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GREEN CHEMISTRY MEETS ONCOLOGY: EXPLORING GLORIOSA SUPERBA AS A NATURAL ANTICANCER AGENT

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ABSTRACT

The well-known medicinal herb *Gloriosa superba*, which is highly valued in traditional Indian medicine, is quickly becoming a powerful source of innovative treatments. With an emphasis on the antibacterial and anticancer potential of *G. superba* tubers and seeds, this study examines their extensive phytochemical profile and remarkable biological qualities. Advanced techniques like Thin Layer Chromatography (TLC), Column Chromatography, Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) were used to identify a wide range of bioactive compounds, including fatty acids, phenolics, gloriosine, colchicine and flavonoids. The tuber extract showed a maximal inhibition zone of 20 mm, indicating that the extracts exhibited high antibacterial activity against multidrug-resistant Staphylococcus aureus (MRSA). Simultaneously, Methanolic seed extracts shown exceptional lethal effects on breast cancer cells MDA MB231. Yielding an IC_{50} value of 19.52 µg/mL in MTT assays. These findings highlight *G. superba* as a natural, eco-friendly alternative to synthetic drugs, offering promising avenues for future antimicrobial and anticancer drug development.

Keywords: *Gloriosa superba*, MRSA, FTIR, GC- MS, Anticancer activity

1. INTRODUCTION

Cancer, a multifactorial and life-threatening disease, continues to be a significant global health burden, accounting for millions of deaths each year [1]. Despite considerable progress in early detection and targeted therapies, challenges such as drug resistance, toxicity and the high cost of treatment remain unresolved. Consequently, there is a renewed interest in the use of natural products and traditional medicinal plants, which offer a wide spectrum of bioactive compounds with potential therapeutic effects. The World Health Organization (WHO) estimates that nearly 80% of the global population relies on plant based medicines for primary healthcare needs. These botanicals are increasingly being investigated for their anticancer, antioxidant, anti-inflammatory and antimicrobial properties [2].

India, with its rich biodiversity and traditional medicinal systems like Ayurveda, Siddha and Unani, is home to over 45,000 plant species. Among these, more than 2,500 species are used in traditional medicine and around 800 are commercially cultivated and used for herbal drug formulations [3]. The increasing demand for herbal products has led multinational pharmaceutical companies to explore phytochemicals for the development of novel therapeutics, nutraceuticals and bioactive agents, particularly in the fields of oncology and infectious disease treatment [4].

One such medicinal plant that has garnered substantial scientific attention is *Gloriosa superba* L., commonly known as flame lily, glory lily, or Kalihari [5,6]. It belongs to the family Liliaceae and is a tuberous, herbaceous climbing plant native to tropical Asia and Africa [7]. It grows abundantly in various regions of India, particularly in the Western Ghats, Eastern Ghats and foothills of the Himalayas. In local Indian languages, it is known as "Agnishikha," "Nangulika," and "Kanvalikizhangu," among others [8].

G. superba holds a prominent place in traditional medicine systems due to its wide range of therapeutic uses [9]. It has been used as an abortifacient [10], anti-arthritic [11], anti-inflammatory [12] and anticancer agent [13]. The plant's tubers, seeds and leaves have been employed for treating ailments such as gout, rheumatism, skin infections, snake bites and intestinal worms [14-16]. In veterinary practice, it has also been used to treat helminthic infections in cattle [17]. Phytochemically, *G. superba* is known for its rich concentration of biologically active compounds. The most important alkaloid present is colchicine ($C_{22}H_{25}O_6N$), a well known mitotic inhibitor used in the treatment of gout and as a research tool in cytogenetics to induce polyploidy [18]. Other notable compounds include gloriosin, colchicoside, salicylic acid, benzoic acid, flavonoids, saponins, sterols and resinous substances. Colchicine content is higher in seeds (0.6%) than in tubers (0.57%) on a dry weight basis and recent studies have even identified novel alkaloids like 1,2-didemethyl colchicine [19].

Among the most pressing global health concerns is the emergence and rapid spread of antimicrobial resistance (AMR), particularly involving pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA). MRSA has evolved resistance not only to β-lactam antibiotics but also to several other antimicrobial classes [20]. It is a major cause of hospital acquired and community acquired infections, including pneumonia, bacteraemia, endocarditis and skin infections [21]. The pathogennicity of MRSA is exacerbated by its ability to form biofilms, which protect the bacterial community from host defenses and hinder the efficacy of conventional antibiotics [22]. The growing threat of drug resistant bacterial strains has necessitated the search for novel antibacterial agents, particularly from natural sources that may offer potent bioactivity with fewer side effects. Medicinal plants like *G. superba* are being increasingly explored for their antibacterial potential, especially against Gram positive organisms such as *Staphylococcus aureus*. Previous studies have demonstrated that alkaloids, tannins, saponins, flavonoids and phenolic compounds possess antimicrobial properties, working through mechanisms such as disrupting bacterial cell walls, inhibiting protein synthesis and interfering with nucleic acid replication [23].

The antimicrobial activity of *G. superba* is of special interest not only because of its potent phytochemicals but also due to its traditional usage in treating infections. Its extracts have shown promise against multiple bacterial strains and its ability to combat multidrug resistant (MDR) organisms suggests it could serve as an effective natural alternative or adjunct to conventional antibiotic therapies. Furthermore, unlike synthetic antibiotics, plant based antimicrobials are less likely to cause adverse side effects and may also reduce the risk of resistance development due to their complex mixture of bioactive compounds. This multifactorial mechanism of action is particularly useful against biofilm-producing and antibiotic-resistant bacteria such as MRSA. The present study aims to investigate the phytochemical composition and evaluate the in vitro antibacterial activity of *G. superba* extracts against methicillin resistant *Staphylococcus aureus* (MRSA). The study seeks to validate the plant's traditional medicinal claims and explore its potential as a natural therapeutic agent for treating multidrug resistant infections

2. MATERIALS AND METHODS

2.1. Collection of Methicillin Resistant *Staphylococcus aureus* (MRSA) Organisms

The Sri Lakshmi Narayana Institute of Medical Science in Agaram, Pondicherry, provided the MRSA selected for this investigation.

