**International Journal of Botany Studies**
**ISSN: 2455-541X, Impact Factor: RJIF 5.12**
**www.botanyjournals.com**
**Volume 1; Issue 4; May 2016; Page No. 13-19**

# Phytochemical and antioxidant screening of *Gloriosa superb* L. from different geographical positions of South India

**Gopi Ragupathi**

Department of Botany, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India.

**Abstract**

*Gloriosa superba* Linn. is one of the important medicinal plant now in endangered list. This plant is widely used for several ethano-medicinal purposes by tribal peoples and traditional practitioners. Seeds and tubers contain valuable alkaloids viz., colchine and colchicoside as the major constituents, which are used an antidote for snake bites, gout and rheumatism. Present study was to evaluate the phytochemicals present in leaves, flowers, seeds and tuber samples were collected from four different places of South India Preliminary phytochemical and antioxidant Screening were carried out by standard procedures. Methanol extract of all samples were evaluated for protein, starch, total sugar and total phenol. The antioxidant potential of the extracts was assessed by employing different assays such as DPPH, ABTS, and Superoxide scavenging capacities. The screening tests also were performed for the presence of the following secondary metabolites such as alkaloid, flavonoids, phenols, steroids, tannin and terpenoids. The results were revealed the presence of various classes of compounds in different parts of the plant. Among the five different geographically collected plant samples (Six accessions), the Bhavani (BHA) sample showed the maximum amount of bioactive compounds and antioxidant activities than the other samples. The result of the extract from the tuber and seed samples were yields high amount of various biologically active compounds than the leaves and flowers. These compounds could serve as potential source for traditional medicines. Further research on this plant for the specific part could be used for isolation and characterization in large scale production.

**Keywords:** *Gloriosa superba,* ABTS, DPPH, SOD, phytochemicals, enzymes and alkaloids.

## 1. Introduction

*Gloriosa superba* Linn. is an important medicinal plant belonging to the family Liliaceae. It is a semi-woody herbaceous branched climber reaching approximately 5 meters height, with brilliant wavy-edged yellow and red flowers [1]. It is one of the endangered species among the medicinal plants [2]. It is extensively scattered in the tropical and sub-tropical parts of the India. It is adapted to different soil texture and climatic variation. The plant grows in sandy-loam soil in the mixed deciduous forest in sunny positions [3]. Being native form Indian especially Southern India it is known as glory lily and climbing lily-in English. In the world market glory lily considered as rich source of colchicines and gloriosine [4]. The flower has analgesic, anti-inflammatory potential, antimicrobial, larvicidal potential, antipoxviral potential, antithrombotic potential, antitumor potential, enzyme inhibition potential, and also used in treatment of snake bite, Skin disease, respiratory disorders [5, 6, 7]. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs. Thus a search for new drugs with better and cheaper substitutes from plant origin is a natural choice. The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on human body [8]. Different parts of *G. superba* have wide variety of uses especially in traditional system of medicine. The tuber is used for the treatment of bruises and sprains, colic, chronic ulcers, haemorrhoids, cancer, impotence, nocturnal seminal emission, and leprosy and also for including labour pains and abortions [9]. *Gloriosa superba* also used in wounds, skin related problems, Fever, Inflammation, piles, blood disorders, Uterine contractions, General body toner, Poisoning[10]. Roots are acrid, anthelmintic, antipyretic, bitter, digestive, expectorant, highly poisonous and promoting expulsion of the placenta. Root paste is effective against paralysis, rheumatism, snake bite and insect bites [11]. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical scavengers [12, 13]. In recent years, interest has considerably increased in finding naturally occurring antioxidants for use in food or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity [14, 15]. The present study to analyse The radical-scavenging activity (RSA) like DPPH, ABTS, Superoxide and Hydroxyl radicals against the Mehanolic extract of *Gloriosa superba* methanolic extract (whole plant) was evaluated. This plant has gained the importance in medicine in recent years for the production of colchicine in large scale [16]. The aim of this study was to identify and determine the phytochemicals and antioxidant activities present in *G. superba* Plants.

## 2. Materials and Methods

The plant samples such as leaves, flowers, seeds and tubers of Gloriosa superba were collected six accessions of the different geographical positions of South India. Such as Bhavani (BHA), Nagercoil (NGL), Kottayam (KTM), Kozhikode (KZD), Bangalore (BNG) and Mandya (MDA) in South India These plants were then identified, confirmed and have been deposited in the herbarium Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu for the future reference.

