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Comprehensive Exploration of Phytochemical Composition and Bioactive Potentials (Antioxidant and Antibacterial Activities) in *Gloriosa superba* (Adavinabhi) Rhizomes

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Abstract

Gloriosa superba, commonly recognised as Adavinabhi, is a medicinal plant renowned for its traditional uses in various herbal formulations. This research aimed to explore the phytochemical substances and assess the antioxidant and antimicrobial properties of *Gloriosa superba* rhizomes. The phytochemical analysis involved qualitative and quantitative assessments of various bioactive compounds present in the rhizomes. Standard methods were employed to identify alkaloids, flavonoids, phenols, tannins, terpenoids, and other secondary metabolites. The quantitative estimation revealed significant concentrations of alkaloids and flavonoids, indicating their potential pharmacological relevance. Furthermore, the biological activities of *Gloriosa superba* rhizomes were studied. The antioxidant action was assessed using established assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assays. Results demonstrated potent antioxidant potential, suggesting the plant's capability to neutralize free radicals and mitigate oxidative stress. Additionally, the antimicrobial activity of *Gloriosa superba* rhizomes was assessed against a panel of pathogenic microorganisms, including bacteria and fungi. The plant extract exhibited significant antimicrobial effects, highlighting its potential as a natural source for antimicrobial agents. In the present study revealed that *Gloriosa superba* rhizomes are rich in bioactive compounds, especially alkaloids and flavonoids. The plant demonstrates notable antioxidant and antimicrobial activities, emphasizing its potential in the development of therapeutic agents. Further investigations deserve to isolate and describe specific compounds accountable for these pharmacological activities, paving the way for the development of novel pharmaceuticals and herbal remedies.

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Introduction

Extensive research has been dedicated to the development and advancement of novel antimicrobial compounds, aiming to address microbial infections with both topical and systemic applications. This research spans across various resources, including microbes, animals, and notably, plants. According to literature reviews and ethnobotanical records, plants represent a potentially untapped resource for the pharmaceutical sector. They may offer innovative chemical compounds or serve as natural repositories of antibacterial agents, thereby holding promise for global infection control efforts. Investigations into the antibacterial properties of plants and their constituents have a long history, dating back to the late 1800s. Clinical microbiologists are particularly interested in antimicrobial plant extracts for two primary reasons(Makky *et al.*,2012).

While synthetic organic chemistry achieved significant milestones in the 20th century, the concept of utilizing plants for medicinal purposes is age-old. In industrialized nations, over 25% of prescribed medications have some association with plants, whether actively or passively(Kavitha *et al.*, 2011). However, there remains a notable research gap concerning plants employed in traditional medicine, particularly within the domain of clinical microbiology. This underscores the need for further exploration in this area. The adverse consequences associated with synthetic products have prompted a shift towards biological control mechanisms over chemical antimicrobials in recent years. Naturally occurring antimicrobial compounds present in basic food items play a crucial role in preventing food material deterioration(Balaji *et al.*, 2014). The rise of bacterial strains exhibiting reduced susceptibility to antibiotics and multi-drug resistance underscores the urgency for innovative approaches to combat infections. Plant-based antimicrobials offer a safer alternative with fewer side effects compared to synthetic counterparts. Moreover, they demonstrate promising therapeutic potential in managing various infectious diseases, highlighting their significance in contemporary healthcare practices (Anitha *et al.*, 2018).

Gloriosa superba Linn, commonly known as the climbing lily, belongs to the Colchicaceae family. It is characterized by its perennial herbaceous nature, climbing to heights of up to five meters(Senthilkumar *et al.*, 2013). Emerging from a single fleshy, cylindrical tuber with a V-shaped rhizome, the plant can produce one to four stems. During its flowering period, the perianth segments undergo a striking transformation, with accrescent growth and reflexed orientation. These segments exhibit a captivating coloration, with yellow hues at the borders and proximal regions, complemented by dark red tones in the median area. Originally native to tropical Africa, *Gloriosa superba* now thrives in various regions of tropical Asia, including Malaysia, Burma, India, and Sri Lanka, where it grows abundantly in unmanaged habitats(Nikhila *et al.*, 2014).

Gloriosa superba Linn, known as the climbing lily, holds significant medicinal value in traditional and conventional medicine. Its rhizome and seeds serve as sources of colchicine, a compound utilized in the treatment of gout(Chitra *et al.*, 2009). Additionally, the plant's leaf juice exhibits antimosquito properties and is employed as a remedy for head lice. Moreover, it finds application in the preparation of arrow poisons due to its toxic properties. In certain cultures, the rhizomes of *Gloriosa superba* are believed to possess antidotal properties against snakebites and are placed on windowsills to ward off snakes(Gulcin *et al.*, 2011). Beyond its medicinal utility, the plant holds cultural significance, with many cultures attributing magical characteristics to it. Overall, *Gloriosa superba* embodies a multifaceted botanical resource, offering both medicinal and cultural value across various traditions and practices(Janani *et al.*, 2014).This study aims to comprehensively explore the phytochemical composition and bioactive potentials, specifically focusing on antioxidant and antibacterial activities, in Gloriosa superba (Adavinabhi) rhizomes. Gloriosa superba, commonly known as flame lily, is renowned for its medicinal properties in traditional systems of medicine. However, a detailed investigation into its phytochemical constituents and biological activities,

particularly its antioxidant and antibacterial properties, is lacking.Furthermore, the antioxidant potential of the extracts will be evaluated through assays like DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay. The antibacterial activity will be assessed against a panel of pathogenic bacteria using well-established methods. The findings of this study will provide valuable insights into the phytochemical profile of Gloriosa superba rhizomes and their potential applications in pharmaceutical and nutraceutical industries.

