Plant Archives Vol. 19, Supplement 2, 2019 pp. 398-402 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ENHANCEMENT OF *GARDENIA JASMINOIDES* ELLIS FRIABLE CALLUS GROWTH AND ITS CONSTITUENTS OF SOME BIOACTIVE COMPOUNDS

Siham A. Salim*, Sumaya Y. Hamza and Mustafa S. Habeeb
Al-Musaib Technical College, University of Al-Furat Al-Awsat Technical, Iraq
*Corresponding author: dr.sihamabdalrazzaq@yahoo.com

Abstract

The current investigation was done to improve development of the induced callus from the *Gardenia jasminoides* plant using the casein hydrolysate as an elicitor and to determine a component of the bioactive compounds in callus cultures using HPLC system. Callus was stimulated by planting the nodal explants on an MS medium supplemented with a combination of 2 $mg.l^{-1}$ TDZ + 0.3 $mg.l^{-1}$ and the cultures were incubated in the growth room at 25 ±2 °C for four weeks till the increase of the callus was completed. A particular weight of induced callus was taken and subcultured on the same combination of growth regulators with concentrations of 0, 50 and 100 $mg.l^{-1}$ of casein hydrolysate (CH). Cultures have been incubated under the same previous conditions of growth. Results confirmed that there was no significant difference in the fresh weight of the callus between CH treatments. Dry weight showed a significant difference between the treatments. The concentration 100 $mg.l^{-1}$ gave the highest rate of 564 mg. Also, the callus tissue and color transformed from the compact white creamy tissue to the friable colored tissue as a result of invigorating the bioaccumulation of active compounds in it. Bioactive compounds were evaluated in ethanolic extract for dry powdered callus samples using HPLC. Results demonstrated the significant effect of CH concentrations in increasing the amount of active compounds significantly as compared with control for the crocin, geniposide and genipin, while the increase was not significant for the two compounds iridoid glucoside and quanic acid.

***Keywords*:** Bioactive compounds, friable callus, *Gardenia jasminoides*, casein hydrolysate.

Abbreviations: CH: casein hydrolysate, HPLC: High Performance Liquid Chromatography, TDZ: Thidiazuron, NAA: Naphthalene Acetic Acid.

Introduction

Gardenia jasminoides Ellis plants are popular evergreen aromatic shrubs belonging to the family Rubiaceae, with sweet fragrance white flowers, native in China and Japan and recently, they are found in multiple areas in the world (Al-Juboory *et al.*, 1998; Kobayashi and Kaufma, 2006; Xiao *et al.*, 2017). These plants are known for their ornamental value and medical benefits as they are valuable sources of many secondary metabolites such as phenols, iridoids, quinines and carotenes, which are used as pharmaceuticals, antioxidants and anti-pathogenic microorganisms (Lee *et al.*, 2009). Flowers of *Gardenia* are used for preparing perfume tea, which is used for relaxation, improving sleep, and treating colds and flu symptoms in the Chinese medicine. Moreover, the crude extracts of *G. jasminoides* used for treating hemorrhage, vascular disease, nervous disorders, toothache, pancreatitis, hepatitis, jaundice, wounds, burns, muscles injuries, skin irritation, anti-angiogenic and treating Diabetes through blocking UP2(Uncoupling Protein 2) enzyme that depresses the secretion of insulin from pancreas (Yang, 2002; Lelono *et al.*, 2009; Hwang *et al.*, 2010; Manikam *et al.*, 2014).

Secondary metabolites are naturally occurring in plants with simple or complex chemical nature that are called phytochemicals. Hence, and in order to obtain these metabolites, large amounts of plant yield are consumed, leading to the high depletion of huge quantities of cultivated plants without knowing the actual content of these metabolites. Recent advances in plant tissue culture technique demonstrate it as a powerful and an alternative important source for the production of various compounds or *de novo* production of new compounds that did not originally exist in the whole plant. These compounds can be used directly or as raw materials in biosynthesis for many active compounds that are important at the pharmaceutical and industrial levels (Eba, 2005; Ahmed *et al.*, 2013).