**2.1. Preparation of plant extract for phytochemical and Antioxdant Screening**
Fresh leaves, flowers, seeds and tubers were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. They were ground into coarse powder by using mechanical pulveriser. All the samples, about 100 g of the powder were repeatedly extracted with methanol in a 500 mL round bottom flask with 250 mL solvent. The reflux time for each solvent was 25 cycles for complete extraction using soxhlet apparatus [17].The filtrate was collected and concentrated by using rotary evaporator under controlled condition of temperature and pressure. The extracts were concentrated to dryness to yield crude residue. These residues were stored at-20°C, used for preliminary phytochemical screening of secondary metabolites and to check the antioxidants activity used for analysis without further treatment.

**2.3 Chemicals**
ABTS, Diphenylpicryl-hydrazyl (DPPH), 2-thiobarbituric acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, EDTA, hydrogen peroxide, L-ascorbic acid, sodium hydroxide, Folin-Ciocalteu reagent, sodium carbonate and other solvents, were procured from HiMedia laboratories Pvt. Ltd, Mumbai of analytical grade.

**2.4 Phytochemical and antioxidant analysis**
Phytochemical screening were performed to assess the qualitative chemical composition of different samples of crude extracts using commonly employed precipitation and coloration reactions to identify the major secondary metabolites like alkaloids, flavonoids, glycosides, Proteins, phenolic compounds, saponins, starch, steroids, tannins and terpenoids. The phytochemical analyses were carried out using standard procedures [18, 19]. The methanol extracts of *G. superba* were screened for the presence of secondary metabolites using the procedures [20, 21].The observations were recorded for total starch [22], soluble protein [23], steroids using Salkowski test [24], flavonoids and tannins using ferric chloride test [25], alkaloids by Mayer's test [26] and proteins by Biuret test[27] and total phenol [28]. The scavenging ability of the natural antioxidants of the crude extract towards the stable free radical DPPH was measured by the method [29]. ABTS (2, 2-azino-bis-3-ethyl benzothiazoline-6-sulphonic acid) radical cation decolourisation [30]. Superoxide scavenging ability of the crude extract was assessed by the method [31].

**2.5 Screening for Alkaloids**
Meyer's reagent (potassium mercuric iodide) 1.36 gm of mercuric chloride was dissolved in 60 ml of distilled water and 5 gm of potassium iodide was dissolved in 10 ml of water. These two solutions were mixed and diluted to 100 ml with distilled water. To 1 ml of the extract, a few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids.

**2. 6 Screening for Flavonoids**
In a test tube containing 0.5 ml of extract, 5 to 10 drops of diluted HCl and small piece of ZnCl or magnesium were added and the solution was boiled for few minutes. The appearance of reddish pink or dirty brown colour indicates the presence of flavonoids.

**2.7 Screening for Steroids**
To 2.0 ml of extract, 1.0 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. A red colour produced in the chloroform layer shows the presence of steroids.

**2.8 Screening for Phenols**
To 1 ml of the extract 3 ml of distilled water followed by few drops of 10% aqueous Ferric chloride solution was added. Formation of blue or green colour indicates the presence of phenols

**2.9 Screening for Tannins**
In a test tube containing about 5 ml of the extract, a few drops of 1% solution of lead acetate was added. A yellow or red precipitate indicates the presence of tannins.

**2.10 Screening for terpenoids**
To 0.5g of the extract 2ml of CHCl3 was added. 3ml of concentrated H2SO4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

**2.11 Screening for DPPH scavenging activity**
The Crude extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the extracts, served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm.

**2.12 Screening for ABTS scavenging effects**
ABTS radical cations ($ABTS^{+}$) were produced by reacting ABTS solution (7mM) with 2.45mM potassium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the three different extracts were added to 0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm.

**2.13 Screening for Superoxide radicals scavenging activity**
Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the leaf extracts (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560nm. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm.

**2.14 Statistical analysis**
Phytochemical estimation and quantification were performed in five replicates under standard procedures to ensure consistency of all conclusions. Data of all experiments were statistically analysed and expressed as Mean ± Standard Deviation. All the statistical analyses were carried out using the SPSS statistical tool (SPSS for windows, release 16.0. SPSS Inc., Chicago, IL). The DMRT and one-way analysis of variance (ANOVA) were used to assess the differences. P values of $<0.05$ were considered as statistically significant.