Material and methods

Sample collection

The rhizomes of *Gloriosa superba* were acquired from the Eturnagaram Wildlife Sanctuary situated in the Mulugu District of Telangana State. Following procurement, the rhizomes underwent a shade-drying process at ambient room temperature for a duration of fifteen days until achieving a uniform weight. Subsequently, the dried rhizomes were pulverized and sieved through a 0.3mm mesh screen. The resulting material was carefully stored in an airtight and sterile container to maintain its integrity and purity.

Extraction

The sequential extraction process was carried out using a Soxhlet apparatus. Initially, 25 grams of *Gloriosa superba* rhizomes were enclosed within Wattman No.1 filter papers, compacted, and inserted into a Soxhlet thimble. Each round-bottom flask was filled with 250 milliliters of solvent, and the rhizomes were sequentially subjected to extraction using Petroleum ether, Ethyl acetate, Chloroform, and Methanol. The extraction temperatures were adjusted to correspond to the boiling points of the respective solvents. Upon completion of extraction, the resulting extracts underwent cooling and filtration through Wattman No.1 filter papers. Subsequently, the solvents were removed using a rotary evaporator, resulting in the production of the crude extract.

Screening of phytochemical analysis of Gloriosa superba with suitable solvents

Qualitative method

Test for alkaloids

The procedure involved dissolving a sample in a diluted hydrochloric acid solution, followed by filtration of the resulting solution. Subsequently, Wagner's reagent, comprising 2 grams of iodine and 6 grams of potassium iodide dissolved in 100 milliliters of water, was added to the filtrate. The observation of a reddish-brown residue indicated the potential presence of alkaloids.

Test for anthraquinones

To initiate the process, a blend of 0.2 grams of the sample and 2 milliliters of chloroform underwent vigorous agitation for a duration of 5 minutes, followed by extraction. The resulting filtrate was then meticulously mixed with a 10% ammonia solution. The presence of anthraquinones was indicated by the emergence of a vivid pink coloration within the aqueous layer of the mixture.

Test for flavonoids

The procedure began with mixing 0.2 grams of the sample and 2 milliliters of chloroform, which were vigorously agitated for 5 minutes before undergoing extraction. Subsequently, the resulting filtrate was thoroughly combined with a 10% ammonia solution. The appearance of a bright pink coloration within the aqueous layer of the mixture signalled the presence of flavonoids.

Test for phenols

To conduct the test, introduce three or four drops of a 10% ferric chloride solution into a test tube containing 0.5 grams of the sample. The observation of the sample turning bluish-black indicates the potential presence of phenols.

Test for saponins

After boiling 2 grams of the sample in 20 milliliters of distilled water for 5 minutes, the sample was strained using a test tube. Subsequently, to induce continuous foaming, 10 milliliters of the filtrate were vigorously

mixed with 5 milliliters of distilled water in a graduated cylinder. Following vigorous agitation, the mixture was left to stand for fifteen minutes. After this period, three or four drops of olive oil were introduced while shaking the froth. The formation of an emulsion layer confirmed the presence of saponins.

Test for tannins

The substance was combined with a 1% gelatin solution containing 10% sodium chloride (0.5 grams). The presence of tannins was verified by the formation of a white precipitate.

Test for phytosterols

A portion of the substance was blended with chloroform and subsequently filtered. To the resulting filtrate, a few drops of acetic anhydride were introduced, followed by boiling and subsequent cooling. Upon tilting the test tube, the addition of concentrated sulfuric acid resulted in the formation of a brown ring at the junction, potentially indicating the presence of phytosterols.

Quantification of phytochemical content from Gloriosa superba extract

Total phenolic contents

We employed the Folin-Ciocalteu method to quantitatively determine the overall phenolic content using spectrophotometry. The preparation of the extracted sample involved mixing the following materials: 2 milliliters of 20% sodium carbonate solution, 0.1 milliliters of plant extract, 2.8 milliliters of deionized water, and 0.1 milliliters of 50% Folin-Ciocalteu reagent. Subsequently, the absorbance of the reaction mixture was measured at a wavelength of 750 nm after incubating at room temperature for 30 minutes. Gallic acid (GA) served as the reference compound for the standard curve, and the total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight.

Total flavonoid content

The quantification of the materials under investigation was conducted using the aluminum chloride colorimetric method to assess the flavonoids. A solution was prepared by combining 1.5 milliliters of 95% ethanol, 0.1 milliliters of 10% aluminum chloride hexahydrate, 0.1 milliliters of 1M potassium acetate, 2.8 milliliters of distilled water, and 0.5 milliliters of the extract. After a 40-minute incubation period at room temperature, the absorbance of the resulting mixture was measured at a wavelength of 415 nm. The flavonoid content was quantified as milligrams of quercetin equivalency (QE) per 100 grams of dry weight, with quercetin serving as the reference flavonoid for establishing the standard curve.