The ability of explants grown *in vitro* to produce and accumulate various active secondary metabolites that are important commercially and medicinally depends on their ability to grow and multiply, as well as the type and concentrations of elicitors or precursors that are added to the culture medium for the enhancement of plant cultures multiplication and stimulation the metabolic pathways on high productivity of the active compounds, in which the callus and cell suspension cultures are most important techniques used for this purpose (Al-Jobouri *et al.*, 2016; Salim and Habeeb, 2018). Various endeavors have been reported for the enhancement of biosynthesis and accumulation of desired secondary metabolites of different *in vitro* cultures of many plant species through using elicitors or precursors, such as production of ephedrine in callus cultures of *Ephedra alata* (Hegazi and El-Lamey, 2011); phytosterols in callus cultures of *Boerhaavia paniculata* (Souza *et al.*, 2014) and phenols in *Verbascum thapsus* callus cultures (Al-Jibouri *et al.*, 2016). The purpose of the present study was to enhance the growth of friable callus, as well as to increase the production of some bioactive metabolites from it using of casein hydrolysate (CH) as an elicitor and quantitative determination of these metabolites using high performance liquid chromatography (HPLC) technique.

Materials and Methods

Explants source, sterilization and induction of callus

Healthy and fresh shoots of *Gardenia jasminoides* (about 8 cm long) were excised from 2-3 years old plants. Subsequent to expelling of leaves, shoots were divided into nodal explants. The later were washed with liquid cleanser under running tap water. After that, they were submerged for 30 min. in cool antioxidant solution of 1:1; citric acid (100

mg.l^{-1}) and ascorbic acid (150 mg.l^{-1}), followed by rinsing with sterile DH_2O. Nodal explants were then surface sterilized by soaking in aqueous solution of 0.1% mercuric chloride ($HgCl_2$) for 5 min. and rinsed 3 times with sterile DH_2O for 3 min each. For callus induction and multiplication, the sterile nodal explant were shorted to segments of 1.0 cm in length and cultured vertically on the basal medium of Murashige and Skoog (1962) (4.43 g.l^{-1} of MS powder medium, HI Media Ltd., India) enhanced with 30 g.l^{-1} of sucrose and plant growth regulators combination of 2 mg.l^{-1} TDZ+ 0.3 mg.l^{-1} NAA, which was the optimal formula for callus induction (Salim and Hamza, 2017) and solidified with 7.0 g.l^{-1} of agar. Cultures were reserved under growth conditions of temperature at 25 ±2 ºC and 16 hours light (1000 lux) for 4 weeks. Callus appeared on the bases of nodal explants during the first two weeks of culture on the above medium (Figure-1). Subcultures were accomplished at regular intervals in a similar development conditions to gain enough amounts of callus for the next experiment.

Fig. 1(A) : *Gardenia jasminoides* plant

Fig. 1(B) : Callus induction in cut edge of node cultured on MS supplemented with 2.0 mg.l^{-1} TDZ + 0.3 mg.l^{-1} NAA. Bar 1 cm.

Elicitation of metabolites production with casein hydrolysate

Casein hydrolysate (CH), was utilized as an elicitor, added at concentrations of 0.0, 50 and 100 mg.l^{-1} to the MS medium with the same previous formula of TDZ and NAA, in order to enhance the growth of friable callus and accumulation of bioactive metabolites in callus cultures. About 150 mg of initiated callus was taken and inoculated on these media. Callus fresh and dry weights were measured after reservation of cultures under the same above growth conditions.

