



Research Journal of Pharmaceutical, Biological and Chemical Sciences

Phytochemical research of plant extracts and use *in vitro* culture in order to preserve rare wild species *Gladiolus imbricatus*.

**Krvavych AS*, Konechna RT, Petrina RO, Kyrka MS, Zayarnuk NL, Gulko RM,
Stadnytska NE, and Novikov VP.**

National University "Lviv Polytechnic", Department of Technology of Biologically active compounds, Pharmacy and Biotechnology, S. Bandera str.12, Lviv, Ukraine 79013.

ABSTRACT

Gladiolus imbricatus a rare species spread in Central Europe and Mediterranean, included in the Red List of Ukraine as endangered. Also it have medicinal properties. The aim of our study is to establish the content of biologically active compounds in plant material and to develop *in vitro* technologies for *G. imbricatus*. Physico-chemical methods of analysis established the presence of polysaccharides, flavonoids, terpenoids and vitamin C mainly and fewer aromatic and quinones compounds. Have proposed alternative technology receiving biologically active compounds from callus culture.

Keywords: *G. imbricatus*, plants extracts, chemical composition, callus culture

**Corresponding author*

INTRODUCTION

Traditional and folklore medicine play an important role in health services around the world. About three quarters of the world's population relies on herbal medicines for health care [1].

A requirement in medicinal drugs from a plant material remains very high. It is connected with a number of their advantages, namely: possibility of the long-term use, soft therapeutic action, availability and non-toxicity. Secondary metabolites are essentially produced and extracted from plants grown in the field under influence of seasonal variations. As a result of reduction of natural supplies of medical plants, perspective is an alternative biotechnological method of production of BAC from the *in vitro* culture.

Gladiolus imbricatus belonging to Iridaceae family. Grows mainly in dry grasslands at the border of bushes at an altitude between 100 and 1450 m. *G. imbricatus* are almost the most cold-tolerant species of the genus. *G. imbricatus* growing in Ukraine in the Carpathians, in Polissia and Roztochchja (Figure 1). According to literature data they contain in their composition mainly glycosides, vitamins and essential oils and fewer aromatic and quinones compounds. In ethnomedicine the plant is used as anesthetic and lactogenic agent, have bactericidal, astringent, tonic, sedative actions. Infusion of corm used to treat allergies. Chemical composition requires more detailed study [3].



Figure 1

To identify and evaluate the therapeutic potential of medicinal herbs, isolation of active components and structural elucidation of these compounds is very essential in medicinal chemistry and natural product research.

The aim of this paper was to establish phytochemical composition and reproducible *in vitro* culture techniques for the rare wild species *G. imbricatus*. The resulting ethanolic and water extracts was filtered and concentrated and then tested for various BAC by

conventional methods - polysaccharides, flavonoids and glycosides, terpenoids, vitamin C, aromatic and quinones compounds were commonly found.

MATERIALS AND METHODS

Collection of plant materials

The raw material for the study was *G. imbricatus*. The plants have been collected from natural habitat, after flowering period. The plant was air-dried without access to sunlight. Then they were finely crushed and used for extraction.

Extraction of plant materials

Plant extract was prepared by Soxhlet extraction method. Powdered plant material was uniformly packed into a thimble and extracted with different solvents separately. Solvents used were chloroform and hexane, and 70% ethanol and hot water. The process of extraction continues till the solvent become colorless [8].

The resulting extracts were filtered and concentrated by vacuum distillation in a water bath at 40 °C. Output of extracts ranged from 7-19%. Dried extract was kept in refrigerator at 4°C for their future use in phytochemical analysis.

Phytochemical screening procedure

The presence of BAC determined using pharmacopoeial methods [10] as well, as by thin layer chromatography (TLC) [2].

Test for flavonoids

Shinoda test

Four pieces of magnesium fillings (ribbon) are added to the 1 ml ethanolic extract followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoid [5]. Colours varying from orange to red indicated flavones, red to crimson indicated flavonoids, crimson to magenta indicated flavonones.

Ferric chloride test

To 2 ml of the extract, few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group.

