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Evaluation of phytocontents of extract of *Gloriosa superba* Linn. by Thin layer chromatography and their antioxidant activity

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ABSTRACT

The Present paper deals with pharmacological aspects and phytochemical screening of *G. superba* Linn. by Thin layer chromatography. The extract showed the presence of various phytocompounds like alkaloids and flavonoids. TLC profiling of *G. superba* linn extract give an idea about the presence of various phytochemicals. Different Rf (Retention factor) value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals. In vitro antioxidant activity was carried out with the extract using Nitric oxide assay with ascorbic acid as a standard. The extract had shown significant antioxidant activity. The results of the Nitric oxide scavenging activity study indicate a concentration dependent antioxidant activity. The total phenolic and flavonoid content was found to be 22.3 ± 3 µg g^{-1}Gallic acid equivalents (GAE) and the flavonoid was 47 ± 5.4 µg g^{-1} quercetin equivalents. The Nitric oxide scavenging activity of the extract was found to be promising and IC_{50} of extract was 119.06±.19. It indicates that the hydro alcoholic leave extract of the plant has the potency of scavenging free radicals in vitro and may provide leads in the ongoing search for natural antioxidants from Indian medicinal plants to be used in treating diseases related to free radical reactions.

Key words: Thin layer chromatography, *Gloriosa superba* linn., Antioxidant activity.

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INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world as regards genetic resources of medicinal plants. According to estimates, large population of the world about 70 and 80% depends on traditional medicines to meet their demands. They rely on medicinal plants because of their effectiveness, lack of modern healthcare alternatives and cultural preferences[1]. The use of herbal medicines for the treatment of diseases and infections is a safe and traditional therapy[2]. Medicinal plants have been an integral part of life in various regional communities for food and drug both. From the beginning of human civilization the plants and their products particularly ethnomedicinal plants plays a great role[3]. *G. superba* Linn., is one of the endangered species among the medicinal plants, which is a striking tuberous climbing plant with brilliant wavy edged yellow and red flowers that appears from November to March every year. The tuberous root stocks of glory lily, *G. superba* linn. boiled with *Sesamum* oil is applied twice a day on the joints, affected with arthritis reduces pain. It is also used to treat intestinal worms, bruises, infertility, skin problem and impotence. So the art of use of plants medicine is herbalism[4]. *G. superba* Linn. is used for the treatment of skin infection, cancer and urinary tract infections[5]. The root tuber is mixed with babchi seeds (*Psoralea corylifoila*), black cumin (*Nigella sativa*) and purple fleebane (*Vernonia anthelmintica*) and is made to a paste and is applied externally for various skin diseases[6]. Traditionally, the rural women prefer *G. superba* linn. plant for gynecological disorders like abortion, menstrual trouble, conception disorders, sterility, delivery problems, etc rather than modern medicines[7]. The Gond tribe of Madhya Pradesh in case of induced abortion they grind rhizome/tuber of the plant kalihari (*G. superba* linn.) mixed with ghee and used orally[8]. The antioxidant activity[9], antimicrobial activity[10], snake bite potential, hapatoprotective activity[11], Antithrombotic/Anti coagulant potential[12] and Analgesic and anti-inflammatory potential[13] of *G. superba* Linn. has already reported.

The overall thrust of the present research was to investigate the phytochemicals, scavenging antioxidant activity and TLC profiling of hydroalcoholic extract of *G. superba* Linn.

MATERIAL AND METHODS:

Sample Preparation and Extraction:

Plant sample of *G. superba* linn. was collected from the Govt. forest Nursery of Bhopal and identified by CMBT (CMBT/2012/56). The leaves of the plants were properly washed with tap water and then rinsed with distilled water. The rinsed leaves were shed dried and crushed to obtain powder. A quantity of 100g of the dried powder of *G. superba* linn. extracted with

mixture of water and alcohol (50:50) using soxhlet was shown in Figure 1. The soxhletion with hydro alcohol was done for one week to obtain extract. After that, the extract was evaporated in water bath at 50^{o}C to obtain crude for phytochemical analysis, antioxidant assay and TLC profiling.

Figure 1 Extraction by Soxhlet Apparatus

Phytochemical Analysis:

Preliminary phytochemical analysis of the hydro alcoholic extract of *G. superba* Linn. was determined by the following procedures[14,15,16] .

Flavonoids:

To the 2ml of extract, few drops of lead acetate solution were added. Formation of yellow colour precipitate indicates the presence of flavonoids.

Alkaloids:

To the 2ml of extract, 1.5ml of 1% HCL was added. After Heating the solution in water bath, 6 drops of Mayer's reagents/wagner's reagent/dragendroff's reagent was added. Formation of orange precipitated indicates the presence of alkaloid.