Extraction of bioactive compounds from callus

After getting adequate amounts of calli on the different concentrations of casein hydrolysate, the calli samples were dried at 55ºC for 48 hours. Extraction was completed by taking 1.0 g of dried samples and crushed in paste-mortar, followed by suspended in 5 ml of ethanol: water (80: 20, v/v) in glass tubes. The suspension was exposed to ultra-sonication (Branson sonifier, USA) at 60% duty cycles for 25 ºC followed by centrifugation at 7.500 rpm for 15 min. The clear supernatant of each sample was evaporated under vacuum (Buchi Rotavapor Re Type). Dried samples were re-suspended in 1.0 ml HPLC grade methanol by vortexing, the mixture were passed through 2.5 µm disposable filter and stored at 4ºC for further analysis, then 20 µl of the sample injected into HPLC system according to the optimum conditions where separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10A-SPD spectrophotometer, column: capcellpack C-18, 3µm particle size (50 × 2.0 mm I.D). Mobile phase: linear gradient of 100mM phosphate: solvent B acetonitrile, UV detection 240 nm wavelength, flow rate 1.2 ml.min^{-1} at 30ºC (Yang *et al.*, 2009). The standard compounds were purchased from Sigma-Aldrich, USA. Readings were measured and retention time was calculated for standards and samples under study. Bioactive compounds concentrations were determined by comparing the standard peak area with the sample under the same conditions using the following equation:

Concentration of sample (µg.ml^{-1}) = (area of sample / area of standard) × concentration of standard × dilution factor

Statistical analysis

Data were subjected to completely randomized design and means were compared according to least significant difference (LSD) under the probability of ≤ 0.05 (GenStat, 2012).

Results and Discussion

Production of Friable Callus Biomass

Results displayed in Figure-2, demonstrated no significant differences were seen among CH concentrations on callus fresh weight. In contrast, significant differences were noticed in callus dry weight, in which the highest value (564 mg) was recorded in 100 mg.l^{-1} CH, followed by 50 mg.l^{-1} CH which gave 499 mg. Furthermore, the callus tissue was changed over from white creamy color to friable and colored texture when transplanted on medium containing CH concentrations (Figure-3). Casein hydrolysate is a nitrogenic acid which is a source of organic nitrogen which affects the callus growth, Therefore, its presence in the culture medium improves callogenesis (Hegazi and El-Lamey, 2011).

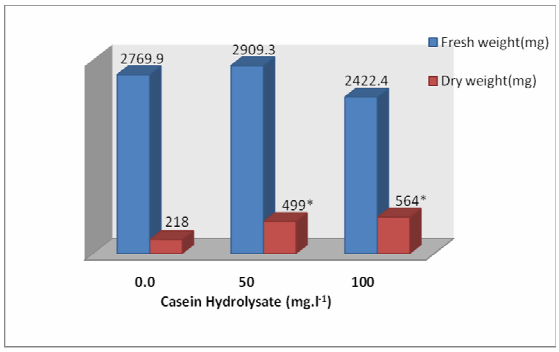

Fig. 2: Effect of casein hydrolysate concentrations on fresh and dry weights of callus. * significant at P≤ 0.05.

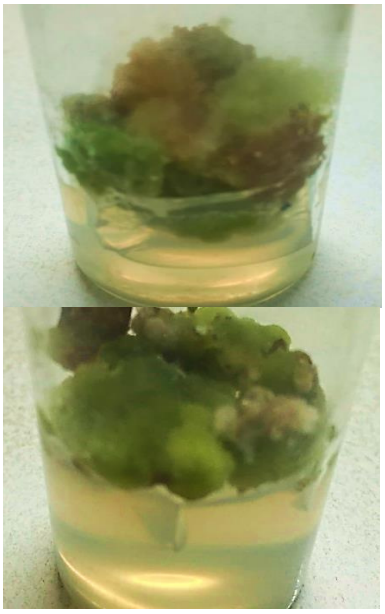

Fig.-3: (A) and (B) callus converted to friable and colored texture when transferred to medium containing CH . Bar 1 cm.