2.2. Plant material collection

G. superba plants were gathered in sufficient numbers in and around the Ariyallur District of Tamil Nadu. After being put in a paper bag, the plants were brought into the lab. Healthy plant materials, such as seeds and tubers, were carefully cleaned with tap water and allowed to shade dry for two weeks at room temperature. In order to eliminate plant fibers, the tuber and seeds were next ground into powder using an electric blender and then sieved separately using a nylon sieve. Gather the powders individually and store them in screw-cap vials for later use.

2.3. Thin Layer Chromatographic Studies (TLC)

Using just progressively ascending procedures, thin layer chromatography was performed on all fractions using TLC pre-coated plates (silica gel 60F 254). The plates were marked with a pencil above 1 centimeter from the bottom after being cut with scissors. Each sample was subtly dissolved in methanol and the dissolved samples were evenly applied on the plates using capillary tubes before being allowed to dry. Hexane (100%), hexane: ethyl acetate (9:1,8:2,7:3) and other solvent systems were used to develop the plates in a chromatographic tank. The plates were dried and

examined using UV light (254 nm and 366 nm), regular daylight and a 10% sulfuric acid strain before being heated in an oven for 5 to 10 minutes at 105°C.

The retention factors for each active compound were calculated for each fraction using the following formula: The Rf value of the chromatogram were recorded.

Rf = Distance moved by the solute / compound
Distance moved by the solvent (solvent front)

2.4. COLUMN CHROMATOGRAPHY

2.4.1. Advance Preparation

Assemble the following glassware and liquids before starting the column. After obtaining and numbering dry test tubes, prepare a dry Pasteur pipette with a bulb attached and then fill the containers with acetone and hexane. Label them clearly.

A tiny piece of plugged cotton should be placed in a pasteur pipette and carefully pushed into place with a glass rod to create a chromatography column filled with silica gel. While gently tapping the column with your finger, clamp it vertically so that its bottom is slightly above the height. To make sure the silica gel is firmly packed, apply all of the gel to the pipette column with your finger for a few seconds. Under the column, place the test tube.

2.4.2. Column Packing

Hexane, a solvent, was used to properly pack silica gel in an appropriate column without creating air bubbles. When the mixture was put on top of the stationary phase, the chemical that was eluting with the solvent began to separate gradually as the order of polarity increased. For the biological analysis, the extracts were subjected to column chromatography. Various percentages of n-hexane and ethyl acetate, methanol and ethyl acetate were used to elute the extract.

n-hexane	:	Ethyl Acetate	80:20%	Fraction 1
n-hexane	:	Ethyl Acetate	20:80%	Fraction 2
n-hexane	:	Ethyl Acetate	100:0%	Fraction 3
Methanol	:	Ethyl Acetate	80:20%	Fraction 4
Methanol	:	Ethyl Acetate	20:80%	Fraction 5
Methanol	:	Ethyl Acetate	100:0%	Fraction 6

Each fraction was collected independently and a rotary evaporator was used to extract the solvent at a lower pressure. TLC analysis was performed on the obtained fraction. To eliminate the solvent, similar fractions were combined and let to evaporate. Several spectrum analyses were performed on the pure chemicals in order to identify them and ascertain their structure.

2.4.3. Running the Column

Slowly add the column's hexanes using a pipette. The solvent needs to completely moisten the column. Grain away any extra hexanes until the gel reaches the top. The top of the column must never be allowed to run dry after adding hexane to the gel. Add dissolved pigments to the column after the hexane level reaches the top of the gel. For the thin layer chromatography process, leave the rest in the test tube. In the test tube, keep gathering the eluent.

To begin eluting the yellow band, add hexanes. Until the yellow band gets close to the top of the column, keep adding hexanes. As soon as the final hexanes have run down to the gel's surface, add methanol. Until the gene band gets close to the bottom of the column, keep adding methanol. Stop the process when the eluent has little to no green color.

Evaporating the solvent from the tubes containing the original pigment, the brown pigment and the yellow pigment is done with a warm water bath. Remove each tube from the water bath as soon as the solvent has completely evaporated. After the solvent has gone, do not leave any of the tubes in the water bath as this could cause the pigment to break down. Close the tubes.

2.5. In Vitro Antibacterial Assay against MRSA

The bactericidal activity of extracts from *G. superba* tubers and seeds was assessed using the Well diffusion method. After preparation, the Mueller Hinton agar medium was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. Following sterilization, the media was put into sterile Petri dishes and let to set. Next, 0.1 milliliters of the test organism were extracted from the broth. After being soaked in the broth, the sterile cotton swab was used to swab the Mueller Hinton agar surface. For five minutes, the cultures were let to dry. The test plant extracts were made in different concentrations (15µl, 30µl, 45µl and 60µl). The plates are incubated for 24 hours at 37 °C and the "zone of inhibition" diameter is measured and expressed in mm.

2.6. Fourier Transmission Infra Red (FTIR) spectroscopic analysis on tubers and seeds of *Gloriosa superba*.

2.6.1. Examination of active principle compounds in the *Gloriosa superba*

The *G. superba's* active principle was qualitatively analyzed using the Fourier Transmission Infra-Red (FTIR) approach, as explained [24].

2.6.2. Preparation of KBr disc

A Thermo Niocolet, AVATAR 330 FT-IR system, Madison, WI 53711-4495 was used to record the FT-IR spectra. To eliminate scattering effects from big crystals, a pure potassium bromide salt was used to grind the air-dried tubers and seeds sample. The spectrometer's beam can then pass through the translucent pellet created by pressing this powdered combination in a mechanical press.