Glycosides:

(Keller-kiliani Test) To 2ml of extract, 1ml glacial acetic acid and 1-2 drops of $Fecl_3$ was added followed by 1ml of concentrated H_2So_4. Green blue colour indicates the presence of cardiac glycosides.

Terpenes/ steroids:

To 2 ml of extract, 5ml of chloroform and 2 ml acetic anhydride was added followed by concentrated H_2So_4 reddish brown colouration of interface indicates the presence of terpenes.

Phenolics:

To 2ml of extract, 5% ferric chloride solution was added. deep blue black colour indicates the presence of phenolics.

Carbohydrates:

(Molisch's test) 2ml of extract were treated with 2 drops of alcoholic α- naphthol solution in a test tube formation of violet ring at the junction indicates the presence of carbohydrate.

Proteins:

2 ml of extracted was treated with few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins.

Saponins:

(Frothing Test) 2 ml of extract was treated with 5 ml of distilled water and shake well. Frothing persistence indicated the presence of Saponins.

Tannins:

To 2 ml of extract, 1% gelatin solution containing sodium chloride was added. Formation of white ppt indicates the presence of tannins.

Estimation of Phyto contents By Thin Layer Chromatography:

This was carried out by TLC method show in Figure. 2. . TLC aluminum sheet was used to separate the active compound present in the extracts. The samples were spotted on thin layer chromatography plates, which were developed in methanol-chloroform (1:20) solvent system. TLC sheet was scanned at 350 nm in UV chamber and revealed that the retention factor (Rf). Then Rf values were calculated by formula and compared with standard values.

Figure 2 Thin Layer Chromatography of *G. superba* linn. Extract

Total phenolic compound assay

Total polyphenols were determined according to the spectrophotometer method with Folin-Ciocalteu's reagent. Gallic acid was used as calibration standard and results were expressed as gallic acid equivalents µg per g dry weight[17]. The extract (200µL) was mixed with 1.5 mL of Folin- Ciocalteau reagent and allowed to stand at 22^0C, for 5 min. Add 1.5 mL sodium bicarbonate solution (20%) was added to the mixture. After 90 min at 22^0C. Absorbance was measured at 725 nm using a UV-Visible spectrophotometer.

Total flavonoid assay

Total flavonoid content was measured by the aluminum chloride colorimetric assay using quercetin as a standard[18]. The plant extract and quercetin was added to 10 mL volumetric flask containing 4 mL of double distilled water. To the flask was added 0.3 mL 5% $NaNO_2$. After 5 min, 0.3 mL 10% $AlCl_3$ was added, 2 mL 1M NaOH was added and the total volume was made up to 10 mL with double distilled water. The solution was mixed well and absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as µg quercetin equivalents (QE)/g dry mass.

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat[19]. Sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25^0C for 180 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylenediaminedihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

RESULTS AND DISCUSSION:

Keeping in view the medicinal importance of *G. superba* linn., this plant was thoroughly investigated for their phytochemical characters including their solvent extractive values. Antioxidants may guard against reactive oxygen species (ROS) toxicities by the prevention of ROS construction, by the description of ROS attack, by scavenging reactive metabolites &

converting them to less reactive molecules [20]. The antioxidant activity *of G. superba* linn. might be due to inactivation of free radicals or complex forming with metal ions or combination thereof. The results of preliminary phytochemical screening of hydro alcoholic extract of *G. superba* revealed the availability of multiple polar and non-polar chemical constituents (Table 1).

Table 1 Phytochemical analysis of *G. superba* linn. Extract

S.Num	Phytochemicals	Tests	Results
1	Carbohydrate	Molish test	+ ve
2	Alkaloids	Mayer's test	+ ve
		Wanger's test	+ve
3	Phenols	With 5% $fecl_3$	+ ve
		Potassium permagnet	+ ve
		Acetic acid	-ve
4	Protein	Millon's	+ ve
5	Flavonoid	Lead acetate	+ ve
6	Steroid	Salkowski test	+ ve
7	Glycoside	Keller-kiliani Test	-ve
8	Organic acid	Iodine test	-ve
		With 5 % lead acetate	+ ve
9	Saponin	Lather formation	-ve

Steroids, terpenoids, flavonoids, phenolic compounds, proteins, carbohydrates were found in extract. Flavonoids and alkaloids are a major group of compounds that act as primary antioxidants or free radical scavengers [21.] The antioxidative characteristics might be attributed to the presence of phytochemical such as flavonoids and other polyphenolic compounds. Poly phenols have been known to show medicinal activity as well as exhibiting physiological activity. The compounds such as flavonoids; which contain hydroxyls are responsible for the radical scavenging activity in plant. The flavonoid contents of the extracts in terms of quercetin equivalent were between 23.15 ± 0.18and 68.3 ± 4.5. The flavonoid contents in the extracts of *G. superba* linn. was (47 ± 5.4 µg g-1). The contents of total phenols that was measured by Folin Ciocalteu reagent in terms of Gallic acid equivalent. The total phenol 22.3 ± 3 µg g-1 in the extract powder. TLC profiling of *G. superba* linn extract give an idea about the presence of various phytochemicals. Different Rf (Retention factor) value of various phytochemicals provide valuable clue about the presence of important phytochemicals. Showed in Table 2.

