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Estimation of Phytochemicals from Mother Plants and *In vitro* Raised Plants of *Gloriosa superba*

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Abstract

Gloriosa superba L. is an important endangered medicinal plant and widely used in Indian system of medicine. All the parts of *Gloriosa superba* keeps several biological activities such as antioxidant, antimicrobial, antibacterial and anthelmintic properties. This is due to the presence of different phytochemicals like alakloids, flavonoids, phenols, tannins, glycosides, saponins, carbohydrates, steroids and minerals in tubers, leaves and stem. Methanol extract of leaves of mother plant showed higher phenolic content (93.80±0.22 mg GAE g^{-1}) and flavanoids (11.47±0.26 mg QE g^{-1}) than in *in vitro* raised plants. Acetone extract of stem of mother plant showed highest concentration of tannins (70.83±0.88 mg TAE g^{-1}).

Keywords: *In vitro*, micropropagation, phytochemicals, secondary metabolites

1. Introduction

Life of a man has always been intensively connected with the nature surrounding him and plants affect every aspect of our lives. Plants have been used by human beings for various purposes like nourishment, defense, protection, food, fiber, medicine and decoration. Human beings are using compounds derived from plants for treating diseases since ancient times. Medicinal plants offer alternative remedies with tremendous opportunities which not only provide access and affordable medicine to poor people but also generate income, employment and foreign exchange for developing countries (Kumbhare et al., 2012). Plants generally produce many secondary metabolites which constitute an important source of microbiocides, pesticides and many other important pharmaceutical drugs (Ibrahim, 1997). From a long period of time, medicinal plants or their secondary metabolites have been directly or indirectly used to cure diseases and recently became of great interest owing to their versatile applications (Wink et al., 2009). Medicinal plants are known to be the richest bio-resource of drugs of traditional system of medicines, modern medicines, food supplements, neutraceuticals, pharmaceutical intermediates and chemical entities or synthetic drugs (Tiwari et al., 2011).

Gloriosa superba L. is an important medicinal plant of Colcicaceae family, commonly known as Kalihari, Creeping lily, Flame lily, Glory lily, Agnishikha, Nangulika and Tiger claw. All the parts of this plant are used for medicinal purposes in Siddha, Ayurveda and Yunani system of medicine due to the presence of toxic alkaloids such as Colchicine and its derivatives like Gloriosin and Colchicocide along with benzon acid, salicylic acid and resinous substances. Medicines of this plant with major secondary metabolism, tropolone type are present in alkaloids, seeds and tubers (Maroyi and Van der Maesen, 2011). The Flame lily exhibits a broad spectrum of functions to get rid of constipation, anti-inflammatory efficacy, antimicrobial, larvisic test, antibacterial energy, anti-depressant energy, enzyme resistance, serpent bite, skin diseases and respiratory disorders (Ade and Rai, 2009; Hemaiswarya et al., 2009). It is found to be suitable for treatment of injuries and sprains, bitterness, chronic injuries, hemorrhoids, cancers, labor pain and abortion (Srivastava and Chandra, 1977). Gloriosa was only found in the wild a decade back but now it has been domesticated for economic gain and all the parts of the plant are utilized in Indian medicine. Being rich in several biologically active compounds this plant species could serve as potential source of drugs that can be used as a complementary source of traditional medicines.

Gloriosa superba is one of the major medicinal plants in India cultivated for its seeds which are exported to developed countries for pharmaceutical use. However, not much is known about the chemical composition of the plant leaves and tubers (Chitra and Rajamani, 2009). The major aim of this work was to perform *in vitro* regeneration and preliminary phytochemical studies among *in vitro* and mother plant of *Gloriosa superba*.

2. Materials and Methods

2.1. Plant material and extract preparation

Fresh parts (leaf and stem) of mother as well as *in vitro* raised plants were used as plant material and then extract was prepared. For extract preparation two solvents, acetone and methanol were used. The samples were washed with distilled water and then dried in room temperature. 100 ml of different solvents (methanol and acetone) were used for homogenization of dried tissues and then extraction was carried out in orbital shaker at 150 rpm for 24 hours. After that, the mixtures were centrifuged at 10000 rpm for 10 min. Supernatant was then filtered through Whatman filter paper (No. 1). UV-Vis spectrometer (Thermo scientific) was used for the estimation of biochemical parameters.

2.2. Quantitative estimation of total phenolic content (TPC)

Folin-Ciocolteu method was used for the estimation of total phenolic content (Singelton and Rossi, 1965). 0.1 ml of extract was mixed with 1.8 ml of Folin-Ciocolteu reagent (ten times diluted) and kept for 6 min at 25°C. Then 1.2 ml of 20% Na_2CO_3 was added to the reaction mixture. It was then kept for one and half hour at room temperature. Absorbance was measured at 765 nm using UV-Vis spectrophotometer (Thermo scientific). Concentration of TPC was determined as mg of gallic acid equivalent (GAE) per gram of tissue using an equation obtained from gallic acid calibration curve.