2.6.3. Infra-Red evaluation

The vibration spectrum was captured as a graphical chart and the frequency of the spectra that were subjected to examination ranged from 4000 to 400/cm wave number. The instrument used to FTIR analysis was instrumentation lab of CSIR-Central Electrochemical Research Institute, Karaikudi.

2.7. Effect on *Gloriosa superba* tubers and seeds compounds analysis by (GC - MS)

2.7.1. Estimation of Photochemical analysis

The plant materials are analyzed using the GC-MS method. This crucial analytical method consists of the combination of mass spectrometry (MS) and gas chromatography (GC). The CSIR-Central Electrochemical Research Institute in Karaikudi offers GC-MS identification analysis for various chemicals.

2.7.2. GC-MS Condition

(i) Capillary column 30m × 0.25 mm coated with 0.25 µM film of HP-5.
(ii) Sample elution using 50: 1 helium was used as carrier gas at 1.0 ml min^{-1}.
(iii) Column temperature 100˚C for 1 minutes increased 10˚C min^{-1} to 275˚C min^{-1} for 20 minutes.
(iv) Time taken for chromatography per sample in 40 mins.

2.7.3. GC-MS approach for phytocomponent analysis of *Gloriosa superba* seeds and tubers

The GC-column received an injection of one microliter of the filtrate. The sample is then separated into distinct fractions after evaporating and being taken away by the helium carrier gas. The mass spectrum of each component was recorded when the sample fraction exiting the column was allowed to enter the mass detector. Data base dictionaries from Annamalai University's central library were used to compare the mass spectrum of the unknown component with the known spectrum.

2.7.4. Component identification

The National Institute of Standards and Technology (NIST) database has been used to interpret GC-MS. The spectrums of the known and unknown components that were kept in the NIST collection were compared. The components structures, molecular formulas and molecular weights were then determined appropriately.

2.8. ANTICANCER ACTICVITY STUDIES ON MTT ASSAY

G. superba seeds anticancer activity analysis at Pondicherry Centre for Biological Science and Educational Trust, Pondicherry.

2.8.1. Cell lines and culture medium:

Breast cancer cell line (MDA MB231) were used in the experiment obtained from National Centre for Cell Science (NCCS), Pune. The cells were maintained in DMEM, supplemented with 10% FBS, penicillin (100µg/ml), streptomycin (100µg/ml) and amphotericin-B (5µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C. Characterization of cell line is essential not only when deriving new lines, but also when a cell line is obtained from a cell bank or other laboratory. Cultures were examined under an inverted phase microscope before starting of experiments and frequent assessments are made for the viability of the cell population throughout the experimental periods. Stock cells of these cell lines were cultured in DMEM, supplemented with 10% FBS (fetal bovine serum). Along with media cells were also supplemented with penicillin, streptomycin and amphotericin-B, in a humidified atmosphere of 5% CO_2 at 37°C until confluence reached. The cells were dissociated with 0.2 % trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock cultures were grown initially in 25 cm^2 tissue culture flasks, than in 75 cm^2 and finally in 150 cm^2 tissue culture flask and all cytotoxicity experiments were carried out in 96 microlitre well plates. 2×10^4 cells/well was added in to each well of 96 well-plates. It was calculated as follow.

Calculation for number of cells in 96 well plates:

For this we need to calculate for no. of cells required for 100 wells = 96 well,

No. of cells / well × 100

= 2 × 104 × 100

= 2 × 106 cells / plate

Total volume of media for 100 wells

= volume of media / well × 100
= 100 µl × 100
= 10 mL
Therefore, a total of 2 × 106 cells in 10 mL of medium, then aliquot the required volume of cell suspension in to each well.

2.8.2. Characterization of cell lines and culture media:

The characterization of cell lines was performed for the detection of microbial growth and cross contamination. Detection carried out using special media like fluid thioglycollate media (TGM) and tryptone soya broth (TSB) and observed using gram staining. Contamination by bacteria, yeast or fungi was detected by an increasing in the turbidity of the medium and/or by decreasing in pH. Cells were inspected daily for presence or absence of microbial growth.

2.8.3. Preparation of media:

2.8.3.1. Preparation of DMEM:

The 9 g of DMEM powder was added in 1 L of distilled water and then it was stirred continuously until clear solution obtain. To this, $NaHCO_3$ was added to maintain pH 7.0-7.2 and then solution was filtered using membrane filtration assembly. It was sterilized using autoclave and stored in reservoir bottle in refrigerator at 4ºC [25].

Preparation of the trypsin solution: 5 mL of trypsin solution was pipette out in to 50 mL falcon centrifuge tube containing 45 mL of PBS using 10 mL pipette.

2.8.4. Preparation of standard and test solutions:

2.8.4.1 Preparation of stock solution of test compounds:

The stock solutions of test compounds were prepared at concentration of 1mg/mL using DMSO and diluted with culture medium to achieve working concentration of 1000 µg/mL.

2.8.4.2. Dilution of test Compounds:

For this, initially 100 µl of complete media was added in to well number 1-9. Well number 10 contained 150 µl test substance only, from that 50 µl was pipette out and added in to well no. 9 which already contain 100 µl of complete media, which lead to 1:3 dilution of test sample. Same procedure was repeated 9 times in order to get final concentration of test sample up to 0.005 µm.

2.8.5. Reference standard stock solution:

Standard stock solution was prepared to achieve the concentration 1000 µg/mL. Stock solution was further diluted using DMSO to obtain the dilutions of solutions.

2.8.6. Design of experiment:

Cell lines in exponential growth phase were washed, trypsinized and re-suspended in complete culture media. Cells were seeded at 2 × 104 cells / well in 96 well microtitre plate and incubated for 24 h during which a partial monolayer forms. The cells were then exposed to various concentrations of the test compounds (as indicated in plate assignment) and standard doxorubicin. Control wells were received only maintenance medium. The plates were incubated at 37 °C in a humidified incubator with 5 % CO_2, 75 % relative humidity for a period of 24 h. Morphological changes of drug treated cells were examined using an inverted microscope at different time intervals and compared with the cells serving as control. At the end of 24 h, cellular viability was determined using MTT assay.