Effect of casein hydrolysate on accumulation of some bioactive compounds in *G. jasminoides* callus cultures

Iridoidglucoside, quanic acid, crocin, geniposide and genipin were chosen as standards for quantitative estimation of bioactive compounds in callus extracts. The standards retention times, areas and peaks are shown in Table-1 and Figure-4.

Table 1 : Retention time, peak area and concentration of standard compounds

Seq.	Compound	Retention time (min)	Peak area	Concentration (µg.ml^{-1})
1	Iridoidglucoside	1.92	109347	25
2	Quanic acid	3.08	105461	25
3	Crocin	4.18	106334	25
4	Geniposide	5.02	109089	25
5	Genipin	6.45	97317	25

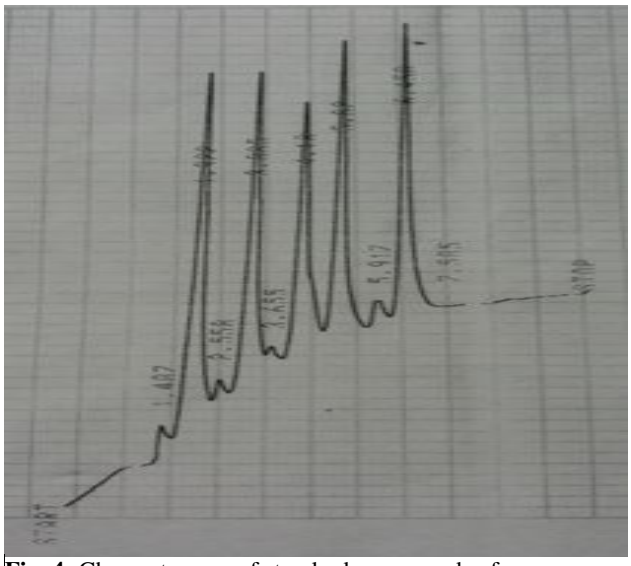

Fig. 4: Chromatogram of standard compounds of Iridoidglucoside, Quinic acid, Crocin, Geniposide and Genipin

The results in Table- 2, Figure- 5 and Figure -6 show that the concentrations of the bioactive compounds within the callus extract and calculable with HPLC, show variations among the concentrations of casein hydrolysate (CH), that found to be vital effective in the induction of bioactive compounds accumulation in callus tissues. It absolutely was found that the rise in the concentration of the compounds Iridoidglucoside and quanic acid at 50 and 100 mg.l^{-1} of CH was not significant as compared to the control treatment. Aadditionally, the crocin compound was significantly exaggerated by increasing the concentration of CH in the culture medium to reach the highest value of 382.16 µg.ml^{-1} (21.53%) at 100 mg.l^{-1} of CH as compared with control (221.50 µg.ml^{-1}, 19.69%), It is noticeable from the amendment in the color of callus to orange (Figure-3 previous) due to the accumulation of this compound.

The accumulation of geniposide was significant in the callus cultures with the increasing of the concentration of CH in the culture medium to reach the maximum content of 81.54 µg.ml^{-1}(4.59%) at concentration 100 mg.l^{-1}, which significantly overcame on control and concentration 50 mg.l^{-1} CH. On the other hand, the results of the same table show that the concentration of the genipin was significantly increased in the callus developed on the medium containing CH to reach its highest value of 795.49 µg.ml^{-1} (44.82%) at the concentration of 100 mg.l^{-1} CH compared to the control treatment and concentration 50 mg.l^{-1} CH.