## 3. Results

In the present study deals with the preliminary phytochemical screening of methanol extracts of *Gloriosa superba* samples from BHA, NGL, KTM, KZD, BNG ans MDA are presented in Tables 1,2and 3. All the samples of leaves, flowers, seeds and tubers showed the enormous occurrence of phytochemicals. The leaf samples showed the presence of proteins, phenols, tannins and terpinoides. The flowers, seeds and tubers samples showed the high content of flavonoids. Alkaloids were found in maximum amount in seed and tubers in all samples, but they were absent in the leaves and flower extracts. When compare to the all four samples collected from different places in which the BHA sample showed the maximum occurrence of bioactive compounds then NGL samples followed by KTM, KZD, BNG and MDA samples.

In summary of phytochemical analysis of *G. superba*, alkaloids and steroids were showed the maximum occurrence in BHA (90.45%, 56.67%) than NGL (87.25%, 54.25%) followed by KTM (80.45%, 51.31%) KZD (75.29%, 50.11%) BNG (85.12%, 52.76%) and MDA (76.18%, 53.17%) respectively. In moderated amount of flavonoids, terpinoids, proteins and tannins were presence in BHA (55.92%, 47.58%, 40.28%, and 36.25%) than NGL (52.47%, 45.72%, 35.22%, 35.65%) followed by KTM (50.87%, 43.14%, 33.11%, and 32.45%) KZD (48.67%, 42.10%, 33.41%, 30.66%) BNG (51.76%, 43.83%, 36.49%, 32.76%) and MDA (56.65%, 41.75%, 34.73%, 31.37%) respectively. In lowest amount of phenols were recorded in BHA (38.64%) than NGL (33.54%) followed by KTM (30.12%) KZD (30.07%) BNG (30.87%) and MDA (33.18%) respectively. The result data were statistically calculated with five replicates of each sample and presented in Table 4.

The free radical scavenging activity of the methanol extracts of *Gloriosa superba* by the DPPH method and the results are shown in table 5. Standard ascorbic acid $IC_{50}$ Value have found in 5.3 µg range (data not shown in the figure). The highest DPPH scavenging activity was shown by methanol extracts of BNG sample (9.90µg) and lowest DPPH activity in KZD samples (25µg) of DPPH scavenging activity under the experimental conditions, respectively. The ABTS scavenging activity with an $IC_{50}$ value of 12.79µg of methanolic extract KZD sample, whereas the other ecotypes had lowest KTM sample 25.48 µg (Table.6). Higher concentrations of the test compounds were more effective in quenching free radicals.

The extract's scavenging capacity for the superoxide anion radicals generated from the photochemical reduction of riboflavin resulted in a decreased the absorbance of the blue formazan solution at 560 nm. The $O_2$ anion radical was inhibited in a dose-related manner as shown in Fig.1. The extract had a significant $O_2$ anion radical scavenging ability in the sample BHA when compared to other samples ($IC_{50}$ value 103.9 µg). The extract was observed to strongly inhibit hydroxyl radical induced deoxyribose degradation in a non-site-specific assay ($IC_{50}$ Value 175.76µg) BNG sample when compared to ascorbic acid ($IC_{50}$ Value 24.50 µg) (Fig.1).

## 4. Discussion

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [32]. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total [33]. In the present study, methanol extracts of all the samples showed the maximum yield of phytochemicals. However, the methanol extract of *G. superba* showed good results for phytochemicals like phenols, alkaloids, flavonoids and tannins [34]. The plant is a well-known ethno medicinal use in Ayurveda for its colchicine content which is used to treat arthritis. Phytochemical studies of tubers or dried roots have showed the presence of colchicines, glycoside, gloriosine, long chain fatty acids, flavonoids, tannins, alkaloids, 3-O-demethylcolchicine-3-O-α-D-glucopyranoside, 1,2-didemethyl colchicine, Glucoside, β and γ Lumicolichicines, β silosterol, Flucoside, 2,3-didemethyl colchicine, luterlin, N-formyl deacetyl colchicines, colchicocide, tannins, superbine, 2-hydroxy-6-methoxy benzoic and salicylic acid [35]. According to [36], the first formed assimilate in the plant will be the simple sugars which will be used for the plant metabolic activities and the excess be stored in their reserve organs. Increased total sugars in tubers may be attributed to the high partitioning efficiency and increased efficiency of the sink to accumulate assimilates in the tubers.