2.8.7. *In vitro* assay for cytotoxic activity (MTT assay):

The Breast cancer cell line (MDA MB231) were plated separately using 96 well plates with the concentration of 1×10^4cells/well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in CO_2 incubator at 37˚C with 5% CO_2. The cells were washed with 200 µL of 1X PBS, then the cells were treated with various test concentration of compound in serum free media and incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37˚C for 4 h using CO_2 incubator. After incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals was dissolved with 100 µL of DMSO and thoroughly mixed. The development of color intensity was evaluated at 570nm. The formazan dye turns to purple blue color [26]. The absorbance was measured at 570 nm using microplate reader.

% viability = (A570 of treated cells – A570 of blank cells) / (A570 of controlled cells – A570 of blank cells) × 100.

Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula % cytotoxicity = 100 – % cell viability.

2.8.8. Data Interpretation:

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes. After 24 h, the cytotoxicity data was evaluated by determining absorbance and calculating the correspondent chemical concentrations. Linear

regression analysis with 95 % confidence limit and R2 were used to define dose-response curves and to compute the concentration of chemical agents needed to reduce absorbance of the formazan by 50 % (IC_{50}). Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula:

% viability = (AT – AB) / (AC – AB) × 100 …… (1)

Where, AT = Absorbance of treated cells (drug)

AB = Absorbance of blank (only media)

AC = Absorbance of control (untreated)

% cytotoxicity = 100 – % cell survival ……(2)

2.8.9. Determination of IC_{50} value:

According to the FDA, IC_{50} represents the concentration of a drug that is required for 50 % inhibition in-vitro. For primary screening, a threshold of 50 % cell growth inhibition as a cut off for compound toxicity against cell lines was used. IC50 values were determined from plot of dose response curve between log of compound concentration and percentage cell growth inhibition. Graph was plotted by keeping log concentration of drug on X axis and % cell growth inhibition or % cytotoxicity Y axis. IC_{50} values were estimated as a concentration of drug at 50 % position on Y axis. The relationship should be sigmoidal, log concentration of the drug on the X axis and response / measurement of the Y axis. The prism web site has some good guides for this. So, we have used this software.

IC_{50} values were calculated using the nonlinear regression program origin. The average of two (duplicates manner) were taken in determination. IC_{50} value has been derived using curve fitting methods with graph pad prism statistical software (Ver. 5.02).

3. RESULT AND DISCUSSION

G. superba L. belongs to the colchicaceae family of flowering plants. The flame lilies are a frequent name for this perennial climber. Because of its possible bioactive components, the plant is employed as a medicinal herb in Ayurveda. Whole plant parts like tubers, flowers, stems and roots contain bioactive chemicals that are commonly employed for biological purposes. Gloriosine and colchicine are the plant's poisonous alkaloids. Numerous illnesses, including gout, arthritis, infertility, STIs, leprosy, kidney issues, typhus, open wounds and snake bites are treated with it. The pharmacological activity of *G. superba* and the phytochemical classes of bioactive substances are updated in this chapter.

In comparison to ampicillin and fluconazole, respectively, Previous study assessed the antibacterial activity of *G. superba* against *E. coli*, *S. aureus*, *A. flavus* and *A. niger*. They discovered that the acetone extract exhibited more antimicrobial activity against *E. coli* [27]. Human pathogens like Escherichia coli, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are all susceptible to the antibacterial activity of acetone, dichloromethane and methanol extracts of the tubers and seeds of *G. superba* L. (*Liliaceae*) when tested using the disc diffusion method. All of the extracts were proven to be effective against five human bacterial species in the current experiment. Against *Proteus mirabilis*, the dichloromethane extract exhibited higher activity. *Proteus mirabilis* in dichloromethane tuber extract (16 mm), *Escherichia coli* in methanol tuber extract (15 mm) and *Staphylococcus aureus* in methanol tuber extract (15 mm) showed the greatest inhibition. *Klebsiella pneumoniae* was shown to be moderately suppressed by 14 mm methanol tuber extract and 14 mm acetone seed extract. The 14 mm methanol seed extract of *Pseudomonas aeruginosa* [28].

According to the current study, *G. superba* tubers and seeds have broad-spectrum antibacterial action, which is reasonable based on the results obtained. The MRSA exhibited stronger antibacterial action. The seeds S3 (16mm) and Tuber T5 (20mm) showed the greatest inhibition. (Table-1 & 2) According to the FTIR study, *G. superba* Plant was examined based on the skeletal form of molecular stretches of active principles with aliphatic aldehyde, alkene, alkane and ether groups [29].

The present study FTIR based on these peaks and the known phytochemicals in *G. superba* tuber, likely active principles include Colchicine (alkaloid with aromatic rings and amide groups), Gloriosine (related to colchicine, contains aromatic and methoxy groups), β-Sitosterol (plant sterol with aliphatic chains and hydroxyl group), Saponins (glycosides with sugar and steroid moieties), Phenolic compounds (like flavonoids, tannins) were present. (Table-3 & Fig-1)

The present study FTIR based on these spectra and known phytochemistry of *G. superba* Seeds, likely compounds include Colchicine (Alkaloid with aromatic rings and amide functionality). Gloriosine (Similar structure to colchicine with aromatic and methoxy groups), β-Sitosterol (A phytosterol with CH_2/CH_3 groups and hydroxyl functionalities), Saponins (Glycosidic compounds with C–O stretches and steroidal cores), Phenolics / Flavonoids (Containing

aromatic C=C and hydroxyl groups), Tannins (Polyphenolic compounds that show strong O–H and aromatic C–H bands), Fatty Acids or Esters (Characterized by C=O and C–H stretches). (Table-4 & Fig-2)

GC-MS study of *G. superba* seeds and tubers. Analysis was done on the colchicines, hexadecanoic acid and pentadecanoic acid [28].