Table 2: Effect of casein hydrolysate concentrations on the production of bioactive compounds from callus tissues

Compound (µg.ml^{-1})	Casein hydrolysate concentrations (mg.l^{-1})			LSD ≤0.05
	0.0	50	100	
Iridoidglucoside	281.54	341.99	361.55	NS*
Quanic acid	109.26	148.16	154.29	NS*
Crocin	221.50	366.77	382.16	112.081
Geniposide	49.08	66.68	81.54	10.997
Genipin	463.46	606.99	795.49	199.696

*NS: not significant

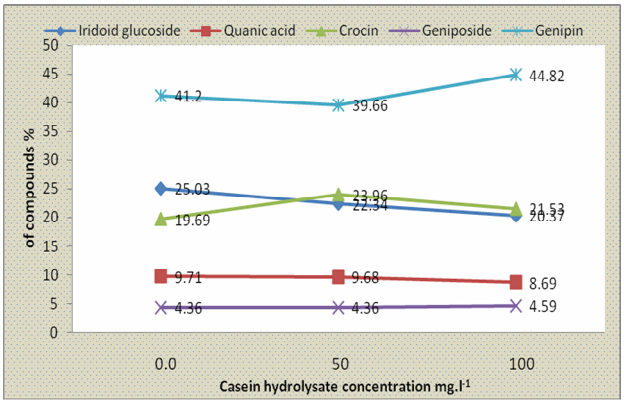

Fig. 5 : Percentages of bioactive compounds from the total extract (%).

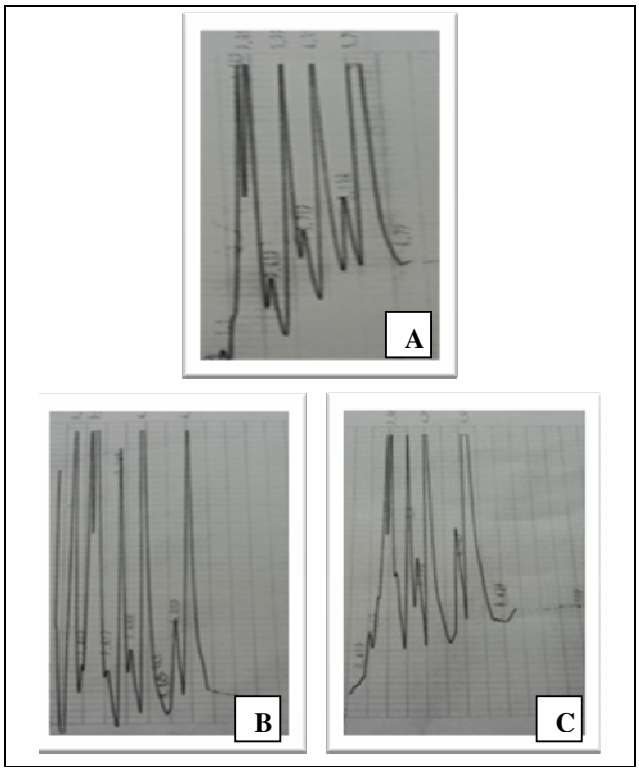

Fig. 6 : HPLC analysis of the crude extracts of *G. jasminoides* callus cultured in : (A) control , (B) 50 mg.l^{-1} CH and (C) 100 mg.l^{-1} CH .

Previous investigations have demonstrated that the utilization of CH in the culture medium increase the development of callus tissues for various species of plants such as induction of callogenesis and the accumulation of alkaloids in *Catharanthus roseus* plant (Ahmed *et al.*, 2000), induction of ephidrine in callus tissue of *Ephedra alata* (Hegazi and El-Lamey, 2011) and indican compound in *Indigofera tinctoria* callus cultures (Rubin-Jose and Nair, 2018). Results of current investigation exhibited obviously the significance of CH in the improvement of friable callus development and bioaccumulation of most significant bioactive and pharmaceutical compounds. *In vivo* and *in vitro* investigations have revealed that iridoids compounds show a board scope of pharmacological impacts such as hepato-protection, cardiovascular, anti-mutagenic, hypoglycemic, viral anti-inflammatory and antitumor (Viljoen *et al.*, 2012).