2.3. Quantitative estimation of total flavanoid content (TFC)

For the estimation of total flavanoid content, the aluminium chloride colorimetric method was used with some modifications (Chang et al., 2002). 0.5 ml of extracts, 1.5 ml of methanol, 0.1 ml of aluminium chloride (10%), 0.1 ml of sodium acetate (1 M) and 2.8 ml of distilled water was mixed for 5 min by vortexing. The reaction mixture was kept at room temperature for 30 min and the absorbance was measured at 415 nm. The calibration curve was prepared for quercetin and the results were expressed as mg of quercetin equivalents (QE) per gram of tissue.

2.4. Quantitative estimation of total tannin content (TTC)

Total tannin content was measured by using the Folin–Dennis method (Singelton et al., 1999). 0.2 ml of extracts was mixed with 0.5 ml of Folin–Dennis reagent. 1 ml of 20% sodium carbonate solution and 1 ml of millipore water was added thereafter. The reaction mixture was incubated at room temperature for 30 min. The absorbance of the reaction was measured at 775 nm. The concentration of total tannin was determined as mg of tannic acid equivalents (TAE) per gram of tissue using an equation obtained from the tannic acid calibration curve.

To calculate the concentration of the content, formula used is as follow:

Concentration (mg)=(X×Total volume×100)/(Aliquot taken×Weight of sample (g))

2.5. Statistical analysis

Experiments were set up in a completely randomized block (CRD) design and each experiment has three replicates (Cochran and Cox, 1963; Gomez and Gomez, 1984). The data was analyzed using one-way and two-way analysis of variance (ANOVA). The statistical analysis was carried out by using MS-Excel and OPSTAT.

3. Results and Discussion

The present investigation was carried out to compare the concentration of different secondary metabolites of mother plant and *in vitro* raised plants of *Gloriosa superba*. The plants for phytochemical estimation were selected according to morphology. Extract of fresh leaves and stem of mother as well as *in vitro* raised plants was prepared by using different solvents (Acetone and methanol).

3.1. Quantitative estimation of total phenolic content (TPC)

Methanolic and acetone extract of leaves and stem of mother and *in vitro* raised plants showed variations in phenolic content. Methanol extract of leaves of mother plant showed higher (93.80±0.22 mg GAE g^{-1}) phenolic content than in *in vitro* raised plants. Lowest phenolic content was recorded in acetone extract of stem of *in vitro* raised plants (19.73±0.33 mg GAE g^{-1}) (Table 1, Figure 1). Similar observations were recorded in *Salacia chinensis*, *Hypochaeris radicata* , where methanol extract of leaf and root parts showed higher phenolic content than that of other solvent extract (Chavan et al., 2012; Senguttuvan et al., 2014).

Table 1: Determination of total phenolic content in *Gloriosa superba*

Extract / Solvents	Mother plant (mg GAE g^{-1})		*In vitro* raised plants (mg GAE g^{-1})	
	Leaf	Stem	Leaf	Stem
Methanol	93.80± 0.31	65.78± 0.41	87.31± 0.22	45.68± 0.16
Acetone	88.61± 0.22	67.85± 0.17	79.90± 0.21	19.73± 0.33
CD (p=0.05)	0.77	0.77	0.56	0.56

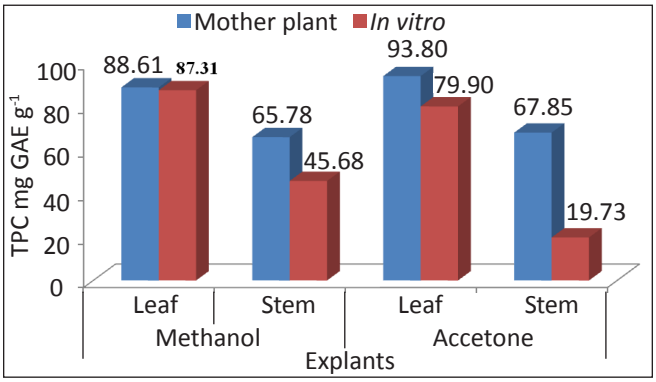

Figure 1: comparison of TPC between mother plant and *in vitro* raised plants

3.2. Quantitative estimation of total flavanoid content (TFC)

Methanolic and acetone extract of leaves and stem of mother and *in vitro* raised plants showed variations in flavanoid content. The solubility of flavanoids was significantly affected by the solvent used for extraction and these findings are in accordance with the results obtained for *Asimina tribloba* and *S. Chinensis* (Harris and Brannan, 2009; Chavan et al., 2012). Methanol leaves extract of mother plant showed higher concentration of flavanoids i.e. 11.47±0.26 mg QE g^{-1}. Acetone extract of stem of *in vitro* raised plants showed lowest concentration of flavanoids i.e. 1.50±0.00 mg QE g^{-1} (Table 2, Figure 2). The same findings were also reported in *Dendrobium nobile* where methanol leaf extract showed higher flavanoid content (Bhattacharya et al., 2014).