The interest of medicinal plants exploration as a source of pharmacologically active compounds has increased worldwide [37]. In most developing countries of the world, plants are the main medicinal sources used in treating infectious diseases. The various phytochemical compounds detected are known to exhibit medicinal activity as well as physiological activity [38]. There are records that show the benefits of these compounds detected from *G. superba*. For example: Many of the previous reports show that the isolated pure compounds with biological activity were alkaloids. Naturally occurring alkaloids are nitrogenous compounds that constitute the basic active principles of flowering plants. Alkaloids are formed as metabolic products and have been reported to be responsible for pharmaceutically active [39]. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Phenolics are the largest group of and have been said to account for most of the antioxidant activity of plant extracts. Phenolics and alkaloids detected in the extracts are compounds that have been documented to possess medicinal properties and health-promoting effects [40, 41].

Plant steroids are known to be important for their cardiotonic activities; they possess insecticidal and anti-microbial properties. They are routinely used in medicine because of their profound biological activities. Glycosides are non-volatile and lack fragrance and serve as defence mechanisms against predation by many microorganisms, insects and herbivores [42]. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knock out some kinds of tumour cells, particularly lung and blood cancers. These compounds served as natural antibiotics, which help the body to fight infections and microbial invasion [43].

Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea. Plant tannins have been recognized for their pharmacological properties and are known to make trees and shrubs. Flavonoids are widely distributed group of polyphenolic compounds, characterized by a common benzopyrene ring structure. The biological functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and

tutors. Flavonoids reduced cancers by interfering with the enzymes that produce estrogen [44].

The strong DPPH scavenging activity of tea could be attributed in part to the tea catechins and some low molecular polyphenols. The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods [45]. The Percentage of DPPH radical scavenging activities of all the extracts were dose dependent [46]. Methanolic extract powders of acerola, passion fruit and pineapple industrial residues, including pulp, seeds and peel, altogether (except for acerola) devoid of seeds, were screened for antioxidant capacity. The total phenolic contents (TPCs) of the extract powders were compared with their radical-scavenging activities (RSA) against both DPPH and superoxide anion ($O_2$) radicals, and their protective effect against liposome peroxidation, triggered by peroxyl radical.

The scavenging properties of the two compounds seem to be interesting, and the differences between DPPH and ABTS tests could be explained by the different mechanism of the reactions for the two radicals [47], as has been reported for other compounds that possessed ABTS scavenging activity, but did not exhibit DPPH scavenging activity [48]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [49].

## 5. Conclusion

Plants are natural sources of bioactive compounds to treat life threatening diseases. This plant *Gloriosa superba* has showed various phytochemicals which means that it can use for treating diseases. The plant methanol extracts revealed the presence of different types of phytoconstituents of *Gloriosa superba* ecotypes has more free radical scavenging activity. The BHA sample having strong capacity scavenge the free radicals like DPPH, ABTS of NGL sample have to scavenge the superoxide radicals. The result obtained from the present study clearly stated that BHA samples excelled in the accumulation alkaloids, flavonoids, soluble protein and total phenol in tubers. Considering all the aspects it can be concluded that the sample BHA might serve as ideal parent for developing hybrids with high seed and tuber yield. Further studies are going on these plants in order to isolate, identify, characterized and elucidate the structure of the bioactive principles to develop new antibacterial and antifungal medications.

## 6. Acknowledgment

The author is thankful to University Grants Commission, New Delhi, and Government of India: Grant. No. F.30-1/2014/RA-2014-16-OB-TAM-5130 (SA-II) dated 20.02.2015 for financial support.

