 822.6* for standard and for sample it was shown correctly as *m/z 822*.(Table 2)

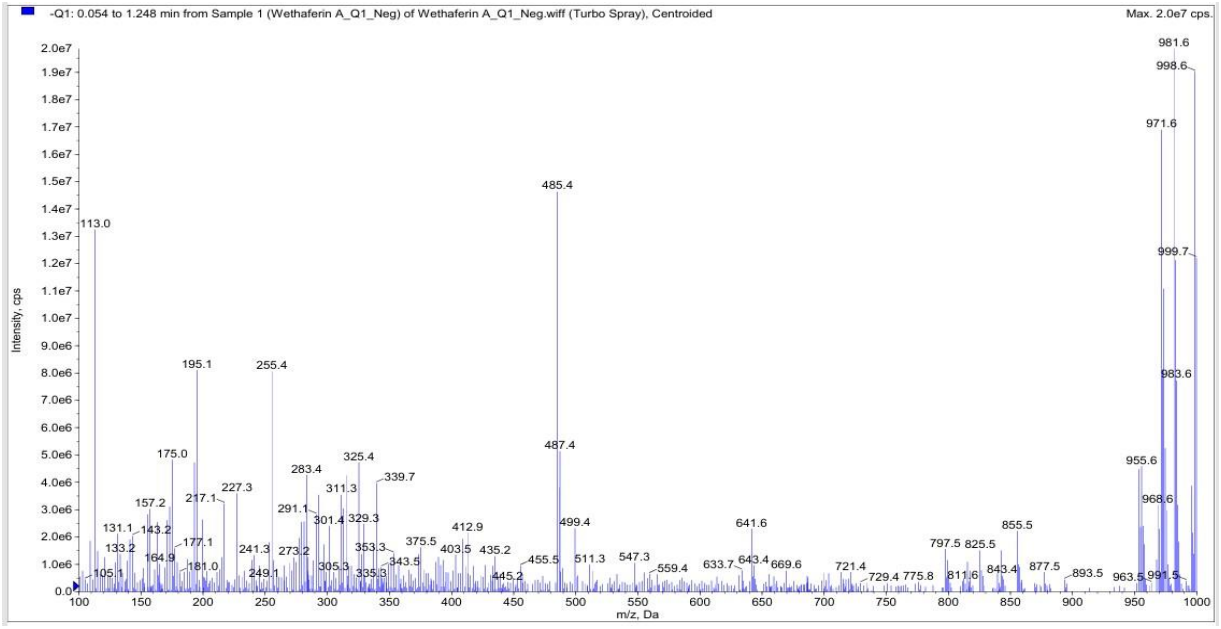



**Figure 4 :Product ion mass of *m/z* for the withaferin-A from the aqueous extract of *W. somnifera***

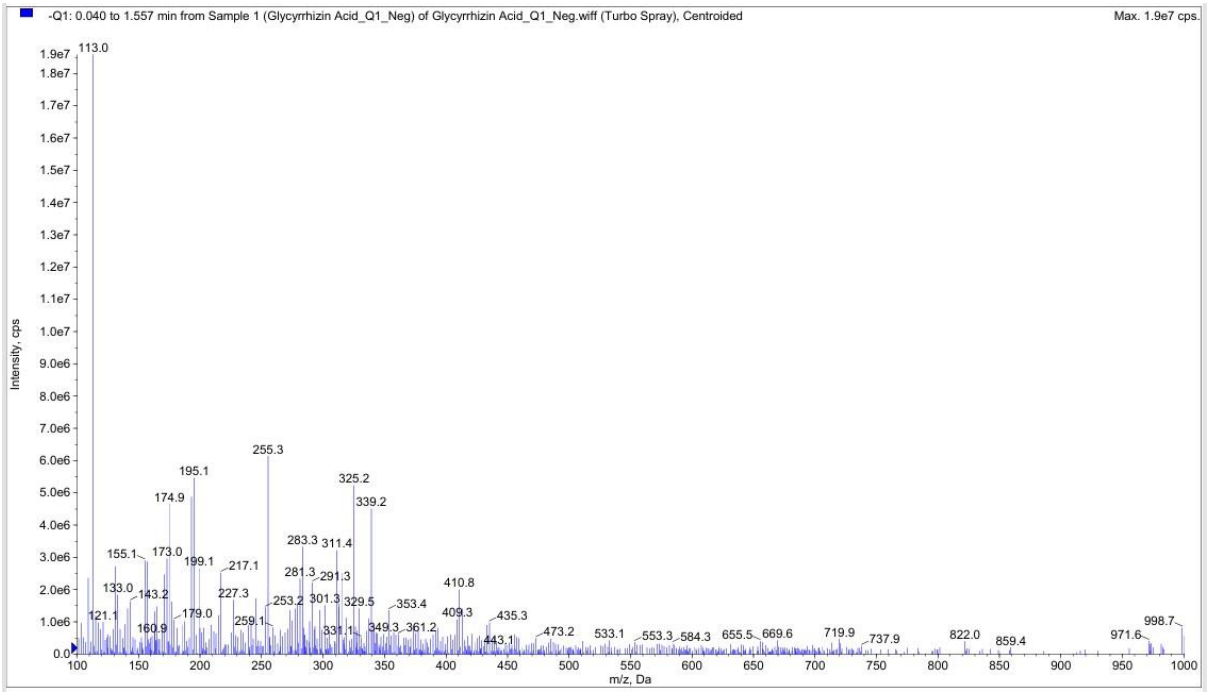



**Figure 6: Product ion mass of *m/z* for the Glycyrrhizic acid**

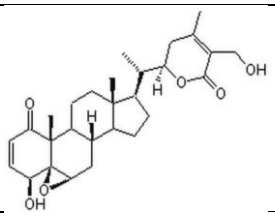
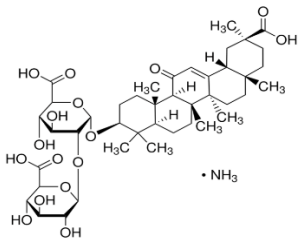
| Sr.NO | Structure | Molecular weight | *m/z Ratio* | Compound |
|---|---|---|---|---|
| 1. |  | 470.60 | 476 | Withaferin A |
| 2. |  | 822 | 822.4 | Glycyrrhizic acid |

**Table 2: Details of the active ingredients studied under LCMS.**

## 5. DISCUSSION:

The ayurveda and ethnobotanical uses of *Withania somnifera* and *Glycyrrhiza glabra* as medicinal herbs are well-established, although there is a dearth of scientific evidence supporting these claims [36]. Attrition occurs at every stage of medication development due to toxicity. The development of new drugs relies heavily on in vitro cytotoxicity testing. One easy, predictable, and low-cost way to determine whether a drug is harmful is to conduct in vitro cytotoxicity tests. In the MTT experiment, a spectrophotometer is used to quantify the formation of insoluble purple formazan crystals, which are produced when live cells' mitochondrial dehydrogenase decreases MTT dye. In order to assess cytotoxicity and cell growth, the MTT test is widely used in high-throughput drug discovery screening [37]. The results show that the extracts were quite active at low doses, but that cell viability dropped off sharply as concentrations rose.

The onset and progression of cancer diseases, cardiovascular, neurological, aging, and are all influenced by free radicals. Consequently, there is a growing interest in antioxidant research. The isolates were screened for their ability to scavenge free radicals using the DPPH assay [38]. A single electron transfer is employed in the DPPH assay to reduce a diverse array of compounds, including metal ions, carbonyls, and radicals, in order to ascertain the efficacy of an antioxidant. The DPPH assay exhibited robust, meaningful activity at low concentrations, which subsequently diminished as the concentrations were increased, similar to viability [39].

