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**Research Article**

# *IN-VITRO* ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF SUPERCRITICAL FLUID EXTRACT OF LEAVES OF *GLORIOSA SUPERBA* LINN

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**A R T I C L E I N F O**

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**A B S T R A C T**

The antioxidant and antidiabetic activity of supercritical CO2 extracts of *Gloriosa superba* Linn leaves obtained from plants grown in different areas of Satara. The antioxidant activity was made by the different assay DPPH, nitric oxide scavenging activity methods and *In vitro* A-Amylase Inhibitory Assay. The supercritical extracts of dried leaves of *Gloriosa superba* Linn (100-1000µg/ml) exhibited the highest antioxidant activity.



## INTRODUCTION

Nature has proved to be an inexhaustible source of remedies for various ailments. Natural products from plant, animal and mineral origin have been the basis of treatment, such traditional medicine is still being practiced widely today in India and across the globe. Due to their safe, effective and inexpensive nature, indigenous remedies are popular among the people of both urban and rural area. About 80% of the world population is still dependent on the traditional medicines.[1]

The knowledge of certain herbs, animals and minerals that have curative and palliative effects were transmitted from one generation to another and it is the outcome of bold experimentation through trial and error method over hundreds of years. Ethnomedicine is the mother of all other systems of medicine such as Ayurveda, Siddha, Unani, Nature cure and even modern medicine. The traditional herbalists are part and parcel of the community and are often familiar with the details of each family and its environs, so that they are in a better position to deal with their day-to-day problems. In fact the native healers take care of the common ailments of the folk in their home setting.[2]

*Langali,* a drug of herbal origin, has been used by the *Ayurvedic* physicians, for the management of different disease conditions. The botanical source of the plant is *Gloriosa superba* Linn. of the family liliaceae.

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It is a climber with very attractive or glorious flowers hence the name as such. It is a branched herbaceous climber common in low jungles almost throughout India upto an altitude of 6000 feet and in Andaman Islands. Texts of *Ayurveda* systematically recorded the part use, pharmacological properties, actions, indications and contraindications of the plant drug. Its use as an ingredient of compound formulations was recorded in *samhitas* (treatise) as well as *samgraha granthas* (compendia of *Ayurveda*).[3]

*Gloriosa superba* derives its name *Gloriosa* from the word "Glorious", which means handsome and *superba* from the word "superb "Means splendid or majestic kind. This plant has been a source of medicine right from the ancient time. So many books and articles have been written so far on the medicinal and other values of this plant. It is one of the most popular herbs. *Gloriosa superba* also known as Glory lily belongs to the family Liliaceae and is known by various vernacular names. In Hindi it is known as Karihari, Languli; In English, Glory Lily; In Kanada, Gowrihoo, Akkutangiballi; In Sanskrit, Langali, Visalya; In Tamil, Kalappai killanku, Nabhik kodi. It is an herbaceous, climbing perennial, growing between 3.5 to 6m in length, but usually trained at 1.5m above ground level.[4]

A common metabolic disorder known as Diabetes mellitus with micro-and macrovascular complications those results in significant morbidity and mortality. It is considered as one of the cause among five leading causes of death in the world. Diabetes is in the top five of the most significant diseases in the developed world and is still gaining significance. Since the existing drugs for the above said disorders encounter many

side effects and need for prolonged treatment including questionable efficacy in the treatment, these reasons force the area of research to find improved treatments which will counteract the side effects and drawbacks of the existing treatment. Herbal drugs are having diversified uses are always an alternative option to the synthetic drugs which are well known for their side and adverse effects. Hence under these conditions exploring new cures from plants source will always be beneficial because of less side effects. On the above facts the objective of this study to evaluate *Gloriosa superba* a possible cure to above said disorders.[5]

## MATERIAL AND METHODS

The leaves of *Gloriosa superb Linn*. were collected from Kas plateu and The plant authentication was done by botanical survey of India, Pune.

### *SC CO2 Extraction*[6]

SC CO2 extraction was conducted in SFXTM -220 (ISCO) extractor system consisting of a 10 mL extractor vessel, a restrictor, an extractor temperature controller (SFXTM -200 controller), and a restrictor temperature controller (ISCO). The SC CO2 set-up includes a pump for CO2 (ISCO syringe pump model 260D) and a chiller (cooling unit Hubbler-chiller control). The temperature and pressure of the extraction system were adjusted and controlled at the desired conditions. The CO2 flow rate was automatically adjusted with the temperature and pressure setting. *Gloriosa superba Linn* powder (~5 g) was loaded into the extraction vessel at each experiment for 3 h. The vessel was then placed in the heating chamber to maintain the operating temperature.

