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ORIGINAL ARTICLE

Media Standardization for Enhanced Production of Bacoside of *Bacopa monnieri In situ* Condition

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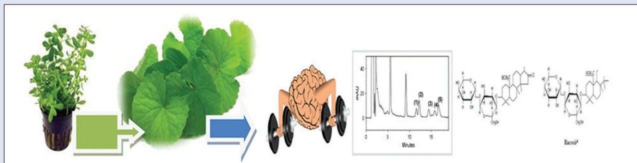
ABSTRACT

Background: Secondary metabolites are known to not only play a major role in the adaptation of plants to their environment but also represent an important source of active pharmaceuticals Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. The present study describes the enhancement of active principal bacoside of *Bacopa monnieri* by using stress and precursor compound. **Materials and Methods:** The study was done *in situ* condition. With incorporation of precursor compounds (phenylalanine and tyrosine [Ty]) and stress-inducing compounds ($ZnCl_2$, $CoCl_2$, and $CuSo_4$). **Results:** The maximum content of bacoside recorded in 7-day-old plant treated with 25 µM $CoCl_2$ (5.313%) separately followed by 15-day-old plants treated with 60 mg/100 ml Ty (5.15%) and 15-day-old plants treated with 50 µM of $CoCl_2$ (5.15%). **Conclusion**: Enhancement of bacoside in *B. monnieri* was observed more than 2% than *in vivo* condition by giving $CoCl_2$ stress *in situ* condition. This kind of experiment is not well documented till date.

Key words: *Bacopa monnieri*, bacoside, enhancement, *in situ*, metal stress, precursor

SUMMARY

- Secondary metabolites are known to play a major role in the adaptation of plants to their environment, but also represent an important source of active pharmaceuticals. Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Present study describes the enhancement of active principal Bacoside of *Bacopa monnerrie* by using stress and precursor compound. After analysing the results we can conclude that Tyrosin incorporation is the best enhancer treatment than phenylalniine in Bacopside production. $CoCl_2$ is effective stress generating compound that favours the Bacoside biosynthesis in Bacopa monerrie [Table 1].Tyrosin is comparable cheap compound than cobalt chloride that gives the same results in 7 days.



Bacopa monnerrie in situ Enhanced Production of Bacopside *(by stress)*:A Memory enhancer

Abbreviations used: BM :Bacopa monerrie; Ty: Tyrosine; Phy: Phenylalanine

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INTRODUCTION

Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors, and industrially important biochemicals. These molecules are known to not only play a major role in the adaptation of plants to their environment but also represent an important source of active pharmaceuticals Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Environmental factors, namely, temperature, humidity, light intensity, the supply of water, minerals, and CO_2 influence the growth of a plant and secondary metabolite production.

Oxidative responses of plants to pathogens and other environmental stresses have received considerable recent attention, which in turn enhance the phenylalanine ammonia lyase enzyme activity that has a key role in the production of various secondary metabolites. Medicinal plants serve as a natural resource for the production of these valuable secondary metabolites and are important to maintain the ecosystem balance; hence, there is a requirement to develop a technique to enhance the production of secondary metabolites with economic feasibility with the minimum use of natural resources. Many scientists have worked on aspect of medicinally valuable moieties production using plant tissue culture (PTC) technique (Chaturvedi 2012, 2013, 2013a, 2013b, 2013c, 2014, 2014a, 2014b, 2014c, 2014d, 2014e, 2014f, 2015, 2017).[1-14] Although PTC serves as an alternative mean to produce the valuable secondary metabolites but to maintain the cultures *in vitro* is costly and difficult task; hence, there is a need to develop a technique that provides a mean to maximize the bioactive principal in economic way using *in situ* condition.

Bacopa monnieri (BM) has a primarily triterpenoid saponins called bacosides, which exhibits minimal observable adverse effects at standard dosages. BM demonstrates antioxidant,[15] hepatoprotective,[16] and neuroprotective[17] According to Liu *et al.*,[18] bacopaside I exhibits neuroprotective, antioxidant, and cerebral adenosine triphosphate-increasing effects postcerebral ischemia in rats. There is also evidence for potential attenuation of dementia, Parkinson's disease, and epilepsy. Current evidence suggests that BM acts through the following mechanisms, i.e., antioxidant neuroprotection (through redox and enzyme induction), acetylcholinesterase inhibition and/or choline acetyltransferase activation, β-amyloid reduction, increased

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cerebral blood flow, and neurotransmitter modulation (acetylcholine, 5-hydroxytryptamine, and dopamine). BM appears to exhibit low toxicity in model organisms and humans.[19] Metal ions (lanthanum, silver, and cadmium) and oxalate are also influenced secondary metabolite production.[20] Effective accumulation of metals (Cr, Fe, Zn, and Mn) also produced an increase of oil content up to 35% in *Brassica juncea*.[21] Cu^{2+} and Cd^{2+} have been shown to induce higher yields of secondary metabolites such as shikonin.[22] Co^{2+} and Cu^{2+} having the stimulatory effect on the production of secondary metabolites.[23]

Keeping these facts, the recent approach was done to develop an economically affordable technique for enhanced production of bacoside in BM *in situ* condition. In the present investigation, we have tried two precursors compounds (tyrosine [Ty] and phenylalanine [PA]) and stress-producing compounds (copper sulfate, zinc chloride, and cobalt Chloride) in various concentration by incorporating them on basal media (HiFoliar media purchased from Hi-media) separately, to maximize bacoside production.

