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Study on Estimation and Antioxidant activity of Gloriosa superba L. Whole Plant Extract

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***Abstract*---**Gloriosa superba is a alkaloid plant, which contains alkaloid components such as colchicine and gloriosine. In this study, Total Phenol content (TPC), Total Flavonoid (TF), Free radical scavenging activity (DPPH) using whole plant (Shoot, Flower, Tuber (SFT) extract derived from G. superba were tested for cancer. G. superba plants are identified and collected. The collected plants were separated into (SFT) G. superba whole plants are dried under the laboratory conditions. The temperature associated with the solvent used in the powder sampling will be followed by extraction using the different conical flask for 1 week and filtered what Mann No.1 paper. The filtrate was stored and used for further experimentation. Analysis of solvent extracts was subjected to phytochemical and antioxidative activity of DPPH assay. Different concentrations such as 20µg, 40µg, 60 µg, 80 µg and 100 µg were taken for all studies and the values are entered in terms of Mean±SD. In case of DPPH assay IC50 values are calculated using ANNOVA. Methanolic extraction from each solvent contains a high value of antioxidant properties.

***Keywords*-** *Total Phenol Ccontent, Total Flavonoid, DPPH, G. superba*

I.INTRODUCTION

India's medicinal and aromatic crops are real emporium. About 9,000 civilians have benefited from 15,000 medicinal plants in India. 7,500 drugs, 3,900 cooking ingredients, 700 culturally important 525 fibers, 400 diseases, 300 pesticides, insecticides, 300 gum, adhesive and dye and 100 for incense and perfume. Based on plant materials for traditional medicine, local communities are estimated to be used by 7,500 plant species [1]. Herbal medicines are highly developed and highly demand in their primary health care in developing countries, with their efficacy, safety and low side effects. The rich traditional heritage, tradition and big biodiversity medicines in India have the worst part of the world market [2].

Polyphenolic compounds are usually occurring eatable and inedible plants, which have many biological effects, which have antioxidant activity [3,5]. Flavonoids are plant phytochemicals and six classes such as (flavonones, flavones, flavonols, isoflavonoids, anthocyanins and flavans) different from their structural individuality around the heterocyclic oxygen ring. Structurally, flavonoids are usually classified by C & S carbon skeletons. Flavonoids can cause aglycones (without sugar moieties) and glycosides (with sugar moieties).

Oxidative pressure is an imbalance between reactive oxygen species (ROS) production and penetrating ability to eliminate ROS. DNA, RNA, fatty tissues, carotenoids, vitamins, and proteins are more damaging to all biomolecules, such as microorganisms [6]. Oxydative stimulating free radicals can result in cell membrane and membrane protein degeneration and mutation, which can continue to developed in many diseases such as lipofuscinosis, oxygen toxicity, aging, atherosclerosis, and liver injury [7,8]. Free radicals are not only a human disease but also lipid oxidation in the food system. The lipids oxide is the main cause of a quality decline in many dietary practices, the creation of undesirable reproduction, some toxic compounds, and reduces food quality and nutritional values.

Antioxidants are compounds that can prevent oxidative or oxidative damage of free radicals, so they are potential carriers of free radicals or reactive oxygen species. Antioxidants act as one or more of the following: reduction of free radical activity, free radicals cleaning, pro-oxidative metals potentially complex and quenching single oxygen [9]. Artificial antioxidants such as butylated hydroxytoluen (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) have been used throughout the world for decades [10], but are restricted used in food products as they are suspected of being cancer [11].

Gloriosa superba L. (Glory Lily) belongs to the family Liliaceae and also an important medicinal plant. G. superba is creeping lily or flame lily, and it's native of tropical Africa and also available in tropical Asia, like India, Sri Lanka, Myanmar and Bangladesh [12]. In south Indians are commonly used as medicinal plants, despite it is also very poisonous plants2. It is one of the endangered species between the medicinal plants [13]. G. superba is a semiwoody herbaceous, branched climber, reaching approximately 5m in height. One to four stems form from one V-shaped fleshy cylindrical tuber. G. superba is a major medicinal plant in India and also cultivated for seeds and tubers are exported to developed countries for medicinal uses. There are various types of traditional medicine in various parts of the plant, especially in tropical Africa and Asia. Tubers are commonly used as a treatment of Livestock injuries and sprains are traditionally used to stimulate pain, chronic ulcers, hemorrhoids, cancer, leprosy, and labor pain. Due to such pharmacological activity, the plant is sometimes used as a mixture of aconite (Aconitum sp.). Glory lily on the global market is considered a rich source of lily colchicines and gloriosine [14]. In the seeds and tubers, valuable alkaloids, like colchicine and colchicoside are important components that it is used for gout and rheumatism. The plant has been identified as anti-cancer drug due to colchicoside action on spin fibers during cell division. Colchicine is a strong antimitotic agent that prevents cell division or controls the cell division with inhibiting mitosis, the divisions of a cell nucleus. The paste of the tubers is morphologically applied for parasitic skin diseases [15]. Traditional medicine of Indians, G. superba tubers are used against tonic, antiperiodic, and also snake bite. [16]. Medically, the tuber is used as an abortifacient, and in small quantities, it functions like diarrhea, tonic, anthelmintic and also feeds to cows and goats [17].

