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COMPARATIVE PRELIMINARY PHYTOCHEMICAL STUDIES OF *IN VIVO* AND *IN VITRO* EXTRACTS OF *GLORIOSA SUPERBA* L.

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ABSTRACT

Gloriosa superba L. (Liliaceae) is known for its valuable alkaloid content colchicine. It is highly used in traditional and modern therapies. Seeds and tubers of this plant contain medicinally potential alkaloid colchicine and have been traditionally used for treatment of ulcer, cancer, gout etc. The present study was done to screen various phytochemicals by using two different solvents viz. Water and Methanol. Methanolic and aqueous extracts of *in vivo* (seed, leaf, tuber) and *in vitro* (shoots, tuber) plant samples were used for preliminary phytochemical screening. The results revealed that both extracts contain all most all type of phytochemicals like Alkaloids, Glycosides, Steroids and Tannins, but the methanolic extracts of *in vitro* plant samples gave better results than other samples in aqueous system.

KEY-WORDS: ***Gloriosa superba*** **L., Phytochemicals, Alkaloids.**

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INTRODUCTION

Plants are rich source of phytochemicals and have been used as herbal medicines to cure various diseases from ancient era. Currently, these plant based herbal medicines were in demand because of low cost and less side effects to human health. Use of plant-based remedies is also widespread in many industrialized countries and numerous pharmaceuticals are based on or derived from plant compounds[1]. *Gloriosa superba* L. is an important medicinal plant belong to Liliaceae. It is one of the endangered species among the medicinal plants. Seeds and tubers of this plant contain valuable alkaloid colchicines. This plant is traditionally used for treatment of skin infection, cancer, gout and urinary tract infections. Due to its ability to cure various diseases, it is highly exploited[2,3]. Plant tissue culture plays an important role to conserve medicinally important plants by produced multiple copies within short period and limited space. The conventional method of propagation of *G. superba* through tubers is a generally followed practice but is considerably slow with a poor multiplication ratio of 1: 1 every year[4]. In nature, less seed germination with poor viability is responsible for its small population size. The poor propagation coupled with over-exploitation by the chemical and pharmaceutical companies has put this plant into acutely threatened species. So, it has been affirmed as an endangered plant by IUCN Red Data Book[5]. Due to above problem, urgent, rapid and reliable protocol for multiplication and conservation is needed for this important species. *In vitro* techniques of propagation provide an alternate and effective means for rapid multiplication of species by the continuous production to meet the demand for commercial exploitation[6]. Tissue culture derived plant material can be used as source of raw material for secondary metabolites which have medicinal potential. So, the present study was undertaken to develop an effective protocol for *in vitro* multiplication and to screen the presence of various phytochemicals in *in vivo* and *in vitro* plant parts of *Gloriosa.*

MATERIALS AND METHODS

Collection of Plant Materials

In vivo collection

Seeds, Leaves and Tubers of *G. superba* were collected from local field of Patan District, North Gujarat. The plant materials were identified (HNGU/LS/BOT/LAB/HS/36/2015) and authenticated by Faculty of Botany, Department of Life Sciences, Hemchandracharya North Gujarat University, Patan, Gujarat. Fresh disease free explants were collected in the early morning for the tissue culture work. For phytochemical screening, each plant sample was washed, dried and powdered for the sample preparation.

In vitro collection

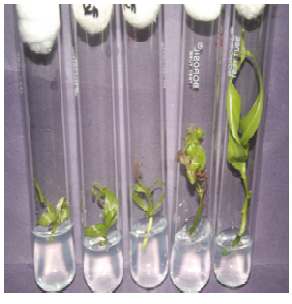
In vitro Shoots, which were developed on MS medium containing BAP or Kinetin (1-4 mg/l) and *in vitro* developed tubers, MS medium supplemented with BAP(6-Benzylaminopurine)+IAA(Indole-3-acetic acid) (2+1.5 mg/l) from shoot apex explants[7]. Both the samples were collected after 12 weeks of inoculation for extraction and screening of phytochemicals.