**Table 1:** Preliminary phytochemical tests for the presence of compounds in *Gloriosa superba* leaves, flowers, seeds and tubers of Bhavani (BHA) and Nagarkoil (NGL)

| S. No | Compounds | Bhavani (**BHA**) | | | | Nagarkoil (**NGL**) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Leaves | Flowers | Seeds | Tubers | Leaves | Flowers | Seeds | Tubers |
| 1 | Alkaloids | - | + | ++ | +++ | - | + | ++ | +++ |
| 2 | Flavonoids | + | +++ | ++ | + | + | +++ | ++ | + |
| 3 | Protein | +++ | ++ | ++ | +++ | +++ | ++ | ++ | +++ |
| 4 | Phenols | ++ | ++ | +++ | +++ | ++ | ++ | +++ | +++ |
| 5 | Steroids | + | + | +++ | +++ | + | + | +++ | +++ |
| 6 | Tannin | - | - | ++ | ++ | - | - | ++ | ++ |
| 7 | Terpenoids | ++ | +++ | +++ | +++ | ++ | +++ | +++ | +++ |

(- = negative; + = slight; ++ = moderate; +++ = High)

**Table 2:** Preliminary phytochemical tests for the presence of compounds in *Gloriosa superba* leaves, flowers, seeds and tubers of Kottayam (KTM) and Kozhikode (KZD)

| S. No | Compounds | Kottayam (**KTM**) | | | | Kozhikode (**KZD**) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Leaves | Flowers | Seeds | Tubers | Leaves | Flowers | Seeds | Tubers |
| 1 | Alkaloids | - | - | + | ++ | - | - | + | ++ |
| 2 | Flavonoids | 1 | ++ | ++ | + | + | ++ | ++ | + |
| 3 | Protein | +++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| 4 | Phenols | + | + | + | ++ | + | + | + | ++ |
| 5 | Steroids | + | + | + | + | + | + | + | + |
| 6 | Tannin | - | - | ++ | ++ | - | - | ++ | ++ |
| 7 | Terpenoids | + | ++ | ++ | +++ | + | +++ | ++ | ++ |

(- = negative; + = slight; ++ = moderate; +++ = High)

**Table 3:** Preliminary phytochemical tests for the presence of compounds in *Gloriosa superba* leaves, flowers, seeds and tubers of Bangalore (BNG) and Mandya (MDA)

| S. No | Compounds | Bangalore **(BNG)** | | | | Mandya **(MDA)** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Leaves | Flowers | Seeds | Tubers | Leaves | Flowers | Seeds | Tubers |
| 1 | Alkaloids | - | - | + | ++ | - | - | + | ++ |
| 2 | Flavonoids | 1 | ++ | ++ | + | + | ++ | ++ | + |
| 3 | Protein | +++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| 4 | Phenols | + | + | + | ++ | ++ | ++ | +++ | +++ |
| 5 | Steroids | + | + | + | + | + | + | +++ | +++ |
| 6 | Tannin | - | - | ++ | ++ | - | - | ++ | ++ |
| 7 | Terpenoids | + | ++ | ++ | +++ | + | +++ | ++ | ++ |

(- = negative; + = slight; ++ = moderate; +++ = High)

**Table 4:** Total percentage of bioactive compounds in *Gloriosa superba* in different Geographical Positions of South India.

| S. No. | Compounds | BHA | NGL | KTM | KZD | BNG | MDA |
|---|---|---|---|---|---|---|---|
| 1 | Alkaloids (%) | 90.45 ± 7.12 | 87.25 ± 8.41 | 80.45 ± 8.12 | 75.29 ± 7.24 | 85.12 ±8.14 | 76.18 ±7.32 |
| 2 | Flavonoids (%) | 55.92 ± 4.21 | 52.47 ± 3.12 | 50.87 ± 4.45 | 48.67 ± 4.26 | 51.76 ± 3.10 | 50.65 ± 3.01 |
| 3 | Protein (%) | 40.28 ± 2.89 | 35.22 ± 3.85 | 33.11 ± 3.15 | 33.41 ± 4.35 | 36.49 ± 3.72 | 34.73 ± 3.38 |
| 4 | Phenols (%) | 38.64 ± 3.55 | 33.54 ± 3.15 | 30.12 ± 2.85 | 30.07 ± 3.12 | 30.87 ± 3.02 | 33.18 ± 3.07 |
| 5 | Steroids (%) | 56.67 ± 4.10 | 54.25 ± 3.95 | 51.31 ± 5.12 | 50.11 ± 5.37 | 52.76 ± 4.11 | 53.17 ± 3.81 |
| 6 | Tannin (%) | 36.25 ± 2.65 | 35.65 ± 2.25 | 32.45 ± 3.64 | 30.66 ± 3.12 | 32.76 ± 2.13 | 31.37 ± 2.95 |
| 7 | Terpenoids (%) | 47.58 ± 3.34 | 45.72 ± 4.05 | 43.14 ± 4.11 | 42.10 3.67 | 43.83 ± 3.85 | 41.76 ± 3.73 |