Quanic acid has a wide spectrum of anti-inflammatory, antioxidant and hepato-protective activities (Pero and Lund, 2009). Also, it is a potent drug for treating prostate cancer (Inbathamizh and Padmini, 2013). Furthermore, crocin which is important natural carotenoids revealed to be progressively powerful as antioxidant, anti-proliferative, bio-surfactant, brain neurodegenerative disorders, Alzheimer disease disorder and enhancer for learning and memory (Wenhao *et al.*, 2010; Singla and Bhat, 2011). Moreover, geniposide a compound displays numerous pharmaceutical activities including anti-diabetic, neuro-protective, anti-inflammatory, hepato-protective, antidepressant, analgesic, antioxidant, cardio- protective, immune regulatory , anti-Alzheimer's disease activity and anti-tumoral effects (Zhou *et al.*, 2019). On the other hand, genipin, another important compound found in the callus extract of *G. jasminoides* which is used to treat diabetes through significant inhibition of uncoupling 2(UP2) enzyme, cancer prevention agent, antioxidant, anti-inflammatory, anti-phlogistic, antithrombotic agent (Manikam *et al.*, 2014).

Conclusion

Active phytochemicals are one of the groups of chemical compounds occur naturally in plants. The most significant bioactive compounds that have been analyzed in callus cultures of *Gardenia jasminoides* plant are iridoid glucoside, quanic acid, crocin, geniposide and genipin. Just as increment the production of these compounds in the callus cultures using casein hydrolysate as an elicitor, which was added to the culture medium of developing callus, showing the importance of plant tissue culture technique in the production of effective compounds of various plant species in reasonable quantities and within a brief timeframe and in a restricted and controlled space.

Acknowledgment

The authors would like to thank the staff of the laboratories of the Ministry of Science and Technology for their invaluable assistance in estimating the effective compounds of the HPLC.

References

Ahmed, F.A.; Abdel-Fatah, O.M.; Kobeasy, M.I. and Ahmed, O.K. (2000). Factors affecting growth and indole alkaloids content of *Catharanthus calli* (*Catharanthus reseus* L.): amino acids, casein hydrolysate and irradiation. Biotechnology, 5: 379-384.

Ahmed, S.; Garg, M.; Tamboli, E.T.; Abdin, M.Z. and Ansari, S.H. (2013). *In vitro* production of alkaloids: factors, approaches, challenges and prospects. Pharmacog. Rev., 7(13):27-33.

Al-Jibouri, A.J.M.; Abed, A.S.; Ali, A.A. and Majeed, D.M. (2016). Improvement of phenols production by amino acids in callus cultures of *Verbascum thapsus* L. Amer. J. Plant Sci., 7: 84-91.

Al-Juboory, K.H.; Skirvin, R.M. and Williams, D.J. (1998). Callus induction and adventitious shoot regeneration of Gardenia (*Gardenia jasminoides* Ellis) leaf explants. Sci. Hort., 72: 171-178.

Eba, A. (2005). Isolation and Characterization of Bioactive Compounds from Suriname and Madagascar Flora. II. A synthetic Approach to Lucilactaene, A PhD. in Chemistry Dissertation Submitted to the Faculty of the Virginia

Polytechnic Institute and State University Blacksburg, Virginia, Pp.1-2.

GenStat. (2012). GenStat Procedure Library Release PL 18.2. Edition.4.VSN International Ltd. Roth Amsted Experimental station. UK.

Hegazi, G.A. and El-Lamey, T.M. (2011). Callus induction and extraction of ephedrine from *Ephedra alata* Decne. cultures. American-Eurasian. J. Agric. Environ. Sci., 11(1):19-25.

Hwang, S.M.; Lee, Y.J.; Yoon, J.J. and Lee, S.M. (2010). *Gardenia jasminoides* inhibits tumor necrosis factor-alpha-induced vascular inflammation in endothelial cells. Phytother. Res., 24-26.