Determination of proanthocyanins content

To establish a calibration curve, test tubes were filled with varying volumes (0.1, 0.2, 0.3, 0.4, and 0.5 milliliters) of catechin, which were then diluted with ethanol. In the sample solution, 0.5 milliliters of the sample was mixed with 1.5 milliliters of hydrochloric acid and 3 milliliters of a methanol solution containing 4% vanillin. The resulting mixture underwent refluxing, followed by thorough blending. After allowing the mixtures to stand undisturbed at room temperature for 15 minutes, absorbance measurements were taken at a wavelength of 500 nm. The total proanthocyanin content was quantified and reported in milligrams per gram of catechin equivalent using the calibration curve.

Methodology of GCMS analysis:

The methanolic extract of *Gloriosa superba* L underwent GC-MS analysis at the Center Analytical Facility of the University College of Technology, Osmania University, following specific parameters outlined by Kumaravel *et al.* (2010). The analysis utilized a GCMSQP2010 SHIMADZU instrument interfaced with gas chromatography equipped with an Elite-1 silica capillary column (30 mm x 0.25 mm ID x M µM df composed of 100% Dimethylpolysiloxane), operating in electron impact mode at 70 eV. Helium (99.99%) was employed as the carrier gas at a split ratio of 10:1, with an injection volume of 0.5 µl and a constant flow rate of 1 ml/min. The injector and ion source temperatures were set at 250°C and 280°C,

respectively. The temperature program for the oven began at 110°C, followed by a 10°C per minute increase to 200°C, then a 5°C per minute decrease to 280°C, and concluded with a 9-minute isothermal period at 280°C. Mass spectra were acquired with a 0.5-second scan interval at 70 eV, yielding mass spectra ranging from 45 to 450 Da. The GC analysis typically lasted for three and a half hours. Following analysis for various components after dilution in methanol, the plant extract underwent filtration using a polymeric solid-phase extraction (SPE) column, as described by Priya *et al.* (2011).

Antibacterial activity by using Gloriosa superba rhizome extract

Nutrient agar plates were prepared, and laboratory cultures of pathogenic bacteria, including *Pseudomonas fluorescens* (MTCC 9768), *E. coli* (MTCC 424), *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC 272), and *Bacillus subtillis* (MTCC 3053), were evenly distributed on the agar plates. Subsequently, activated samples were applied using the paper dip method, and the plates were then incubated for 24 hours. Following the incubation period, any clear zones indicating bacterial inhibition surrounding the samples were observed, measured, and recorded. Samples demonstrating antibacterial activity were selected for further investigation, as described by Sivakumar *et al.* (2015).

(2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity assay

The DPPH free radical scavenging activity of the samples was evaluated following the protocol outlined by Kibiti and Afolayan (2015). A methanol solution containing DPPH at a concentration of 0.135 millimolar was prepared. Subsequently, 1 mL of the DPPH solution was mixed with 1 mL of each plant component or reference medication (BHT, Vitamin C) at concentrations ranging from 5 µg/mL to 80 µg/mL. Additionally, a control sample containing only the DPPH solution and methanol was prepared. After vortexing the mixture, it was allowed to stand at room temperature in the dark for half an hour. The absorbance at 517 nm was then measured using a spectrophotometer filled with methanol. The entire experiment was conducted in triplicate. The scavenging ability of the plant fractions and the standard was determined using a specific equation.

$$\text{DPPH Scavenging activity (\%)} = \left\{ \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \right\} \times 100$$

Total antioxidant capacity (phosphomolybdenum assay)

The Total Antioxidant Capacity (TAC) of the plant fractions was evaluated using the phosphomolybdenum technique, following the methodology outlined by Olugbami *et al.* (2015). In brief, a reagent solution comprising 3 mL of 0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate was combined with 0.3 mL of each solvent fraction and standard drug. The concentrations of the standard drug varied from 25 µg/mL to 400 µg/mL, and the resulting mixture was transferred into test tubes. These test tubes were then covered and incubated for ninety-five minutes at 95°C in a water bath. Subsequently, the absorbance at 695 nm was measured after allowing the samples to cool to room temperature. For the control mixture, distilled water was utilized in place of the samples, and common medications like gallic and ascorbic acid were included. Higher absorbance readings indicated a greater potential for total antioxidants

$$\text{The percentage inhibition (\%)} = \left\{ \frac{(\text{absorbance of sample} - \text{absorbance of control})}{(\text{absorbance of sample})} \right\} \times 100$$

Results and discussion

Qualitative analysis of phytochemical content from Gloriosa superba extract:

The biological activity of certain plant extracts can be attributed to the presence or absence of various phytochemicals. Hexane, chloroform, and methanol were utilized to extract the consecutive Soxhlet extract of the seeds and tubers in ascending order of polarity.

Table 1. Phytochemical analysis of *Gloriosa superba* Rhizome.