A Previous study evaluated the ethanol was used to extract the phyto-components from the tubers of *G. superba* L cultivars from Sirumalai (GA1), Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyan (GA5). Gas Chromatography – Mass Spectrometry (GC-MS) analysis was used to determine the chemical composition and concentration in the tubers. In terms of the phyto-components, GA1 displayed fifteen, GA2 thirteen, GA3 eight, GA4 fourteen and GA5 thirteen. The ecotypes GA1, GA2, GA4 and GA5 had more phyto-components. *G. superba* L's key alkaloid, colchichine, was present in good concentrations in the GA2, GA3, GA4 and GA5 accessions [30].

Nine bioactive chemicals were found in the tuber and seventeen in the seeds of *Gloriosa superba*, according to the current study's GC-MS analysis. Colchichine, a significant alkaloid of *G. superba* L., was detected in high concentration in the tubers and seeds. (Table- 5 &6) (Fig- 3 & 4)

G. superba extracts can be regarded as the best extracts against breast cancer cells since they show greater anticancer activity of 60-80% against MDA MB 231 cells at a concentration of 50 ug/ul [31].

According to Singh *et al*. [32], HepG2 cell lines (human liver cancer cells) were used to test the anticancer activities of *G. superba* tuber extract. Methanolic extract was shown to have the strongest anticancer effect, resulting in 50% cell death at a concentration of 100 ug/ul. Hep-G2 cell death rates can be caused by a number of factors, including protein binding, receptor binding inhibitors, DNA replication interaction, etc.

The current study examined the anticancer properties of *G. superba* methanol tuber extract (Table-7) (Fig-5). Using the MTT assay on a breast cancer cell line (MDA MB231), the anticancer efficacy of chemomile extract was evaluated in vitro. Values for IC50 (µg/ml) were computed. The findings demonstrated that, in comparison to the common medication doxorubicin, the extract from *G. superba* seeds exhibited strong anticancer activity (IC50 value 19.52 µg/ml).

TABLE-1 Antibacterial activity of tuber extract of *Gloriosa superba* against MRSA

Plant	Extract	Compound	Zone of inhibition (mm)		
			20 µl	30 µl	40 µl
Gloriosa superba	Tubers	T1	-	10	10
		T2	12	14	16
		T3	10	12	15
		T4	10	14	16
		T5	18	19	20
		T6	-	10	10

TABLE-2 Antibacterial activity of seeds extracts of Gloriosa *suberba* against MRSA

Plant	Extract	Compound	Zone of inhibition (mm)		
			20 µl	30 µl	40 µl
Gloriosa superba	Seeds	S1	-	10	10
		S2	10	12	14

		S3	10	12	16
		S4	-	10	10
		S5	-	10	12

TABLE-3 Molecular stretches of active principles isolated from *Gloriosa superba* tubers through FT-IR Spectroscopic analysis

PEAK NUMBER	WAVE NUMBER X (cm^{-1})	Probable Functional Group	Possible Active Compound Class
1.	3381.59	O–H or N–H stretch (broad)	Alcohols, Phenols, Amines
2.	2929.23	C–H stretch (alkane)	Alkanes, Fatty acids
3.	2150.01	C≡C or C≡N stretch	Alkynes or Nitriles
4.	1646.19	C=C or C=O stretch	Alkenes, Amides, or Ketones
5.	1461.34	CH_2 bending	Alkanes, Lipids
6.	1421.23	CH_3 bending	Alkanes, Terpenoids
7.	1246.04	C–O stretch	Esters, Ethers, Phenols
8.	1163.54	C–O or C–N stretch	Alcohols, Ethers, Amines
9.	1082.06	C–O or C–N stretch	Carbohydrates, Glycosides
10.	985.20	=C–H out-of-plane bending	Alkenes
11.	928.06	=C–H out-of-plane bending	Aromatics or Alkenes
12.	859.77	Aromatic C–H bend	Aromatic Compounds
13.	765.78	Aromatic C–H bend	Aromatic Compounds
14.	708.05	Aromatic substitution patterns	Benzene derivatives
15.	574.37	C–Br or C–I stretch	Halo-compounds
16.	527.82	C–Br stretch	Alkyl halides
17.	436.43	Metal–O or C–I stretch	Organometallics or Halogenated compounds

TABLE-4 Molecular stretches of active principles isolated from *Gloriosa superba* seeds through FT-IR Spectroscopic analysis

PEAK NUMBER	WAVE NUMBER X (cm^{-1})	Probable Functional Group / Vibration	Possible Compound Type
1.	3864.53	O–H stretch (free, sharp)	Alcohols, Phenols
2.	3782.68	O–H or N–H stretch (sharp)	Phenols, Amines
3.	3367.13	H-bonded O–H or N–H stretch	Alkaloids, Flavonoids
4.	2921.72	C–H (asymmetric stretch, CH_2)	Fatty acids, Alkanes
5.	2853.13	C–H (symmetric stretch, CH_3/CH_2)	Lipids, Steroids
6.	2362.28	CO_2 overtone or impurities	Atmospheric CO_2 or trace gases
7.	2335.78	C≡C or C≡N (weak)	Alkynes or Nitriles
8.	2270.74	N=C=O or terminal alkyne stretch	Isocyanates, Alkynes
9.	1741.93	C=O stretch (ester, aldehyde, or ketone)	Esters, Fatty acids, Saponins
10.	1613.56	C=C stretch (aromatic ring) or N–H bend	Flavonoids, Alkaloids
11.	1539.98	N–O or N–H bending	Amines, Amides
12.	1437.42	CH_2 bending or aromatic ring vibration	Steroids, Aromatic compounds
13.	1260.54	C–O or C–N stretch	Glycosides, Esters, Ethers
14.	870.39	=C–H out-of-plane bending (aromatic or alkene)	Flavonoids, Aromatic compounds
15.	817.50	C–H deformation (aromatics)	Aromatic rings