Because there are a series of urgent and emerging need to detect existing antimicrobial compounds in new operations of the treatment of infectious diseases caused by various chemical structures and multi-functional stress and disorders. Therefore, the current work evaluates the total phenol content, the total Flavonid content and the antioxidant activities of whole plant extract (chloride, flower, tuber) of Gloriosa superba.

II. MATERIALS AND METHODS

Collection of the Plant Material

The fresh entire plants (Shoot, flower, tubers) were collected at Bharathiar University, Coimbatore Tamilnadu, India. This plant was identified by the Department of Botany at the Annamalai University of Tamil Nadu in 2014-2015. New plants are collected and divided into shoots, flower, tuber and washed in water, dried in shade and stored in airtight containers. The Plant parts were finely powdered and soaked in 300 ml methanol for 48 hrs. The separation was filtered using WhattmannNo.1 filter paper and the filtrate was concentrated and inhibited pressure in vacuum at 40º C for 30min using a rotary evaporator and kept into lyophilization to reduce the traces of solvent.

Total Phenolic Content

Total phenol content of G. superba was assayed by modified Dewanto et al 2002 [18] procedure. The different concentrations of 10µg, 20µg, 40µg, 60µg, 80µg, and 100 µg were using an aliquot of diluted extract and added to 0.25mL of Folin-Ciocalteu reagent. The elucidation was adjusted with distilled water to a final volume of 3mL and shaken thoroughly. The solution was incubated and kept in the dark placed and read at 760nm was read against prepared blank. The total phenol content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight. The total sample was analyzed in three replicates.

Total Flavonoid Content

Total Flavonoid content in G. superba whole plant extract was analyzed by the aluminum chloride colorimetric system [19]. 0.5ml of entire plant extract of at different concentrations like 10µg, 20µg, 40 µg, 60 µg, 80 µg, and 100 µg were taken and the final volume was made up to 3mL with methanol. After that, 0.1ml AlCl3 (10%), 0.1ml of potassium acetate and 2.8ml of distilled water were added continuously and test solution was vigorously shaken. After 30 minutes for the incubation periods, absorbance was recorded at 415 nm. The concentration of flavonoids in test samples was calculated and expressed as the equivalent of quercetin (QE) / g of sample. The entire sample was analyzed in three replicates.

Free radical scavenging activity (DPPH) 1,1 - diphenyl- 2-picrylhydrazyl radicals

The antioxidant activity of methanolic whole plant (shoot, flower, tuber) extract of G. superba was analyzed with stable 1,1-diphenyl -2-Picryl hydrazyl radical (DPPH) (Sigma-Aldrich) photospectrometrically as stated by [20]. Stock solution with each plant extract of methanol solution in a concentration of 1 mg / ml. Since the stock solutions. Different concentrations like 10µg, 20µg, 40 µg, 60 µg 80 µg and 100 µg were taken and mixed with equal volume of Methanolic solution of DPPH (0.1 mM). Each sample was added in 0.5ml of methanol solution and mixed with 2.5 ml of 0.5 mM of Methanolic DPPH solution. The mixture was vortexted vigourously and kept in 30 minutes in a dark place under room temperature. The absorbance was analyzed at 517nm against a blank using a UV spectrophotometer. The positive control was using ascorbic acid and also experiments were conducted in three triplicates.

Statistical analysis

One-way ANNOVA was used to statistically analyze SPSS 17.0. The variation was considered significant when $p<0.005$. Triplicate ratings were made for each set of test conditions. All values were expressed as Mean±SD (Standard Deviation). The IC50 value is calculated for all the test conditions.

III. RESULT AND DISCUSSION

Total phenol contents

Total phenol content of whole plant extract (shoot, flower, tuber) of G. superba was estimated different concentrations as 25 µg , 50 µg , 75 µg , 100 µg and 125 µg concentrations was 1.752 µg/ml, 1.101 µg/ml and 2.021 µg/ml respectively. Gallic acid was taken as positive control which has 0.934 µg/ml of total phenol contents are shown in (Fig 1). Polyphenols and phenols found in plants are two secondary metabolites considered as natural antioxidants. They are frequently quantified with Folin's reagent. They are found to be an effective hydrogen donor widely distributed throughout the plant kingdom. There is a broad difference between phenolic compounds as a result of antioxidants [21]. Owing to the existence of phytochemicals that might have radical scavenging activity, specifically polyphenols which are a probably hydrogen atom donor [22].