S. No.	Phytochemicals	Results
1	Alkaloid	Present
2	Anthraquinones	Present
3	Flavonoids	Present
4	Phenols	Present
5	Saponins	Absent
6	Tannins	Present
7	Phytosterols	Present

According to preliminary phytochemical screening conducted by Senthilkumar (2013), the extracts contain tannins, terpenoids, steroids, glycosides, and alkaloids. Further analysis of the bioactive chemicals found in *Gloriosa superba* rhizomes revealed the presence of alkaloids, anthraquinones, flavonoids, phenols, tannins, phytosterols, and coumarins, while saponins were absent.

Table 2. Phyto-components identified from methanolic extracts of *Gloriosa superba* rhizome by GC-MS analysis

Peak	R.Time	Area%	Name
1	1.047	0.01	2-Butynoic acid
2	1.146	0.01	Butane, 2-methyl-
3	1.294	0.32	Pentane, 2-methyl-
4	1.331	5.33	Pentane, 3-methyl-
5	1.383	31.78	cis-2,3-Epoxyoctane
6	1.472	62.36	S-Methyl-l-cysteine
7	25.776	0.07	Tetratriacontane
8	27.166	0.07	Nonacosane
9	28.486	0.05	Docosane

Based on numerous phytochemical tests, it was determined that the plant contains a wide range of biologically active compounds that could potentially serve as a source of raw pharmaceuticals to complement traditional treatment methods (Banu and Nagrajan, 2012). Additionally, a study conducted by Venkatachalam MR *et al.* in 2010 found that the extract obtained from the shoots and flowers of Gloriosa superba did not contain any terpenoids or saponins, in contrast to Solanum torvum. On the other hand, the tuber extract from sweet potatoes, Asparagus racemosus, elumpotti, Ormocarpum cochinchinense, Datura, Camellia sinensis, and Guazuma ulmifolia showed positive results for terpenoids, alkaloids, saponins, and phenols (Muthukrishnan, S.D *et al.*, 2012). Rehana B *et al.* (2012) reported on the phytochemical analysis of Gloriosa superba methanol tuber extract, and the current study nearly confirmed the earlier findings, with the exception of phenolics. All of the phytochemicals detected in this study are present in the prior study, with the exception of alkaloids.

Table 3. Zone of inhibition of *Gloriosa superba rhizomea extracts* in methanol.

Bacteria name	Ampicillin	*Gloriosa superba* in methanol
	Zone of inhibition in mm	
E. coli (MTCC 424)	12	10
Klebsiella pneumoniae (MTCC 272)	13	10
Bacillus subtillis (MTCC 3053)	11	11
Staphylococcus aureus (MTCC 96)	9	9
Pseudomonas fluorescens (MTCC 9768)	10	8

Quantification of phytochemical content from Gloriosa superba extract

Various solvent extraction techniques were employed to quantify important phytochemicals such as total phenolic concentration, flavonoids, and proanthocyanins. Petroleum ether, ethyl acetate, chloroform, and methanol solvents were utilized for this purpose.

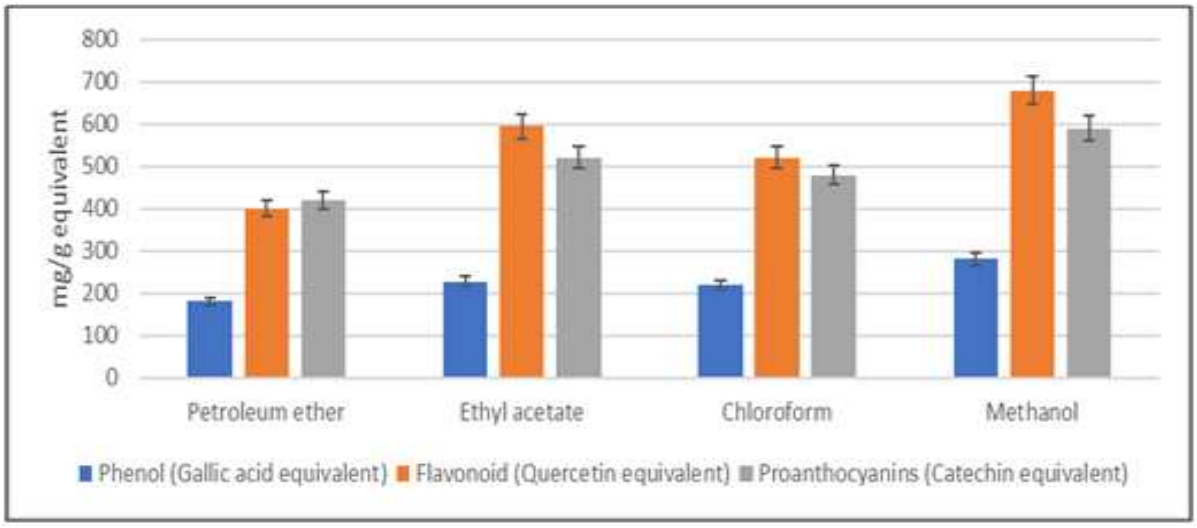

Fig. 1. Quantification of phytochemical content from gloriosa superba rhizome extract with different solvents.