16.	780.95	C–H out-of-plane (aromatic substitution)	Phenolic compounds
17.	699.78	Mono-substituted benzene ring	Aromatic compounds
18.	599.32	C–Br or ring deformation	Halogenated organics
19.	535.68	C–Br / skeletal vibrations	Alkaloids or Steroidal backbones
20.	502.26	C–I or ring vibrations	Organohalogens or complex rings
21.	471.31	Metal–O or ring torsion	Possibly organometallics
22.	425.69	Ring deformation or skeletal vibrations	Aromatics or Steroidal nucleus

TABLE-5 Bioactive compounds present in tubers of *Gloriosa superba* by GC-MS

S. No	Component RT	Compound Name	Molecular Weight	Formula	Component Match	Area %
1	5.8301	1,2-Cyclopentanedione	98.037	C_5H6O_2	25387.8	5.073
2	11.8942	m-Guaiacol	124.052	$C_7H_8O_2$	199334.1	39.83
3	12.6161	Salicyl alcohol	124.052	$C_7H_8O_2$	32547.5	6.504
4	14.0195	Cyclohexene, 3-methyl-6-(1-	136.125	$C_{10}H_{16}$	8557.0	1.71
5	15.1572	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	151.048	$C_4H_9NO_5$	50493.9	10.09
6	17.1844	4-(1-Hydroxyallyl)-2-methoxyphenol	180.079	$C_{10}H_{12}O_3$	9220.8	1.843
7	17.2825	Methyl 2-methyl-3-phenyl-prop-2-enoate	176.084	$C_{11}H_{12}O_2$	5768.7	1.153
8	22.0991	n-Hexadecanoic acid	256.24	$C_{16}H_{32}O_2$	26517.4	5.299
9	40.8516	.alpha.-Lumicolchicine	798.336	$C_{44}H_{50}N_2O_{12}$	142611.0	28.5

TABLE-6 Bioactive compounds present in Seeds of *Gloriosa superba* by GC-MS

S. NO	Component RT	Compound Name	Molecular Weight	Formula	Component Match	Area %
1	3.1734	2,2'-Bioxirane	86.037	$C_4H_6O_2$	28529.4	1.51
2	4.1610	Glyceraldehyde	90.032	$C_3H_6O_3$	122723.8	6.497
3	4.9291	Propanoic acid, 2-nitro-, methyl ester	133.038	$C_4H_7NO_4$	48142.9	2.549
4	5.2005	Dihydroxyacetone	90.032	$C_3H_6O_3$	126034.0	6.672
5	5.8185	1,2-Cyclopentanedione	98.037	$C_5H_6O_2$	151184.9	8.003
6	6.9447	2-Propen-1-ol	58.042	C_3H_6O	7015.2	0.3714
7	7.0140	2H-Pyran-2,6(3H)-dione	112.016	$C_5H_4O_3$	8511.4	0.4506
8	10.0749	(S)-(+)-2-Amino-3-methyl-1-butanol	103.1	$C_5H_{13}NO$	40063.2	2.121
9	10.1673	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-	144.042	$C_6H_8O_4$	8760.3	0.4637
10	11.3859	Phenol, 4-chloro-	128.003	C_6H_5ClO	6165.9	0.3264
11	12.1713	Ethanamine, 2-propoxy-	103.1	$C_5H_{13}NO$	12838.8	0.6796
12	15.2438	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	151.048	$C_4H_9NO_5$	112419.9	5.951
13	16.3180	Pentanoic acid, 5-hydroxy-, 2,4-di-t-	306.219	$C_{19}H_{30}O_3$	4166.4	0.2206
14	17.2767	Tetrahydro-4H-pyran-4-ol	102.068	$C_5H_{10}O_2$	42695.7	2.26
15	22.0933	Propanedioic acid, propyl-	146.058	$C_6H_{10}O_4$	7604.0	0.4025

16	32.7603	Bis(2-ethylhexyl) phthalate	390.277	$C_{24}H_{38}O_4$	985994.5	52.19
17	43.6409	Colchicine	399.168	$C_{22}H_{25}NO_6$	176211.4	9.328

TABLE-7 Anticancer activity of *Gloriosa superba* Tubers (MTT Assay)

Tested concentration(µg/ml)	**OD at 570nm(triplicate values)**		
500	0.098	0.096	0.092
250	0.122	0.117	0.115
100	0.177	0.162	0.157
50	0.222	0.211	0.2
25	0.265	0.271	0.259
Control	**0.558**	**0.563**	**0.580**

Tested concentration(µg/ml)	**OD at 570nm(triplicate values)**		
500	0.098	0.096	0.092
250	0.122	0.117	0.115
100	0.177	0.162	0.157
50	0.222	0.211	0.2
25	0.265	0.271	0.259
Control	**0.558**	**0.563**	**0.580**