**Table 5:** The DPPH radical scavenging activity of *Gloriosa superba* methanolic extract from different Geographical Positions of South India.

| | | % of Inhibition | | |
|---|---|---|---|---|
| | Ascorbic acid | **2 µg** | **4 µg** | **8 µg** |
| | | $16.86^{a}$ ± 0.045 | $38.44^{b}$ ± 0.095 | $94.98^{c}$ ± 0.076 |
| S. No | Samples of Methanolic extract | **10 µg** | **20 µg** | **30 µg** |
| 1 | Bhvani (BHA) | $20.21^{f}$ ± 0.100 | $50.46^{g}$ ± 0.065 | $68.86^{g}$ ± 0.054 |
| 2 | Nagarkoil(NGL) | $17.29^{e}$ ± 0.038 | $40.95^{a}$ ± 0.090 | $62.00^{b}$ ± 0.041 |
| 3 | Kottayam (KTM) | $11.86^{c}$ ± 0.062 | $44.84^{c}$ ± 0.080 | $65.57^{e}$ ± 0.049 |
| 4 | Kozhikode (KZD) | $13.93^{d}$ ± 0.063 | $42.65^{b}$ ± 0.078 | $64.96^{d}$ ± 0.085 |
| 5 | Bangalore (BNG) | $09.19^{a}$ ± 0.062 | $45.91^{d}$ ± 0.061 | $64.07^{c}$ ± 0.079 |
| 6 | Mandya (MDA) | $13.47^{d}$ ± 0.158 | $42.14^{b}$ ± 0.069 | $67.94^{f}$ ± 0.045 |

Values are given as Mean ± S.D of five replicates in each and expressed µg/g Crude extract. Values, that are not sharing a common superscript (a,b,c,d,e,f,g) differ significantly at P≤ 0.05 (DMRT).

**Table 6**: The ABTS scavenging activity of *Gloriosa superba* methanolic from different Geographical Positions of South India.

| | | % of Inhibition | | |
|---|---|---|---|---|
| | Gallic acid | **5 µg** | **7.5 µg** | **10 µg** |
| | | $32.78^{a}$ ± 0.170 | $54.38^{b}$ ± 0.057 | $70.38^{c}$ ± 0.037 |
| S.No | Samples of Methanolic extract | **10 µg** | **20 µg** | **40 µg** |
| 1 | Bhvani (BHA) | $26.36^{g}$ ± 0.102 | $60.74^{f}$ ± 0.058 | $73.92^{f}$ ± 0.034 |
| 2 | Nagarkoil(NGL) | $12.38^{c}$ ± 0.268 | $52.79^{c}$ ± 0.048 | $83.19^{h}$ ± 0.046 |
| 3 | Kottayam (KTM) | $05.28^{a}$ ± 0.029 | $44.22^{a}$ ± 0.049 | $69.98^{c}$ ± 0.082 |
| 4 | Kozhikode (KZD) | $09.21^{b}$ ± 0.119 | $47.53^{b}$ ± 0.045 | $64.06^{a}$ ± 0.043 |
| 5 | Bangalore (BNG) | $14.86^{d}$ ± 0.050 | $58.07^{e}$ ± 0.079 | $72.94^{e}$ ± 0.041 |
| 6 | Mandya (MDA) | $19.16^{e}$ ± 0.048 | $64.05^{h}$ ± 0.048 | $68.66^{b}$ ± 0.045 |

Values are given as Mean ± S.D of five replicates in each and expressed µg/g Crude extract. Values, that are not sharing a common superscript (a,b,c,d,e,f,g,h) differ significantly at P≤ 0.05 (DMRT).