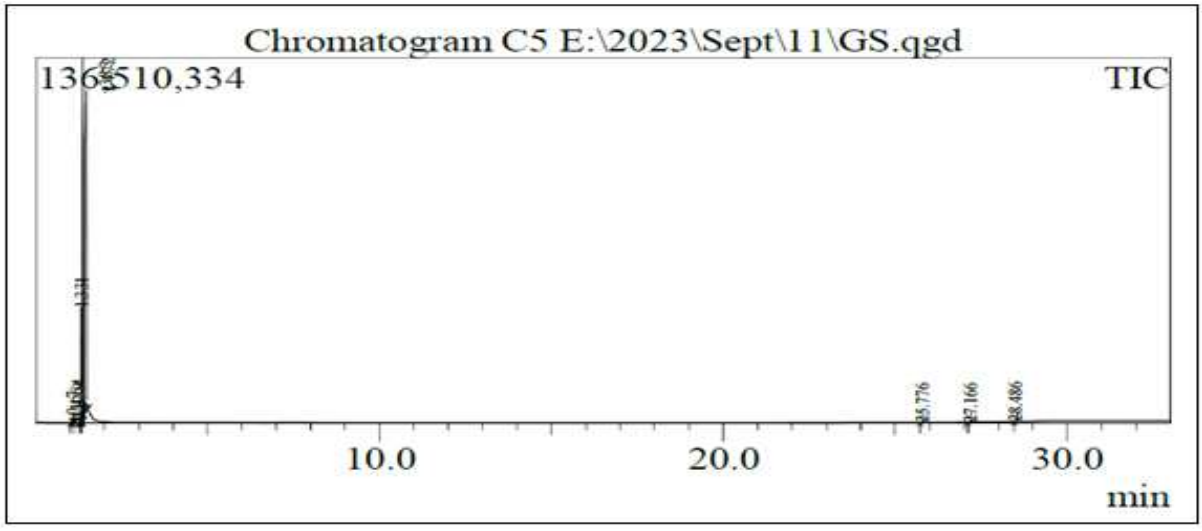

Fig. 2. GC-MS chromatogram of methanol extract of *Gloriosa superba* rhizome.

The results revealed that the phytochemicals extracted from methanol showed the highest concentrations (Figure 1). Specifically, the total phenolic concentration from methanol extraction was found to be 280 mg/g, while flavonoids and proanthocyanins were found to be 660 mg/g and 580 mg/g, respectively. The second-highest concentrations were observed in the ethyl acetate solvent extracts, with 220 mg/g of phenolic compounds, 600 mg/g of flavonoids, and 520 mg/g of proanthocyanins. In contrast, the extracts from petroleum ether and chloroform exhibited lower quantities of the phytochemicals. Specifically, phenolic compounds were found at concentrations of 190 mg/g and 220 mg/g, flavonoids at 400 mg/g and 520 mg/g, and proanthocyanins at 420 mg/g and 480 mg/g, respectively.

Similar studies conducted by Muthukrishnan S.D. *et al.* (2012) and Nadkarni KM *et al.* (2011) have indicated that Gloriosa superba seeds comprise phenolics, steroids, alkaloids, flavonoids, terpenoids,

and carbohydrates. Additionally, terpenoids, steroids, alkaloids, flavonoids, and carbohydrates were identified in G. superba leaves. Furthermore, the tubers of *Gloriosa superba* were found to contain minerals, sugars, alkaloids, flavonoids, vitamins C and E, phenols, glycosides, and saponins (Nikhila, G.S. *et al.*, 2014; Senthilkumar, M. *et al.*, 2013). Shanmugham H. *et al.* (2009) reported that *Gloriosa superba* leaves and tubers exhibit a variety of chemical groups, including alkaloids, flavonoids, glycosides, saponins, steroids, phenols, and tannins. Moreover, these studies collectively highlight that the *Gloriosa superba* plant harbors numerous physiologically active compounds, indicating its potential as a source of crude pharmaceuticals to complement traditional medical treatments in the future.

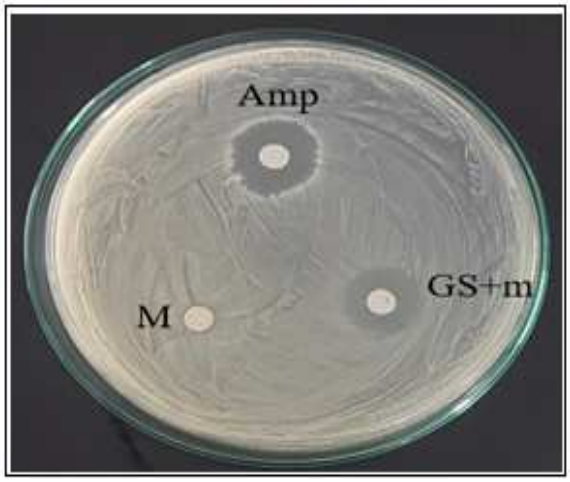

Fig. 3. Antibacterial activity of *Gloriosa superba* in *E. coli* (MTCC 424).
Amp: Ampicillin, GS+M: *Gloriosa superba* in methanol and M: methanol

In a study conducted by Paul John Peter *et al.* (2012), a tabulated analysis of the chemical name, molecular formula, molecular weight, and peaks of unknown compounds from the GC-MS spectrum was undertaken. The *Gloriosa superba* rhizome methanol extract was found to contain nine different chemicals. The primary constituents identified in the methanol extract of *Gloriosa superba* rhizome were S-Methyl-l-cysteine (62.36%), cis-2,3-Epoxyoctane (31.78%), Pentane, 3-methyl- (5.33%), and Pentane, 2-methyl- (0.32%).This analysis represents the initial step in elucidating the composition of active ingredients in this medicinal plant, highlighting the significance of GC-MS analysis in such research endeavors.