Following treatment with hydrogen peroxide and exposure to ultraviolet C, an in vitro proliferation experiment was carried out on HT 29 cells in order to gain a better understanding of the impact that this treatment has on the cell population. Through both prevention and treatment, plant extracts are able to assist in restoring the damage that was caused by hydrogen peroxide and ultraviolet C [40].

To corroborate the establishment and presence of active chemical ingredients, an LCMS investigation was done to collect more accurate data, which was then used to authenticate the existence of active compounds. The presence of active compounds such as withaferin A and glycyrrhizic acid, on the other hand, suggests that the potential for action has been confirmed [41].

Through the protective effect on the mucin layer, which is damaged by chemotherapeutic drugs, the purpose of this article is to investigate the potential of these medications in tolerating gastrointestinal disbalance. In addition, the antioxidant capabilities of these substances are investigated, which may help patients with difficulties such as muscle fatigue and muscle wasting, as well as improve their overall quality of life [42]. It has been established that these compounds are effective and bioactive through the use of antioxidant and proliferation assays. Additionally, the MTT assay has been utilized to evaluate the safety levels and toxicity ranges of these medications.

## 6. CONCLUSION:

Based on the findings of the study, it can be concluded that Ashwagnadha and Liquorice have the potential to counteract the effects of chemotherapeutic agents, specifically with regard to enhancing the health of the gut and the protection of the cell population as analysed on HT 29 cell line. Additionally, the study also found that varied concentrations of WS and GG can be beneficial. Additionally, the antioxidant and free radical scavenging properties exhibit good and different concentrations, and they have the potential to assist in improving the quality of life of cancer patients who are undergoing chemotherapy [43]. The safety levels that were examined by the MTT assay provide strong support for all these efficacy studies when performed on MCF7 cell line.

The presence of all active constituents Withaferin A and Glycyrrhizic acid was confirmed by LCMS. Consequently, these herbs have the potential to be further compounded and administered in an effective amount to both support chemotherapy and contribute to an improvement in the patient's quality of life.

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