Extraction procedure

The powdered *in vivo* and *in vitro* plant materials (2 gm) were extracted by simple maceration method with two different solvent (20 ml) viz. Water and Methanol. Samples were kept on shaker (200 rpm) at room temperature for 24 hrs. The extracts were then filtered using whatman filter paper no. 41, after that evaporated to obtained crude sample, which was used for preliminary phytochemical screening[8].

A

B



C

D

Figure A *Gloriosa* Plant
Figure C *In vitro* developed Shoots

Figure B *In vivo* Tuber
Figure D *In vitro* developed Tuber

Phytochemical screening

Phytochemical tests were carried out for all the extracts[9,10]. All the tests are done thrice to check its consistency for presence or absence of various phytochemicals.

1. Alkaloids

To 1 ml of each extracts, a few drops of Mayer's, Dragendroff's and Wagner's reagents were added. Formation of white or pale precipitate showed the presence of alkaloids.

2. Flavonoids

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

3. 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.

4. Saponins
In a test tube containing about 5 ml of the extract, few drops of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. A honeycomb like froth was formed and it showed the presence of saponins.

5. 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.

6. Tannins
To 2ml of extract, 0.1% $Fecl_3$ was added to the filtered samples and observed for brownish green or a blue black colouration which shows the presence of tannins.

7. Steroids
2.0 ml of extract, 5.0 ml of chloroform and 2.0 ml of acetic anhydride was added followed by 1.0 ml of concentrated sulphuric acid. A reddish brown colour produced in the chloroform layer shows the presence of steroids.

RESULTS AND DISCUSSION

The results of preliminary phytochemical screening in two different extracts viz. Water and Methanol of *in vivo* and *in vitro* plant materials of *G. superba* are shown in Table. 1. The results of each type of phytochemical tests reveal that Alkaloids were present in both the Aqueous and Methanol extracts of *in vivo* and *in vitro* Plant materials. Flavonoid was present in both the Aqueous and Methanolic extracts of *in vivo* Leaf, Tuber and *in vitro* shoots. Phenol was present in both the Aqueous and Methanolic extracts of *in vivo* Leaf, Tuber, *in vitro* shoots and tuber, but absent in seed extracts. Saponin was present in aqueous extracts of *in vivo* leaf and seed and in both Aqueous and Methanol extracts of *in vitro* shoots and Tuber. Glycosides were present in only *in vitro* parts (Shoots, Tuber) in both the extracts. Tannin was present in Methanol extracts of *in vivo* Leaf and *in vitro* Shoots and the aqueous extracts of *in vivo* and *in vitro* tuber. Steroid was present in only in *in vivo* Leaf in both aqueous and Methanol extracts. Similar type results in *in vivo* preliminary phytochemical analysis of various parts Viz. Seed, Tuber and Leaves were reported in *Gloriosa*[11,12,13,14] but no results for *in vitro* developed shoots and tuber were reported so far. *In vitro* developed shoots have all phytochemicals except steroid and tannins in water extract. *In vitro* developed tuber which is an important source of Colchicine (Alkaloid) shows the presence of alkaloids through the spot test in both the solvent along with flavonoids, phenols and saponins. The tannin was reported only in methanol along with glycosides and steroids. The presence of various phytochemicals was reported in *in vivo* plant parts of *Celastrus paniculatus* Willd., *Piper longum* L. and *Ziziphus mauritiana* Lam. *Fruits*[15,16]. Preliminary screening of methanolic extract of different plant parts showed the presence of flavonoids, glycosides, steroid, and alkaloids in both *in vivo* and *in vitro* plant parts. The present study can be useful in further standardization for medicinally important Phytochemicals.

Table 1
Phytochemical screening of in vivo (Seed, Leaf, Tuber) and in vitro (Shoots, Tuber) samples of G. superba

Sr. No.	Phytochemicals	*In vivo* plant samples						*In vitro* plant samples			
		Seed		Leaf		Tuber		Shoots		Tuber	
		WE	ME	WE	ME	WE	ME	WE	ME	WE	ME
1.	Alkaloids										
	i) Dragondroff's	+	+	+	+	+	+	+	+	+	+
	ii) Mayer's	-	-	+	+	-	-	+	+	-	-
	iii) Wagner's	+	+	+	+	+	+	+	-	+	+
2.	Flavonoids	+	-	+	+	+	-	+	-	+	-
3.	Phenol	-	-	+	+	+	+	+	+	+	+
4.	Saponins	-	-	+	-	+	-	+	+	+	+
5.	Glycosides	-	-	-	-	-	-	+	+	-	-
6.	Tannin	-	-	+	+	+	-	-	+	+	-
7.	Steroids	-	-	+	+	-	-	-	-	-	-