Table 2: Determination of total flavanoid content in *Gloriosa superba*

Extract / Solvents	Mother plant (mg QE g^{-1})		*In vitro* raised plants (mg QE g^{-1})	
	Leaf	Stem	Leaf	Stem
Methanol	11.47± 0.16	2.68± 0.09	10.33± 0.16	2.27± 0.14
Acetone	11.17± 0.26	2.66± 0.07	4.33± 0.16	1.50± 0.00
CD (p=0.05)	N/A	0.38	0.32	0.32

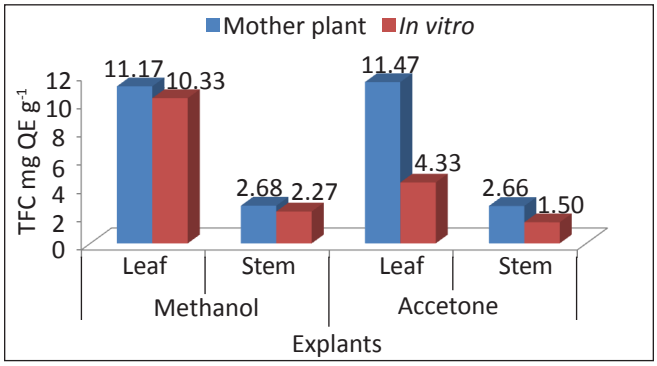

Figure 2: comparison of TFC between mother plant and *in vitro* raised plants

3.3. Quantitative estimation of total tannin content (TTC)

Methanolic and acetone extract of leaves and stem of mother and *in vitro* raised plants showed variations in tannin content. Acetone extract of stem of mother plant showed highest concentration of tannins (70.83±0.88 mg TAE g^{-1}), whereas methanol extract of leaves of *in vitro* raised plants showed lowest concentration of tannins (14.90±0.20 mg TAE g^{-1} DW) (Table 3, Figure 3).

In the present investigation, phenolic, flavanoid and tannin contents showed variations among mother plant and *in vitro* raised plants in different plant parts (leaves and stem) in different solvents. These variations may be due to the hormonal content, specific metabolic as well as endogenous

Table 3: Determination of tannin contents in *Gloriosa superba*

Extract / Solvents	Mother plant (mg TAE g^{-1})		*In vitro* raised plants (mg TAE g^{-1})	
	Leaf	Stem	Leaf	Stem
Methanol	60.00± 0.57	47.20± 0.25	60.13± 0.53	14.90± 0.20
Acetone	56.33± 0.32	70.83± 0.88	56.40± 0.21	28.63± 0.33
CD (p=0.05)	1.33	N/A	0.79	0.79

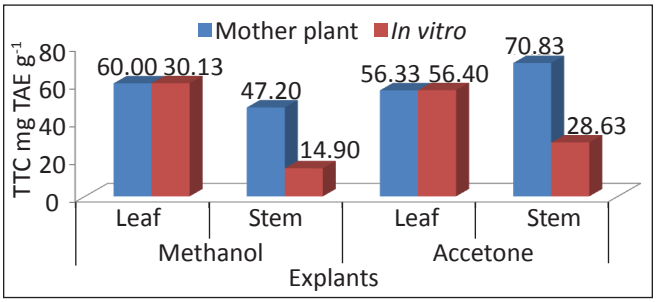

Figure 3: Comparison of TTC between mother and *in vitro* raised plants

physiological changes taking place in the plants. Similar variations of phenolics and flavanoid content within plant parts were reported in 12 medicinal plants of the families *Asclepiadaceae* and *Periplocaceae* (Surveswaran et al., 2010). A number of factors, such as somaclonal variation, plant-to-plant variation in chemical content and variation in agroclimatic conditions are responsible for overall discrepancy between field and *in vitro* plants (Ciddi, 2006). It appears to be pertinent to accept the *in vitro* system which may serve as alternative source of metabolites and thus may be exploited for efficient generation of such substances throughout the year, which are pharmacologically promising but are severely limited in production.

4. Conclusion

Among *in vitro* and mother plants, mother plants showed highest phenolics, flavanoids content whereas tannin content was high in *in vitro* raised plants. Moreover, preliminary phytochemical analysis can further be utilized for identification of best provenances for large scale production through biotechnological intervention. A study to promote mass multiplication and quantification of active constituents is recommended in order to achieve maximum benefits from high value medicinal plants in the region.

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