Table 2 Rf values of TLC spots

S. Num.	Rf value of TLC Spot
1	.14
2	.18
3	.21
4	.28
5	.64

The extract of this plant was then evaluated for antioxidant activity with nitric oxide NO. The hydro alcoholic extract of *G. superba* linn. showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentration 100µg/ml. the extract showed 42.46±.64% inhibition, but Ascorbic acid showed 68±0.57% of inhibition (figure 3). The IC_{50} value of drug was 119.06±.19. The available nitric oxide radical is linked with various carcinomas and inflammatory conditions [22]. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract directly competes with oxygen to react with nitric oxide and thereby inhibits nitrite formation. The present study proved that the nitric oxide scavenging activity of the extract is promising.

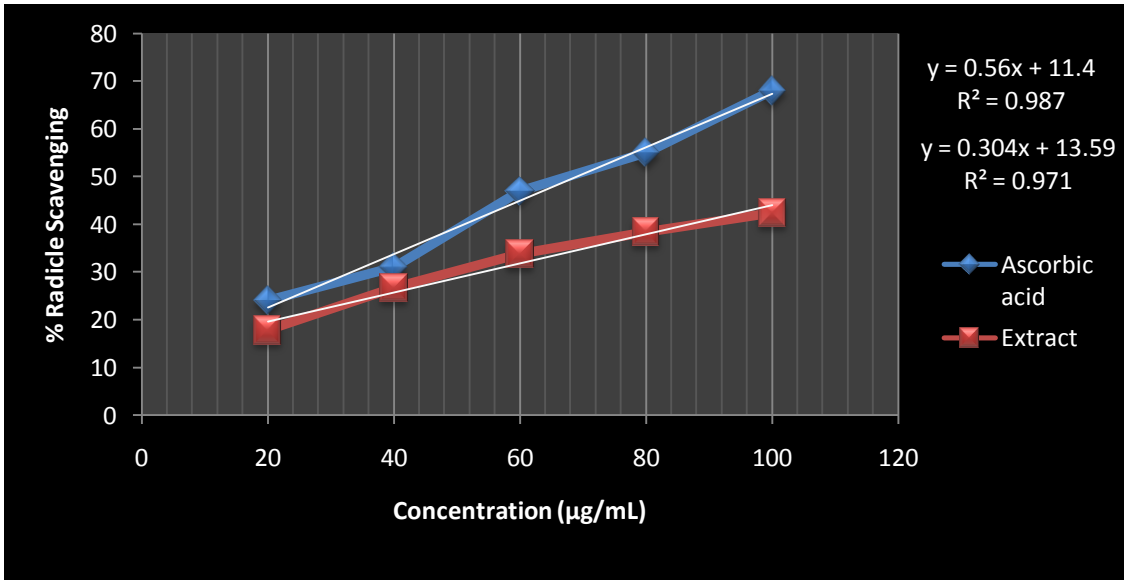

Figure 3 Nitric oxide scavenging

CONCLUSION:

In conclusion, the results of the present study suggest that tested plant materials have moderate to potent antioxidant activity or free radical scavenging activity. The extract showed the presence of various phyto compounds like alkaloids and flavonoids. TLC profiling of *G. superba* linn extract give an idea about the presence of various phytochemicals. Different Rf (Retention factor) value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals. In vitro antioxidant activity was carried out with the extract using Nitric oxide assay with ascorbic acid as a standard. The extract had shown significant antioxidant activity. The results of the Nitric oxide scavenging activity study indicate a concentration dependent antioxidant activity. The total phenolic and flavonoid content was found to be 22.3 ± 3 µg g^{-1}Gallic acid equivalents (GAE) and the flavonoid was 47 ± 5.4 µg g^{-1} quercetin equivalents. The Nitric oxide scavenging activity of the extract was found to be

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FUTURE EXPECTS:

However, we do not know what components in the plant extracts show these activities. More detailed studies on chemical composition of the plant extract, as well as other in vivo assays are essential to characterize them as biological antioxidants which are beyond the scope of this study. It should also be kept in mind that antioxidant activity measured by in vitro methods may not reflect in vivo effects of antioxidants. Many other factors such as absorption/metabolism are also important. The findings of this study support this view that *G. superba* linn. Plant is promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. The providing data can just enrich the existing comprehensive data of antioxidant activity of plant material. In the present state of affairs, TLC profiling of *G. superba* linn plant extract different solvent system indicated the presence of diverse type of phytochemicals. Different Rf values of the compound also reflects an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from this plant extract for their structural analysis.

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