Note:- WE- Water extracts, ME- Methanolic extract,'-'=Absent, '+'=Present

CONCLUSION

Preliminary screening of methanolic extract of different plant parts showed the presence of flavonoids, glycosides, steroid, and alkaloids in both *in vivo* and *in vitro* plant parts. The present study can be useful in further standardization for medicinally important Phytochemicals.

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REFERENCES

1. Kumar J., Nino, Amala lourthuraj A. *In vitro* regeneration and phytochemical analysis of *Justicia gendarussa.* Indian J. Innovations Dev., (2): 106-111, (2012)
2. Kirtikar K.R., Basu B.D. "Indian Medicinal Plants". 2nd edition. Bishen Singh and Mahendra Pal Singh publication: Deharadun, p.33, (1975)
3. Badola H.K. Endangered medicinal plant species in Himachal Pradesh. A report on the International Workshop on *'Endangered Medicinal Plants Species in Himachal Pradesh',* organized by G. B. Pant Institute of Himalayan Environment and Development at Himachal Unit, Mohal-Kullu during 18-19 2002. Curr.Sci.,; 83: 797-798,(2010)
4. Krause J. Production of Gloriosa tubers from seeds. Acta Hortic., 177: 353–360, (1986)
5. Sivakumar G., Krishnamurthy K.V. *In vitro* organogenetic responses of *Gloriosa superba*. Russ. J. Plant Physiol., 51: 790-798, (2004)
6. Yadav K., Singh N. Factors influencing *in vitro* plant regeneration of Liquorice (*Glycyrrhiza glabra* L.). Iranian J. Biotechnol.,10: 161-167, (2012)
7. Murashige T., Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497, (1962)
8. Krishnaiah D., Devi T., Bono A., Sarbatly R. Studies on Phytochemical constituents of six Malaysian medicinal plants, Journal of Medicinal Plant Research, 3(2): 067-072, (2009)
9. Tiwari P., Kumar B., Kaur M., Kaur G., Kaur H. Phytochemical screening and Extraction: A Review. Internat. Pharmaceutica Sciencia., 1: 98-106, (2012)
10. Singh D., Mishra M., Yadav A. Evaluation of phytocontents of extract of *Gloriosa superba* Linn. by Thin layer chromatography and their antioxidant activity, Am. J. Pharm. Tech. Res., 2(4): 582-591 (2012)
11. Muthukrishnan S., Subramaniyan A: Phytochemical Constituents of *Gloriosa superba* Seed, Tuber and Leaves, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3(3): 111-117, (2012)
12. Dadsena R., Sahu N., Agrawal S., Kumar A. Phytochemical analysis of three endangered plants (*Costus specious, Gloriossa superba* Linn and *Rauvolfia serpentine (*Linn. Benth) from Kanker District of Chhattisgarh, India, The Bioscan, 8(2): 655-659, (2013)
13. Senthilkumar M. Phytochemical screening and Antibacterial Activity of *Gloriosa superba* Linn. International Journal of Pharmacognosy and Phytochemical Research, 5(1); 31-36, (2013)
14. Suttee A., Bhandari A., Bais C., Sharma A. Pharmacognostical and phytochemical evaluation of *Celastrus paniculata*, International Journal of Pharmacognosy and Phytochemical Research, 4(4): 227-233, (2013)
15. Sharma V., Kalyani, Vyas P., Harisha C.R., Prajapati P.K. Pharmacognostical and phytochemical study of *Piper longum* L. and *Piper retrofractum* Vahl., Journal of Pharmaceutical and Scientific Innovation, 1(1): 62-66, (2012)
16. Rathore S.K., Bhatt S., Dhyani S., Jain A. Preliminary Phytochemical Screening of medicinal plant *Ziziphus mauritiana* Lam. *Fruits,* International Journal of Current Pharmaceutical Research, 4(3):160-162, (2012)