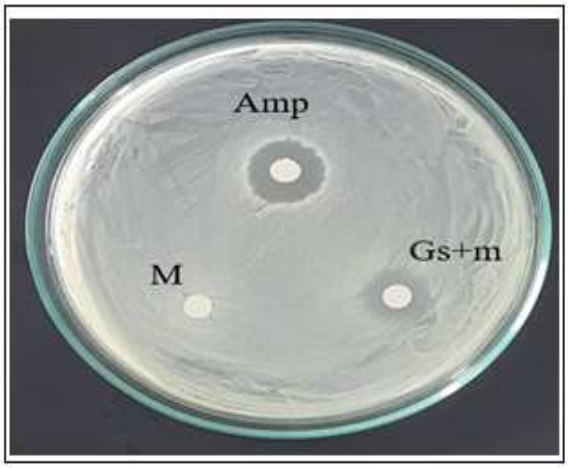

Fig. 4. Antibacterial activity of *Gloriosa superba* in *Klebsiella pneumoniae* (MTCC 272).
Amp: Ampicillin, GS+M: *Gloriosa superba* in methanol and M: methanol

The findings suggest avenues for further investigation into the pharmacological significance, diversity, and detailed phytochemistry of *Gloriosa superba* rhizomes.

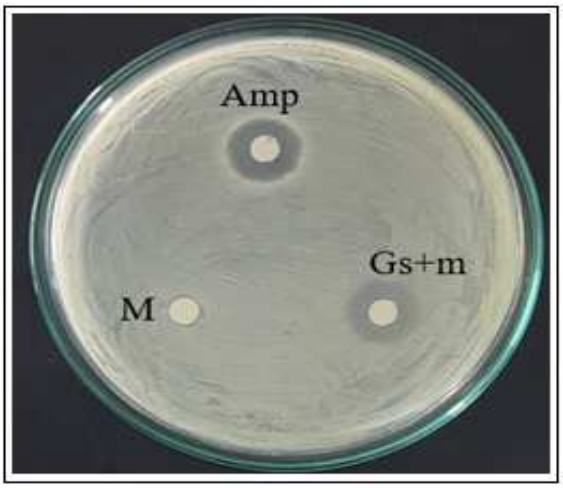

Fig. 5. Antibacterial activity of *Gloriosa superba* in *Bacillus subtillis* (MTCC 3053).
Amp: Ampicillin, GS+M: *Gloriosa superba* in methanol and M: methanol.

Our investigations (Kala, C. *et al.*, 2004) revealed through GC-MS analysis of pharmaceuticals the presence of a diverse array of phytochemicals, notably alkaloids and other structurally recognized compounds. Previous research conducted by Banu H. *et al.* (2012) utilized GC-MS analysis to examine the

leaves of *Gloriosa superba*, identifying eight distinct chemicals. Similarly, Abhishek M. *et al.* (2011) documented the phytochemical substances present in the rhizomes of Nervilia aragoana using GC-MS analysis. Insights from studies by Bhakuni, D.S. *et al.* (2009) and Elgayyar, M. *et al.* (2001) suggest that the phytochemicals identified through GC-MS possess diverse biological functions. These findings hint at the potential to develop potent medications for the treatment of various serious illnesses leveraging the phytochemicals sourced from *Gloriosa superba*.

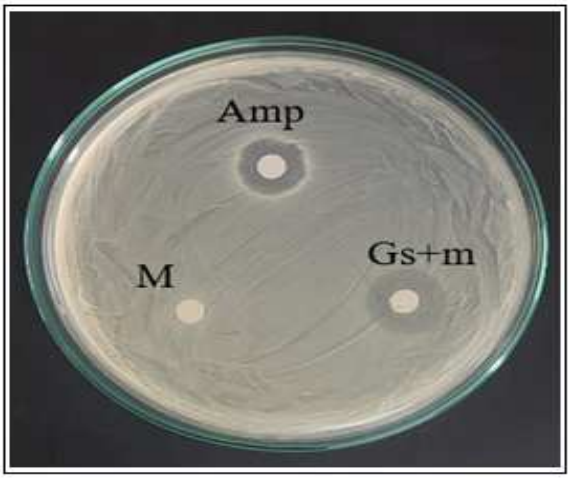

Fig. 6. Antibacterial activity of *Gloriosa superba* in *Staphylococcus aureus* (MTCC 96).
Amp: Ampicillin, GS+M: *Gloriosa superba* in methanol and M: methanol.

Antibacterial activity by using Gloriosa superba rhizome extract.

The antibacterial activity of rhizome extracts in organic solvents such as methanol was observed. Combining these extracts with antibiotics like Ampicillin resulted in extended antibacterial activity. Notably, the rhizome extract of *Gloriosa superba* in methanol exhibited increased antibacterial effects compared to methanol alone. The maximum zone of inhibition (13±0.22 mm) was observed with Ampicillin, followed by the rhizome extract of G. superba (10±0.22 mm). Figure 5-9 illustrates the confirmed maximum area of inhibition by the *Gloriosa superba* methanol extract. This enhanced activity may be attributed to the ability of secondary metabolites to form complexes with external proteins and the microbial cell wall (Trease, S.E. *et al.*, 1983). *Gloriosa superba's* inhibitory effect on bacterial growth in methanol extract may stem from increased availability of these secondary metabolites, which are known to contain a wide variety of antibiotic compounds (Ikigai H *et al.*, 1993).