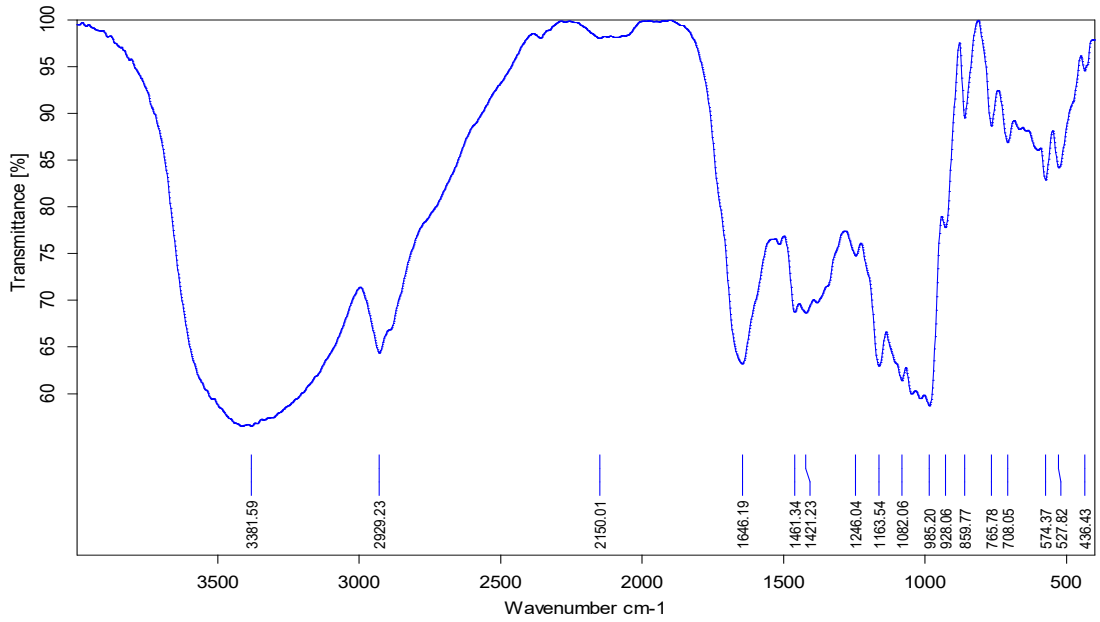

Fig-1 : The molecular stretches of active compounds present in the *Gloriosa superba* tuber

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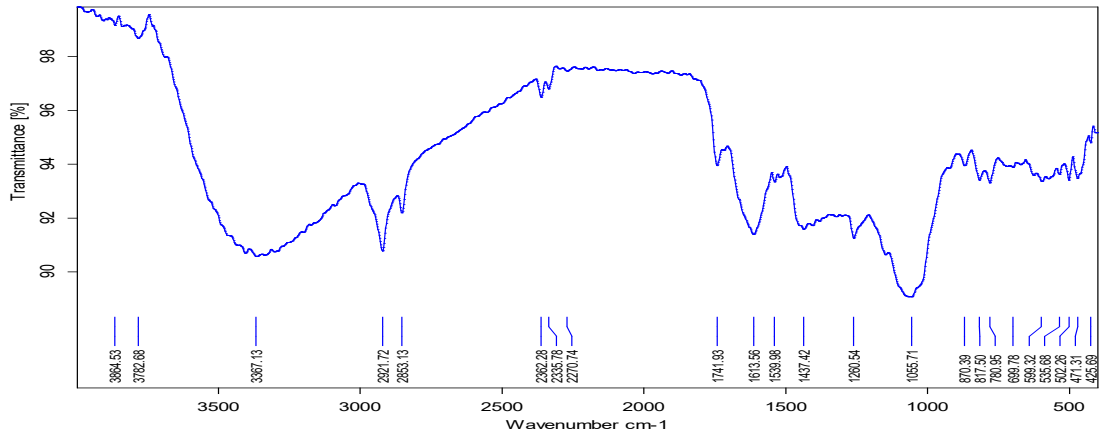

Fig-2 : The molecular stretches of active compounds present in the *Gloriosa superba* Seeds