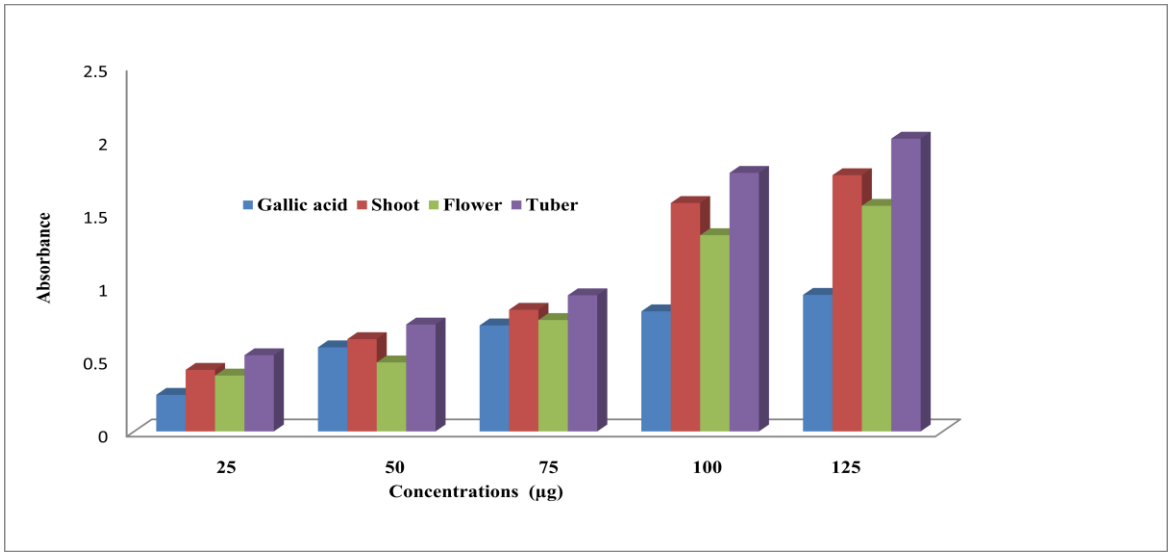

Fig1. Estimation of Total phenol content of whole plant extract of G. superba

Total flavonoids

Total flavoinoid content of different part of whole plant extract of G. superba was estimated different concentrations as (25 µg , 50 µg , 75 µg , 100µg and 125 µg) was shoot(1.654 µg/ml), flower (2.345 µg/ml) and tuber(1.987 µg/ml) while standard Gallic acid was taken as positive control which has (0.911 µg/ml) of total flavonoid contents are shown in (Fig 2). Lee 2007 [23] defined flavonoids are the plant pigments that is responsible for the color of floral parts. Flavonoids are ketonic compounds that can induce anti inflammatory activity and inhibits the oxygen compounds, enzyme cyclo oxygenase dependent pro inflammation activity. Furthermore, flavonoids have a powerful anti inflammation activity as they inhibit prostaglandin synthesis. Flavonoids in higher plants are inseparable with cardiovascular diseases and antioxidant potentials that can treat cancer disease [24,25]. Flavonoids and antioxidants origin of vitamins A, C, E and plant source diets [26,27,28,29,30].