Sodium hydroxide test

To 2 ml of the ethanolic extract 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

Test for Quinones

- About 0.5 g of plant extract was taken and added 1 ml of extract and 1 ml of con. H_2SO_4 was added formation of red colour shows the presence of quinones.
- One drop of ethanolic test solution is placed on a filter-paper, followed by 1 drop of 0-2 percent ethanolic phenylacetone nitrile solution and 1 drop of 0.1 N sodium hydroxide. A positive response is indicated by the appearance of a blue or violet stain edged by a yellow ring.

Molisch's test for Carbohydrates

Few drops of Molisch's reagent were added to the portion of sample dissolved in distilled water; this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test [6].

Test for terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids [9].

Thin Layer Chromatography

TLC of flavonoids

Extract was subjected to TLC analysis [10], to find the presence of flavonoids to support the chemical test. Solution of quercetin was used as a standard. A volume of 1 ml of solutions of standards and investigated extracts was spotted on the plates with a thin layer of silicahel. Plate is placed in a chamber with a solvent system butanol: acetic acid: water in volume ratio 3:1:1. After uplift the front of the solvent to a height of 10-12 cm and dried chromatograms exhibit with concentrated ammonia solution, spots of flavonoids were observed under UV light (yellow color).

TLC of Carbohydrates

0.02 ml of water extract was applied to the base line of the chromatogram "Silufol". Plate is placed in a chamber with a 90% solution of ethanol and chromatographed. The chamber previously saturated by solvent vapors for 30 min. After passing the solvent front about 12 cm plate removed from the chamber and air dried. Spots were visualized by spraying successively the chromatogram with 20% thymol solution and dilute sulfuric acid and heating at 100-150°C for 4 min. The chromatogram shows the basic spot color orange.

TLC of vitamin C

0.5 g of powdered material placed in a flask, add 5 ml of distilled water, and stirred for 15 minutes after the infusion is filtered. Filtrate was applied to the plate "Sylufol", near sample ascorbic acid. Plate is placed in a chamber with a solvent system: ethyl acetate - ice acetic acid (8:2). After chromatography plate was dried in air. Chromatogram spraying 0.04% water solution of Na 2,6-dichloroindophenol. Ascorbic acid is shown with white spots on a blue background.

TLC of Quinones

Silufol UV-254 were used for analytical TLC. The components were visualised under UV/Vis light at 254 and 365 nm in a UV cabinet and by using a solution of phosphomolybdic acid (20 g) and ceric sulphate (2.5 g) in 500 ml of sulphuric acid (5%), followed by heating. Quinones were detected by spraying the plate with a solution of 5% KOH in ethanol followed by heating for 5 minutes at 105°C.

In vitro corm development

In this study, fresh corms of *G. imbricatus* were used as explant. For pre-treatment, the corms were cleaned in running water and outer scales were removed. Corms were surface-sterilized with 70% EtOH for 17 min and with 4.5% Sodiumhypochlorite for 20 min, consecutively, and washed three times with sterilized distilled water [4].

The experiments were maintained on solidified basal medium Murashige & Skoog which contained, mineral salts and vitamins (100 mg/L myo-inositol, 2 mg/L 1glisin, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine HCl, 0.1 mg/L thiamine HCl, 30 g/L sucrose, 8 g/Lagar) and 2,5 mg/L a- naphthaleneacetic acid (NAA) and 0,3 mg/L kinetin. The pH of medium was adjusted to 5.8 before autoclaving for 15 min.

For callus cultures, sterilized corms were divided into two group. First group of corms was cut into 4 mm transverse slices, and the second group was cut into 4 mm longitudinal slices. Callus cultures were grown at a temperature of $24 \pm 2^{\circ}\text{C}$ with illumination provided by cool white florescent lamps at 40 μE with a 16-h light period and subcultured. Subculturing was periodically carried out at 4 weeks intervals. Five callus lumps were inoculated in each 250 ml flask, three flasks in each treatment. All experiments were repeated three times. Cultured at a temperature of 23-25°C in the dark. The frequency callus development assessed in percentage by the number of explants that gave callus from the total eksplants.

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical screening of the *G. imbricatus* extracts showed the presence of flavonones, carbohydrates, vitamin C and also aromatic and quinones compounds as shown in table1.