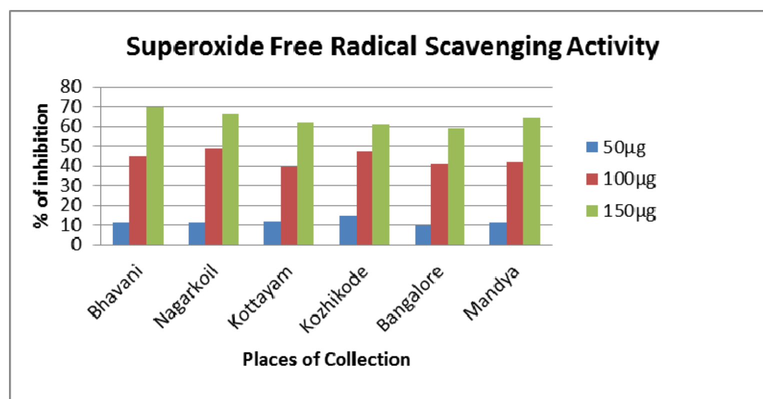


**Fig 1:** The Superoxide free radical scavenging activity of methanol extract of *Gloriosa superba.* Values are means of five replicate determinations (n =5) ± standard deviation.

## 7. References


1. Rajak RC, Rai MK. Herbal Medicines Biodiversity and Conservation Strategies. International Book Distributors, 1990, 75-79.
2. Badola HK. Endangered medicinal plant species in Himachal Pradesh. A report on the International Workshop on "Endangered Medicinal Plant Species in Himachal Pradesh', organized by G.B. Pant Institute of Himalayan Environment and Development at Himachal Unit, Mohal-Kullu during 18-19 March 2002. Current Science. 2002; (83):797-798.
3. Sivakumar G, Krishnamurthy KV. Gloriosa superba L. - a very useful medicinal plant. In: Recent Progress In Medicinal Plants, USA, 2002, 465-82.
4. Trease SE, Evans D. Colchicum seed and corn. In: Pharmacognosy, 12th edn. Balliere Tindall, London, 1983, 593-597.
5. Ade R, Rai MK. Review: Current Advances in Gloriosa superba L. Biodiversitas. 2009; 10(4):210-214.
6. Alagesaboopathi C. Antimicrobial screening of selected medicinal plants in Tamilnadu, India. Journal of Microbiology. 2011; 5(6):617-621.
7. Hemaiswarya S, Raja R, Anbazhagan C, Thiagarajan V. Antimicrobial and Mutagenic Properties of the Root Tubers of Gloriosa Superba Linn. Pakistan Journal of Botany. 2009; 41(1):293-299.
8. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; 4:685-688.
9. Kala CP. Indigenous uses and sustainable harvesting of trees by local people in the Pachmarhi Biosphere Reserve of India. International Journal of Medicinal and Aromatic Plants. 2011; 1(2):153-161.
10. Srivastava UC, Chandra V. Gloriosa superba Linn. (Kalihari)-An important colchicine producing plant. *Journal of Res. Indian Med.* 1977; 10:92-95.
11. Chitra R, Rajamani K. Perise performance and correlation studies for yield and its quality characters in Glory lily Gloriosa superb (L). Academic Journal of Plant Science. 2009; 2:39-43.
12. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. Journal of Agricultural and Food Chemistry. 1994; 42:6-29.
13. Duh PD. Antioxidant activity of burdock (Arctium lappa Linne): its scavenging effect on free radical and active oxygen. Journal of American Oil Chemist's Society. 1998; 75:455-465.
14. Ito N, Fukushima S, Akihiro H, Michiko S, Tadashi O. Carcinogenecity of butylated hydroxylanisole in F 344 rats. Journal of National Cancer Institute. 1983; 70:343-347.
15. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry. 2001; 49:5165-5170.
16. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Nirali Prakashan, Pune, 2004, 506.
17. Didry N, Duberwil L, Tratin F, Pinkas M. Antimicrobial activity of aerial arts of Drossera pelitata Smith on oral bacteria. Journal of Ethnopharmcology. 1998; 60:215-28.
18. Sofowora LA. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibaban, 1993, 55-71.
19. Trease GE, Evans WC. Pharmacognosy. 13th edn. Bailliere Tindal, London. 1989, 176-180.
20. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edition. Chapman and Hall Co. New York.1998, 1-302.
21. Kokate CK, Purohit AP, Gohale SB. Pharmacognosy. Nirali Prakashan publishers, Pune, India. 2003, 1-624.
22. McCready M, Silveria GV, Owens QAC. Determination of starch and amylase in vegetables. Annals of Chemistry. 1950; 29:1156-1158.
23. Lowry DH, Rosenbrough NJ, Far AL, Randal RL. Protein measurement with folin phenol reagent. Journal Biological Chemistry. 1951; 193:265-275.
24. Longanga OA, Vercruysse A, Foriers A. Contribution to the ethno botanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela area, Democratic Republic of Congo (DRC). Journal of Ethnopharmcology. 2000; 71(3):411-423.
25. Martin J, Martin M. Tannin assays in ecological studies: lack of correlation between phenolics, proanthocyanidins and protein-precipitating constituents in mature foliage of six oak species. Oecologia. 1982; 54(2):205-211.
26. Fransworth NR. Biological and Phytochemical screening of plants. Journal of Pharm Science. 1966; 35:225-276.
27. Kumar G, Banu Z, Murugesan A, Pandian Z. Preliminary Toxicity and Phytochemical Studies of Aqueous Bark