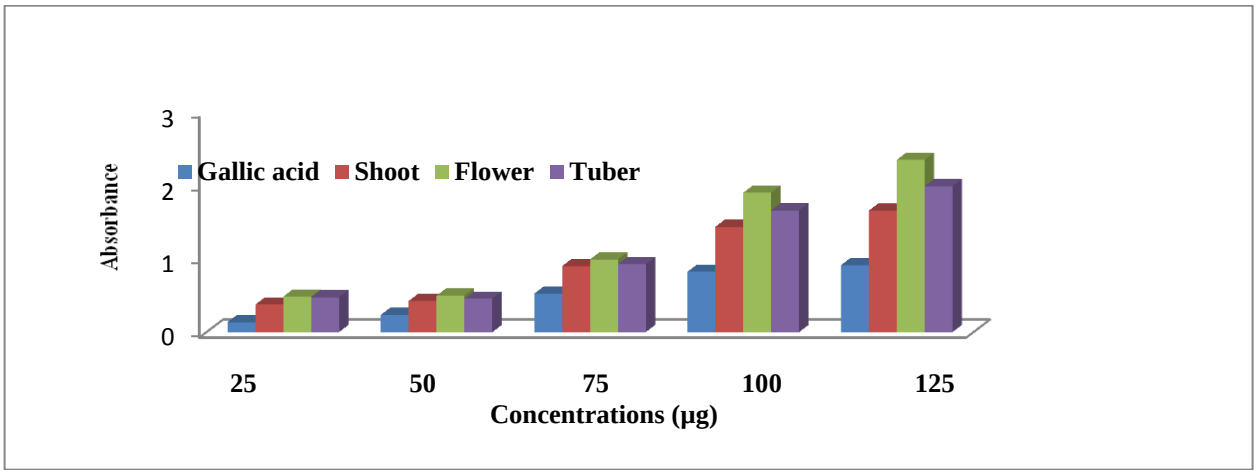

Fig 2. Estimation of total flavonoid content of whole plant extract of G. superba

Antioxidant activity of DPPH assay

According to Huang et al 2005 [31], DPPH that free radical scavenging activity is one of the antioxidant assay, which is based on transfer of electrons, that produces a violet colour solution in the treatment with ethanol. DPPH is stable at a free radical and room temperature, the colorless ethanol solution may reduce the presence of an antioxidant molecule. This is the simplest and fastest way to assess antioxidants through spectrophotometric method. DPPH Radical Scavenging Activity of different concentrations of methanolic extracts 25µg, 50 µg 75 µg100 µg of G. superba whole plant parts such as shoot, flower, tuber was estimated as 65.76µg/ml, 60.34 µg/ml, 80.12 µg/ml, respectively. Ascorbic acid (AA) was taken as positive control which showed activity of 48.16 µg/ml (Fig 3). Superoxide anions are reactive species create with a transfer of single electron and involves in the development of other reactive oxygen species as H_2O_2, hydroxyl radical or singlet oxygen in a living system [32]. Since, scavenging antioxidants activity reduced the power of effective managing within the disease as stomach problems, ulcer, cancer, and AIDS. Antioxidant while reacts with nitric oxide forms peroxynitrite which can produce toxic radicals such as hydroxyl radicals [33,34,35,36].

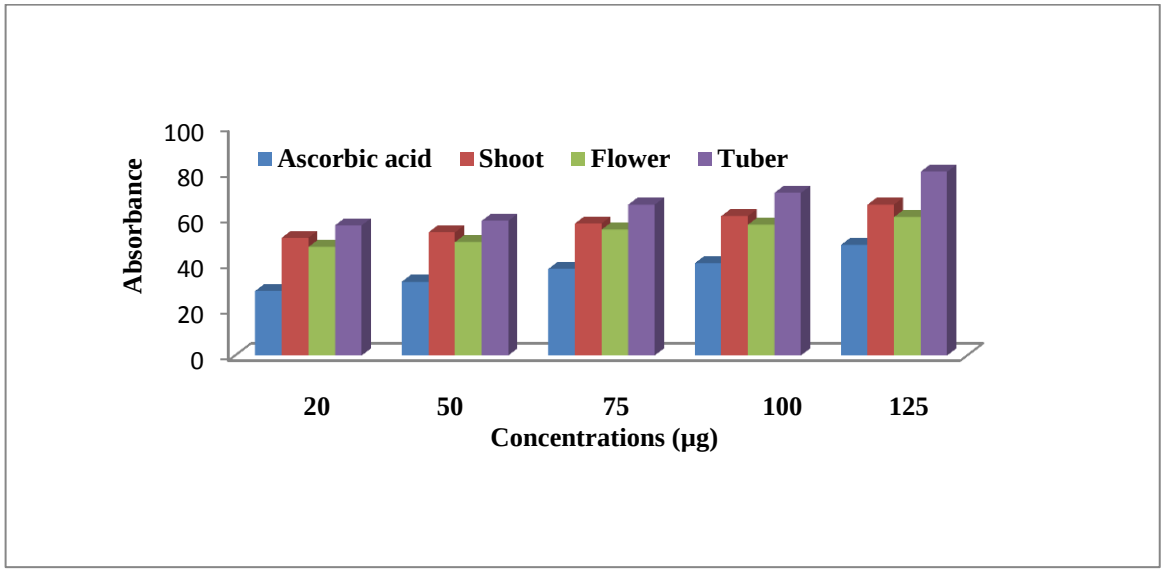

Fig 3. DPPH assay of whole plant extract of G. superba

IV. CONCLUSION

The current study investigate the estimation of total phenol content, flavonoid, and antioxidant assays of whole plant parts of G. supeba. The inhibitory concentration of this extract may be found in the presence of phytochemicals like as phenols and flavonoids. Hence, this valuable phytochemicals are used as a control of various diseases.

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