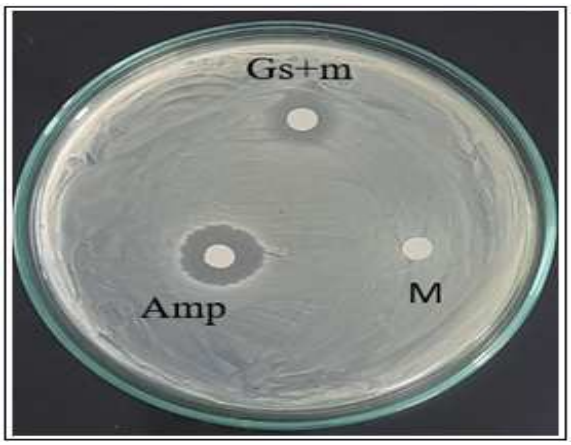

Fig. 7. Antibacterial activity of *Gloriosa superba* in *Pseudomonas fluorescens* (MTCC 9768).
Amp: Ampicillin, GS+M: *Gloriosa superba* in methanol and M: methanol

The stronger antibacterial effect of *Gloriosa superba* in methanol extract compared to methanol alone can be attributed to the polarity of the extraction solvent, its inherent bioactivity, and its ability to diffuse in the assay media (Jigna PA *et al.*, 2005; Sivakumar T *et al.*, 2015). Consequently, various phytochemical compounds abundant in different parts of the plant offer herbal protection against microbial infections. Phytochemical analysis of the tuber, flower, and shoot extracts revealed the presence of proteins, amino acids, carbohydrates, reducing sugars, xanthoproteins, alkaloids, flavonoids, terpenoids, tannins, and saponins, alongside aromatic acids and phenolic compounds. Hence, the antibacterial activity of *Gloriosa superba* in methanol extract may be attributed to these phytochemical compounds.

The antibacterial potential of the phytochemicals extracted from *Gloriosa superba* rhizomes has shown notable effectiveness, particularly against *Escherichia coli*, studies investigating the extracts from G. superba have unveiled impressive antibacterial properties against a spectrum of bacteria including *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas fluorescens*.

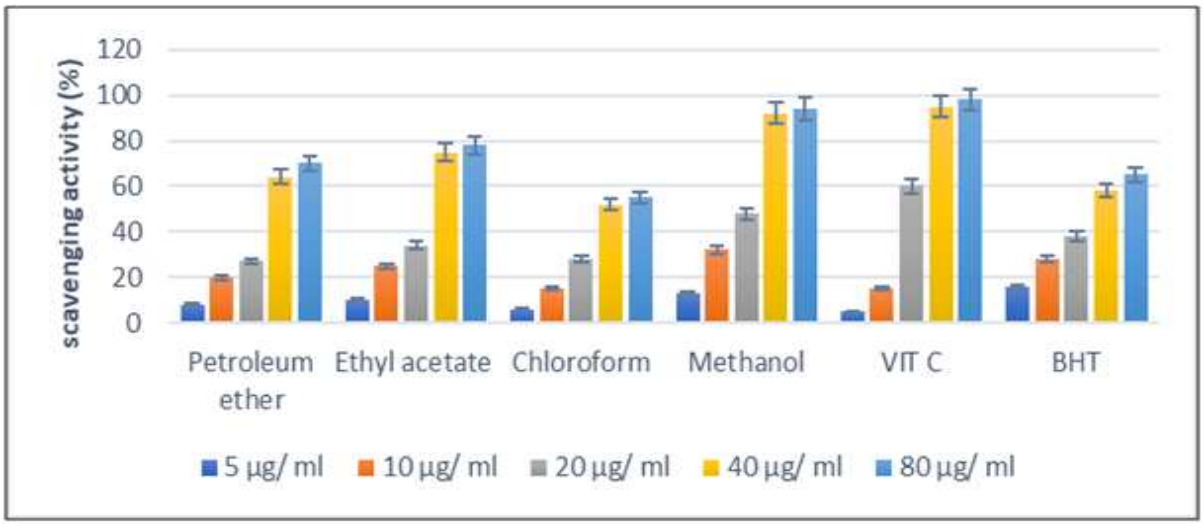

Fig. 8. 2,2 Diphenyl-1-picrylhydrazyl radical scavenging activity of gloriosa superba rhizome extract.

These findings underscore the broad-spectrum antibacterial activity of G. superba extracts, suggesting its potential as a natural antimicrobial agent against various bacterial strains. The observed efficacy against both gram-negative and gram-positive bacteria further highlights the promising therapeutic applications of *Gloriosa superba* in combating microbial infections. Such research not only elucidates the diverse antibacterial effects of G. superba but also emphasizes its significance in the development of novel antimicrobial treatments.