### *Antioxidant Activity*[5]

### *DPPH radical scavenging activity*

The ability of Compounds to scavenge DPPH radical was assessed using S. Ganapaty 2007 *et al* method with modification. Briefly, 1 ml of Different forms of extracts at concentration of 100, 200, 400, 600, 800, 1000µg/ml was mixed with 3.0 mL DPPH (0.5 mmol/L in methanol), the resultant absorbance was recorded at 517 nm after 30 min. incubation at 37$^{O}$C. The percentage of scavenging activity was derived using the following formula, Percentage of inhibition (%) = [(A control – A sample) / A control] x 100
Where A control - absorbance of DPPH

A sample - absorbance reaction mixture (DPPH with Sample).

### *Nitric oxide scavenging activity*

Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of the extract (250–2500 mg/ml) dissolved in methanol and incubated at 25°C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilic acid, 2% phosphoric acid and 0.1% N-1-naphthylethylenediaminedihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilic acid and subsequent coupling with N-1-naphthylethylene diaminedihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to standard.

### *In Vitro A-Amylase Inhibitory Assay*[7]

The 0.5 mg/ml enzyme solution was prepared by dissolving α -amylase in 20 mM phosphate buffer (pH 6.9). The colorimetric reagent is 3, 5 -di nitro salicylic acid (DNS) was prepared by dissolving 1 g of DNS, 30 g of sodium potassium tartarate and 20 ml of 2 N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water. A 1% w/v starch solution was prepared in 20 mM phosphate buffer. Different concentrations of extract (100, 200, 200,400,600,800 and 1000 µg/ml) were prepared for inhibitory assay. Similar concentrations of acarbose solutions were made as a reference standard.

Briefly, α-amylase inhibitory assay was carried out by adding 1ml of enzyme solution to test tubes containing 1ml of various concentrations SFE extract and incubated at 25°C for 10 min. After incubation, 1ml of starch solution was added to the mixture and further incubated at 25°C for 10 min. The reaction was then stopped by adding 2 ml of DNS color reagent and incubated in boiling water for 5 min. After cooling, the test tubes are diluted to 10 ml with buffer solution and the absorbance was measured on UV-visible spectrophotometer at 540 nm. Control, representing 100% enzyme activity was prepared in a similar manner, without extract. The experiments were carried out in triplicate. The % inhibition of α-amylase activity was calculated using the following formula:

% Inhibition = {(Ac - At)/Ac} x 100

Where Ac is the absorbance of the control and At is the absorbance of the test sample. From a plot of concentration against % inhibition.

## RESULTS

### *DPPH radical scavenging activity*

**Table no 1** Scavenging effect of SFE extracts of *Gloriosa Superba L* on (DPPH) free radical

| Sr no. | Extracts | Percentage inhibition | |
|---|---|---|---|
| | | Ascorbic acid | *Gloriosa Superba* L |
| 1 | 100 µg/ml | 32.26 ±1.38 | 10.13±2.17 |
| 2 | 200 µg/ml | 46.34 ±1.18 | 20.13 ±2.25 |
| 3 | 400 µg/ml | 59.54 ±1.16 | 19.32 ±1.19 |
| 4 | 600 µg/ml | 68.92 ±1.15 | 47.52 ±2.36 |
| 5 | 800 µg/ml | 75.26 ±1.08 | 59.13 ±5.13 |
| 6 | 1000 µg/ml | 82.32 ±1.10 | 77.15±2.23 |

Values expressed as mean of three replicates ± S.E.M.