Table 1. Qualitative analysis of the phytochemicals of the *G. imbricatus*.

Compounds	Extracts			
	Ethanol	Chloroform	Aqueous	Hexane
Flavonoids	+	+	-	+
Quinones	+	+	-	+
Vit. C	-	-	+	-
Carbohydrates	-	+	+	+
Terpenoids	-	-	-	-

+ = Presence; - = Absence

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, flavonoids have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory and antitumor activities [7]. Carbohydrates, especially pectin, increase resistance to the effects of many allergens.

Quinone compounds have various bioactivities and play important roles in the nature. Among a lot of quinone compounds including naturally occurring compounds, especially the naphthoquinone derivatives have wide variety of bioactivities. Also potency as anti-inflammatory agent, anti-allergic agent, anti-asthma medicine, anti-dragon gore medicine, anti-psoriasis medicine, bronchodilator, thrombus prevention and hypotension are found in their derivatives.

They can be also utilized for the infection drugs as anti-fungal agents, anti-virus medicines and antibacterial agents against gram-positive bacteria, gram-negative bacteria, etc.

***In vitro* corm development**

Maximum formation of callus (94-95%) was detected at 4 weeks. With the introduction of explants *in vitro* conditions first signs of callus genesis appeared after 1-2 weeks of cultivation. The resulting callus had a bright, slightly yellowish color, characterized by dense texture and low intensity of growth. A visual analysis callusnyh cultures were signs of morphogenesis, callus different relative homogeneity structure.

Further research has to be determine the content of BAC of callus cultures and it's quantitative analysis, and comparing them with the content and biological activity in extracts from medicinal plants *G. imbricatus*.

The above data show that *G. imbricatus* has medicinal properties and can be recommended for clinical study and introduction of public health practice.

CONCLUSION

Extracts obtained by the above method is a yellow liquid with aromatic.



Here a phytochemical analysis of various solvent extract of *G. imbricatus* was conducted. Phytochemical analysis of the plant *G. imbricatus* showed the presence of many BAC, such as flavonoids, carbohydrates, vitamin C and aromatic and quinones compounds.

G. imbricatus studied here can be a potential source of useful drugs exploiting the antimicrobial, antioxidant and antitumor activities of these plants.

The wild plant harbours higher values of the active ingredients than the ornamental plant which needs further research.

Advanced studies are being conducted on these plants in order to isolate, identify, characterize and elucidate the structure of the BAC.

The results on *in vitro* culture will help the stable maintenance *in vitro* with lower costs along with further possible use of callus for research BAC and biological activity.

ACKNOWLEDGEMENT

Authors express thanks to State Fund of Fundamental Researches Ministry of Education and Science of Ukraine for providing of research.

REFERENCES

- [1] Premanathan M, Rajendran S, Ramanathan T, Kathiresan K, Nakashima H, Yamamoto N. Indian J Med Res 2000; 112: 73-7.
- [2] Harbone JB. Phytochemical methods: A guide to modern technique of plant analysis, Chapman and Hall, London, 1998.
- [3] Rakosy-Tican L, Balogh A, Copaci C. Propagation of Ornamental Plants 2000: 88-91.
- [4] Remotti PC, Löffler HJM. Plant Cell Tiss. Organ Cult 1995; 42:171-178.
- [5] Andrew C. A Dorling Kindersley Book, The Encyclopedia of Medicinal Plants, 2002, pp. 157-193.
- [6] Yisa, Jonathan Australian J of Basic and Applied Sci 2009; 3 (4): 3975–3979.
- [7] Rice-Evans C, Miller NJ, Paganga G. Trends Plant Sci 1997; 2:152–9.
- [8] Aiyegroro, OA, Okoh AI. 2010. BMC compl. And Alt. Med10: 21.
- [9] Ody P. The Complete Medicinal Herbal, Dorling Kindersley, New York, 1993, pp. 43-45.
- [10] State Pharmacopoeia of Ukraine 1.4, Ukrainian Scientific Centre Pharmacopoeial quality of drugs, Charkiv, 2011, pp.269-275.