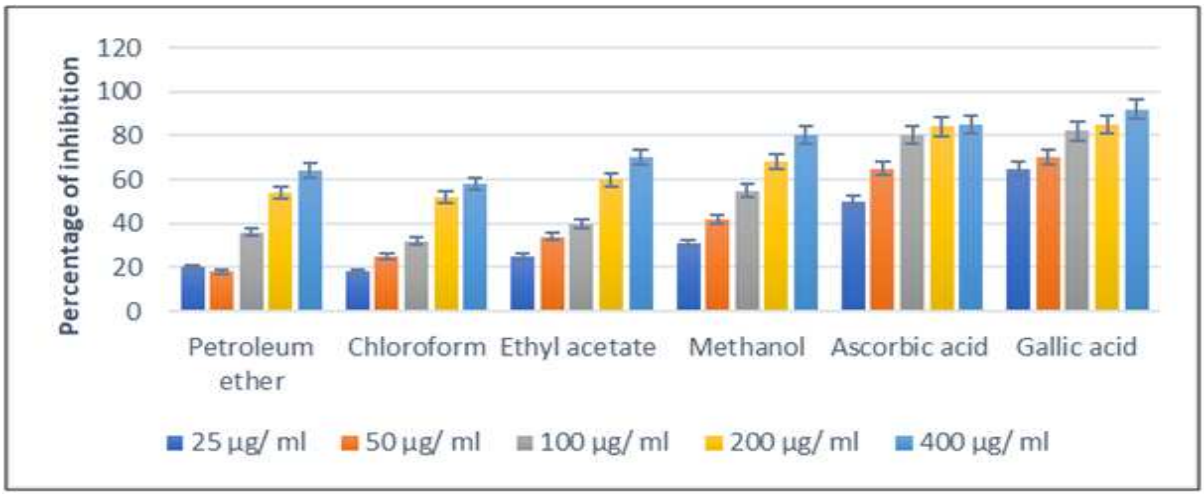

Fig. 9. Total antioxidant capacity of gloriosa superba rhizome extract.

(2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity assay

Various organisms rely on oxidation to generate energy for essential biological functions (Gulcin *et al.*, 2010). During the process of hydrogen or electron donation, the stable nitrogen-centered free radical DPPH turns yellow when reduced. Substances capable of carrying out this reaction are termed antioxidants, acting as radical scavengers (Lavanya *et al.*, 2010). Figure 2 illustrates the results of assessing the antioxidant capacity of G. superba rhizome extract using the DPPH anti-scavenging method. Notably, vitamin C exhibited the highest DPPH anti-scavenging activity (98% at 80µg/ml), surpassing the methanolic extract (95% at 80µg/ml), ethyl acetate (78% at 80µg/ml), BHT (65% at 80µg/ml), petroleum ether (75% at 80µg/ml), and chloroform (55% at 80µg/ml) extracts (Figure 2). Except for chloroform, all extracts demonstrated higher DPPH anti-scavenging values, suggesting that alcoholic extracts

may be more suitable for various medicinal antioxidant applications.

Total antioxidant capacity (phosphomolybdenum assay)

Using the phosphomolybdate method, the total antioxidant capacity of the various extracts was determined and expressed as equivalents of gallic acid (percentage of extract) at a 90% sample concentration. Gallic acid (90%) exhibited the highest level of antioxidant activity, followed by ascorbic acid (82%), methanol (80%), ethyl acetate (75%), and petroleum ether (62%). The lowest level of antioxidant activity was observed in chloroform (58%), with the levels decreasing in the following order: gallic acid > methanol > ethyl acetate > petroleum ether > chloroform.

Conclusion

In conclusion, this study comprehensively examined the qualitative and quantitative phytochemical composition of *Gloriosa superba (Adavinabhi)* rhizomes, revealing a diverse array of bioactive compounds. Significantly high levels of alkaloids and flavonoids were detected, indicating the plant's potential therapeutic value in traditional medicine and pharmaceutical contexts. The GC-MS analysis of *Gloriosa superba* rhizomes revealed the presence of nine distinct bioactive chemicals, each with varying therapeutic potentials. The isolation and utilization of these bioactive compounds hold promise for the development of novel medications with significant therapeutic efficacy. Furthermore, the notable antimicrobial activity against a spectrum of bacteria and fungi suggests *Gloriosa superba* rhizomes' potential as a natural source of antimicrobial agents. These findings warrant further research aimed at isolating and characterizing specific bioactive compounds responsible for these activities. Such efforts could elucidate the mechanisms of action, conduct toxicity assessments, and explore potential synergies with existing medications. Investigation into the biological activities of *Gloriosa superba* rhizomes demonstrated promising antioxidant and antimicrobial properties. The potent antioxidant activity, evidenced by effective scavenging of DPPH and ABTS radicals, highlights the plant's capacity to counteract oxidative stress, particularly relevant in various health conditions where oxidative damage is implicated.Integrating traditional knowledge with scientific evidence may facilitate *Gloriosa superba's* incorporation into mainstream healthcare, promoting sustainable and holistic approaches to health and wellness.

Conflict of interest

The authors declare no conflict of interest to report regarding this research work.

Data availability

All data generated or analysed during this study are included in this article.

Ethical approval

The author confirms that there are no ethical issues in the publication of the manuscript.

Human and animal rights

No animals/humans were used for studies that are the basis of this research.

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