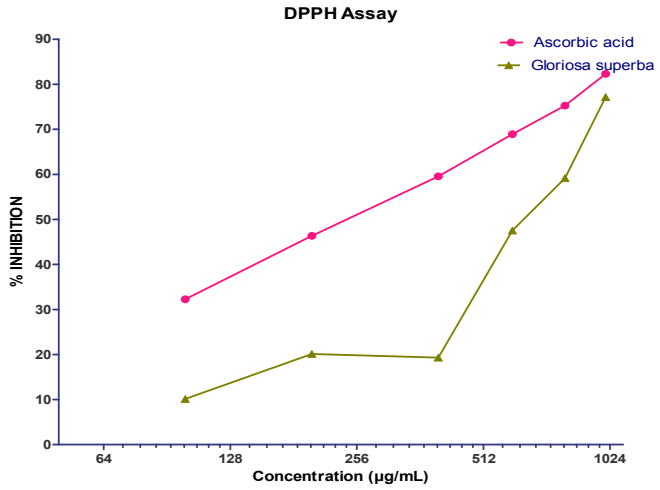


**Fig No 1** DPPH radical scavenging activity

### *Nitric oxide scavenging activity*

**Table no 2** Scavenging effect of SFE extracts of *Gloriosa Superba* L on the Nitric oxide scavenging activity

| Sr no. | Extracts | Percentage inhibition | |
|---|---|---|---|
| | | Ascorbic acid | *Gloriosa Superba* L |
| 1 | 100 µg/ml | 13.33 ±1.32 | 7.23 ±2.11 |
| 2 | 200 µg/ml | 18.26 ±1.35 | 12.15 ±2.19 |
| 3 | 400 µg/ml | 20.00 ±2.36 | 19.33 ±2.31 |
| 4 | 600 µg/ml | 12.00 ±1.28 | 30.15 ±2.30 |
| 5 | 800 µg/ml | 18.66 ±2.16 | 33.25 ±2.15 |
| 6 | 1000 µg/ml | 45.33 ±1.27 | 41.25±2.15 |

Values expressed as mean of three replicates ± S.E.M.

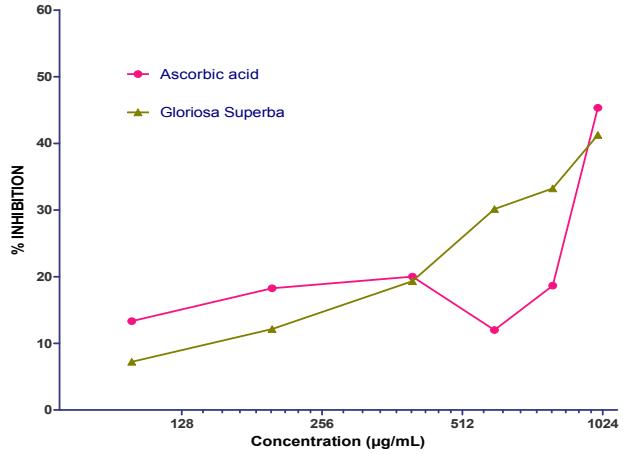


**Fig No 2** Nitric oxide scavenging activity

### *In vitro A-Amylase inhibitory assay*

**Table No 3** *In-Vitro* α-Amylase Inhibitory Assay of *Gloriosa superba* L

| Sr no. | Extracts | Percentage inhibition | |
|---|---|---|---|
| | | Acarbose | *Gloriosa Superba* L |
| 1 | 100 µg/ml | 33.36 ±2.33 | 35.15 ±2.19 |
| 2 | 200 µg/ml | 37.49 ±2.35 | 41.23 ±2.21 |
| 3 | 400 µg/ml | 48.62 ±2.36 | 46.25 ±2.31 |
| 4 | 600 µg/ml | 57.59 ±2.29 | 47.25 ±2.20 |
| 5 | 800 µg/ml | 68.50 ±2.18 | 60.12 ±2.10 |
| 6 | 1000 µg/ml | 76.26 ±2.23 | 65.21 ±2.19 |

Values expressed as mean of three replicates ± S.E.M.

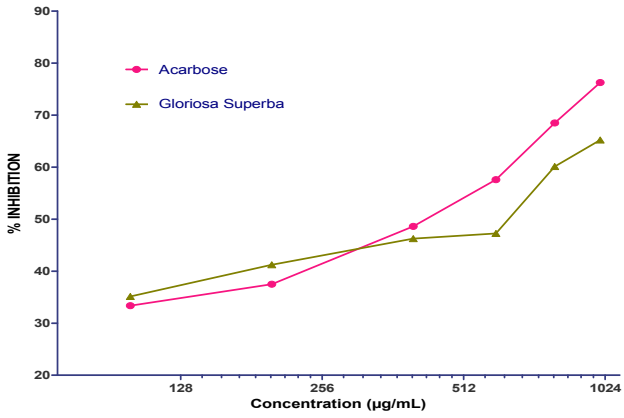


**Fig No 3** *In vitro* A-Amylase inhibitory assay

## CONCLUSION

In the present study, SFE extract of *Gloriosa superba* L proved a potential medicinal plant against free radicals. The antioxidant property of *Gloriosa superb* L was showed 77.15%, 41.25%, 65.21% for 1000ug/ml by the different assay DPPH, Nitric oxide scavenging activity methods and *In vitro* A-Amylase inhibitory assay respectively . Further, an isolation and characterization of bioactive molecules is necessary to reflect underlying mechanism.

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