Inbathamizh, L. and Padmini, E. (2013). Quanic acid as a potent drug candidate for prostate cancer-a comparative pharmacokinetic approach. Asian J. Pharma. Clin. Res., 6(4):106-112.

Kobayashi, K.D. and Kaufma, A.J. (2006). Common Gaardenia. College of Tropical Agriculture and Human Resources. University of Hawaii at Manoa Cooperative Extension Service Ornamental and Flowers. 32:1-7.

Lee, J.H.; Lee, D.U. and Jeong, C.S. (2009). *Gardenia jasminoides* Ellis ethanol extract and its constituents reduce the risks of gastric and reverse gastric lesions in rats. Food and Chem. Toxic., 47: 1127-1131.

Lelono, R.A.; Tachibana, S. and Itoh, S. (2009). Isolation of antifungal compound from *Gardenia jasminoides*. Pakistan J. Biotech. Sci., 12(13): 949-951.

Manikam, B.; Nair, R. and Elumalai, M. (2014). 'Genipin'-the natural water soluble cross-linking agent and its importance in the modified drug delivery systems : an overview. Curr. Drug Deli., 11(1): 139-139.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physio. Planta., 15(3):473-497.

Pero, R.W. and Lund, H. (2009). *In vivo* treatment of humans with quanic acid enhances DNA repair and reduces the influence of life style factors on risk to disease. IJBB, 5(3): 293-305.

Rubin-Jose, A.S. and Nair, G.M. (2018). Production of indican from the callus cultures of *Indigofera tinctoria* Linn. Int. J. Adv. Res., 6(10): 16-22.

Salim, S.A. and Habeeb, M.S. (2018). Elicitation of secondary metabolites production from *Thevetia neriifolia* Juss *in vitro* cultures using chemical elicitors. 10th International Conference on Chemical, Agricultural, Biological and Environmental Sciences (CABES-18) March 19-20, Dubai (UAE):57-66.

Salim, S.A. and Hamza, S.Y. (2017). An efficient protocol for micro propagation of *Gardenia jasminoides* Ellis. Biosci. Biotech. Res. Asia, 14(2):757-766.

Singla, R.K. and Bhat, V.G. (2011). Crocin: an overview. Indo Global J. Pharm. Sci., 1(4):281-286.

Souza, J.M.M.; Bekov, S. and Santos, A.S. (2014). Improvement of friable callus production of *Boerhaavia paniculata* Rich and the investigation of its lipid profile by GC/MS. Ann. Brazilian Acad. Sci., 86(3): 1015-1027.

Viljoen, A.; Mncwangi, N. and Vermaak, I. (2012). Anti-inflammatory iridoids of botanical origin. Curr. Med. Chem., 19(14): 2104-2127.

Wenhao, H.; Xuan, L.; Honggao, X.; Ying, G.; Fang, Y. and Yanxiang, G. 2010. On-line HPLC-ABTS screening and HPLC-DAD-MS/MS identification of free radical scavengers in Gardenia (*Gardenia jasminoides* Ellis) fruit extracts. Food Chem., 123(2):521-528.

Xio, W.; Li, S.; Wang, S. and Ho, C.T. (2017). Chemistry and bioactivity of *Gardenia jasminoides*. J. Food Drug Ana., 25: 43-61.

Yang, B.; Liu, X. and Gao, Y. (2009). Extraction optimization of bioactive compounds (crocin, geniposide andd total phenolic compounds) from Gardenia (*Gardenia jasminoides* Ellis) fruits with response surface methodology. Innov. Food Sci. Emerg. Technol., 10: 610-615.

Yang, Y. (2002). Chinese Herbal Medicine Comparisons and Characteristics. Churchill Livingstone, London.

Zhou, Y.X.; Zhang, R.Q.; Rahman, K.; Cao, Z.X.; Zhang, H. and Peng, C. (2019). Diverse pharmacological activities and potential medicinal benefits of geniposide. Evidence-Based Comp. Alter. Med., 2019:1-15.