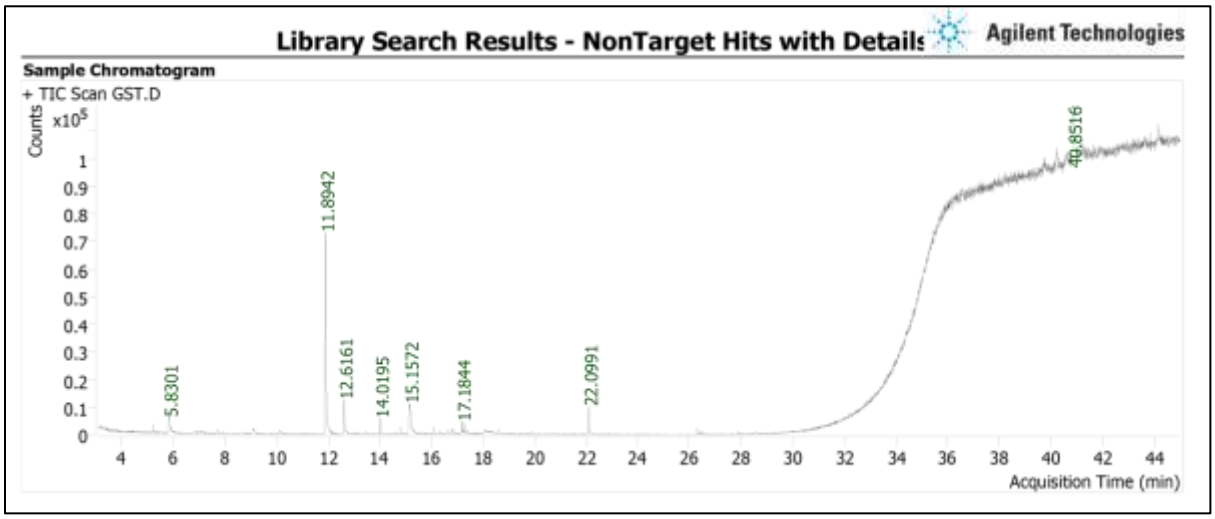

Fig-3 : The bioactive compounds present in the tubers of *Gloriosa superba*

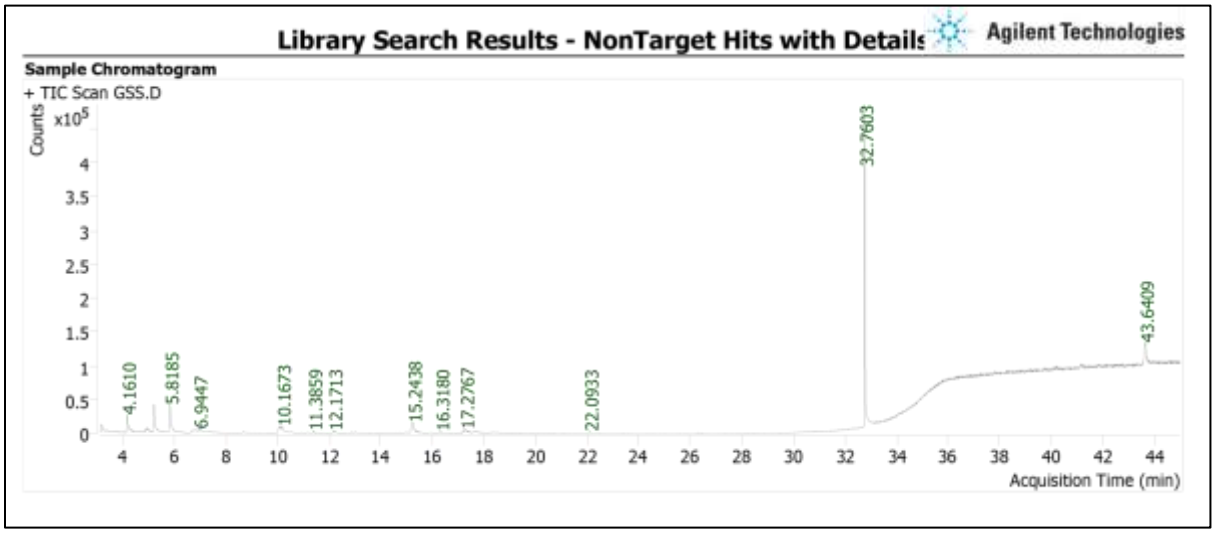

Fig-4 : The bioactive compounds present in the tubers of *Gloriosa superba*

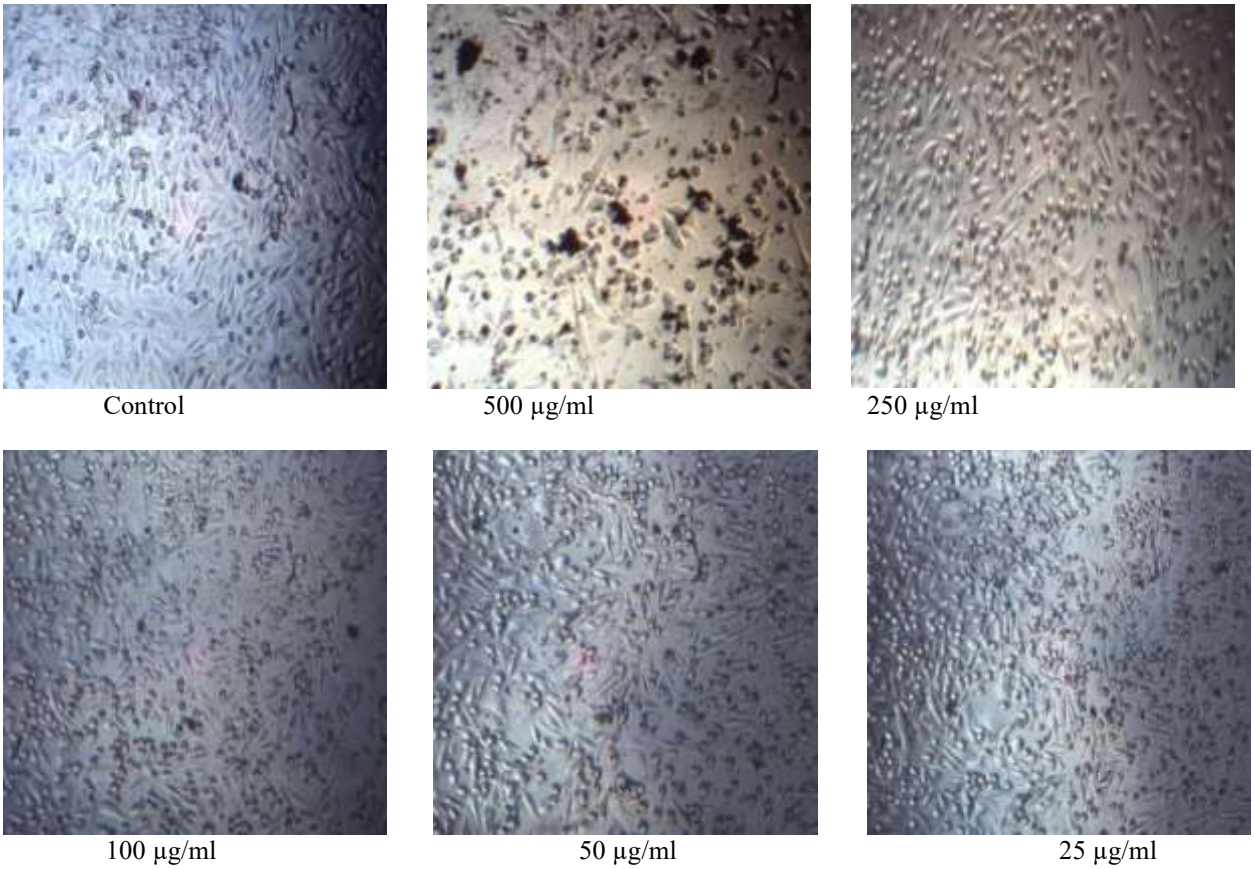

Fig-5 : Anticancer activity of *Gloriosa superba* seeds (MTT Assay)

4. CONCLUSION

These findings not only support the traditional uses of *G. superba* in Ayurvedic and folk medicine but also emphasize its relevance in contemporary pharmaceutical development. Its dual action, combating both infectious diseases and cancer, highlights the plant's multidimensional therapeutic potential. Moreover, its eco-friendly, cost-effective nature enhances its viability as a sustainable alternative to synthetic drugs.

G. superba emerges as a botanical powerhouse with promising implications for the development of novel antimicrobial and anticancer therapeutics. Continued exploration, including clinical validation and formulation studies, is essential to fully harness and translate its bioactive wealth into modern healthcare solutions. This study lays a strong foundation for such future advancements, affirming the enduring value of medicinal plants in the era of precision medicine.

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