Extract of Helicteres isora L. International Journal of Pharmacology. 2007; 3(1):96-100.
28. Malick CP, Singh MB. Plant Enzymology and Histo Enzymology. Kalyani Publishers, New Delhi, 1980, 286.
29. Mensor LL, Fábio S, Menezes GG, Leitão AS, Reis, Tereza C. dos Santos, Cintia S. Coube, LeitãomSG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotheropy Research. 2001; 15:127-130.
30. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radical Biol Med 1999; 26:1231-1237.
31. Winterbourn CC, McGrath BM, Carrell RW. Reactions Involving Superoxide and Normal and Unstable Haemoglobins. Biochemistry Journal. 1975; 155(3):493-502.
32. Geissman TA. Flavonoid compounds, tannins, lignins and related compounds. *In* M. Florkin and E. H. Stotz (ed.), Pyrrole pigments, isoprenoid compounds and phenolic plant constituents. Elsevier, New York, N.Y. 1963; (9):265.
33. Schultes RE. The kingdom of plants. *In* WAR. Thomson (ed.), Medicines from the Earth. McGraw-Hill Book Co., New York, N.Y. 1978, 208.
34. Devi NN, Femina W. GC-MS analysis of Gloriosa superba medicinal plant of Tamilnadu. Journal of Pharmacy Research. 2012; 5(1):343-345.
35. Shanmugam H, Rathinam R, Chinnathambi A, Venkatesan T. Antimicrobial and mutagenic properties of the root tubers of Gloriosa superba linn. (Kalihari). Pakistan Journal of Botany. 2009; 41(1):293-299.
36. Datta SC. Plant Physiology. Wiley Eastern Ltd., New Delhi, India, 1994.
37. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. 2005; 100:80-84.
38. Sofowora A. Medicinal Plants and Traditional Medicines in Africa. Chichester John Wiley & Sons New York. 1993; 97-145.
39. Doughari JH. Antimicrobial activity of Tamarindus indica Linn. Tropical Journal of Pharmacy Research. 2006; 5:597.
40. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. Journal of Nutrition. 2004; 134:3479-3485.
41. Cowan MM. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 1999, 564-582.
42. De M, Krishina De A, Banerjee AB. Antimicrobial screening of some Indian spices. Phototherapy Research. 1999; 13:616-618.
43. Sodipo OA, Akiniyi J, Ogunbanosu. Studies on certain characteristics of extracts of bark of Pansinystalia macruceras (K.Schem) Piere. Exbeile. Global Journal of Pure and Applied Science. 6, 83-87.
44. Ogunleye DS, Ibitoye SF. Studies of antimicrobial activity and chemical constituents of Ximenia americana. Tropical Journal of Pharmacy Research. 2003; 2(2):239-241.
45. Porto CD, Calligaris S, Celloti E, Nicoli MC. Antiradical properties of commercial cognacs assessed by the DPPH test. Journal of Agriculture Food Chemistry. 2000; 48:4241-4245.
46. Oliveira ACD, Valentim IB, Silva CA, Bechara EJH, Barros MPD, Mano CM *et al.* Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. Food Chemistry. 2009; 115:469-475.
47. Naik GH, Priyadarsini KI, Hari Mohan. Free radical scavenging reactions and phytochemical analysis of Triphala, an ayurvedic formulation. Current Science. 2006; 90(8):1100-1105.
48. Wang M, Jiangang L Meera R, Shao Y, LaVoie EJ, Huang TC, Ho CT. Antioxidative phenolic compounds from sage (Salvia officinalis). Journal of Agricultural and Food Chemistry. 1998; 46:4869-4873.
49. Hochestein P, Atallah AS. The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. Mutation Research.1998; 202:363-375.