MATERIALS AND METHODS

Plant material of BM has been procured from Pharmanza herbal Pvt. Ltd. campus. Identification of the plant has been done using Pharmanza herbarium repository voucher no PHPL/HB/006.A. The plant material was grown in a beaker containing basal media (HIFoliar media) at 3 g/l concentration with incorporation with different concentrations of precursors (Ty and PA) and stress-producing compounds (copper sulfate, zinc chloride, and cobalt chloride) at normal light condition (16 h light and 8 h of dark period) pH 5.8 on room temperature in the month of July 2017 for 7 and 15 days of time period [Table 1].

Extraction and quantification of bacoside

The plant material of BM was harvested at the time period of 7 and 15 days of date of treatment separately. The plant material was cut into pieces, and extracts were prepared following methanolic cold extraction method. The samples were kept for 48 h in methanol, filtered, and dried *in vacuo*. Dried and weighed all samples were subjected for high-performance liquid chromatography (HPLC) qualitative and quantitative estimation separately for bacoside content.

High-performance liquid chromatography analysis

HPLC analysis was done using Shim-pack GIST C18, 5 µm, 4.6 mm × 250 mm column with dissolved 0.14 g of anhydrous potassium dihydrogen phosphate in 900 mL of water, add 0.5 ml of phosphoric acid, dilute with water to 1000 mL, mix the content, filter it through 0.2 µm 0 membrane filter and degas mobile phase A, while degassed acetonitrile was used AS mobile phase B. Injection volume was 20 µl used with 1.5 mL/min (Gradient Mode). Detection was done at wavelength 205 nm.

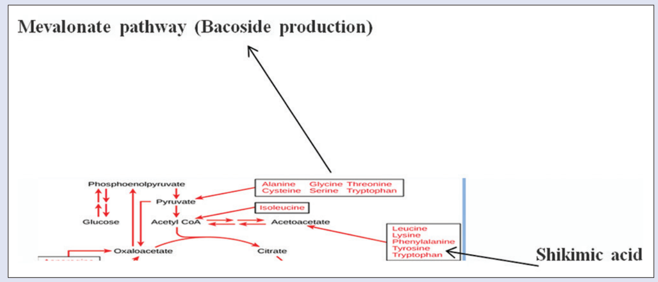

Figure 1: Proposed biosynthetic pathway of bacoside

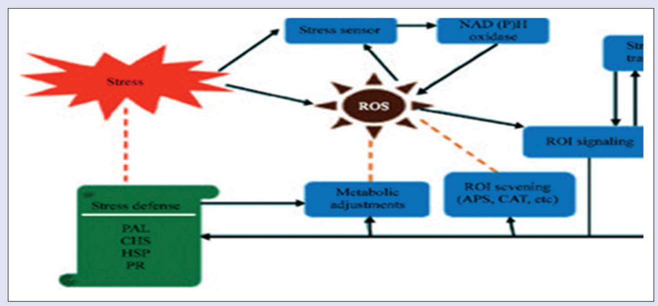

Figure 2: Mechanism of reactive oxygen species production during abiotic and biotic stress that in turn effects the gene expression of phenylalanine ammonia lyase, chalcone Synthase. These enzymes have indirect role in the biosynthesis of saponin

Table 1: Effect of stress on Bacopside production in Bacopa monnerrie (Brahmi)

Media composition	Bacoside content % of 7 days old samples	Bacoside content % of 15 days old sample	Bacpside content % Increase/Decrease with control (±)	
			After 7 days	After 15 DAYS
Basal + 100mg/100ml PA	3.46	4.17	+ 0.42	+ 1.13
Basal + 60 mg/100ml PA	3.26	4.05	+0.22	+1.01
Basal + 80 mg/100ml PA	3.31	4.63	+0.27	+1.59
Basal + 60mg/100ml Tyrosin	3.09	5.31	+0.04	+2.27
Basal + 80mg/100ml Tyrosin	3.54	3.39	+0.5	+0.35
Basal + 100mg/100mlTyrosin	3.65	4.10	+0.061	+1.06
Basal + 50µM CuSo4	4.04	4.59	+ 1	+1.55
Basal + 75 µM CuSo4	4.02	4.23	+ 0.98	+1.19
Basal + 100µM CuSo4	4.39	4.25	+ 1.35	+1.21
Basal + 25µM ZnSo4	3.09	4.89	+ 0.05	+1.85
Basal + 50µM ZnSo4	2.78	2.53	- 0.26	- 0.51
Basal + 75µM ZnSo4	2.99	2.36	- 0.05	- 0.68
Basal + 100µM ZnSo4	2.96	2.11	- 0.08	- 0.93
Basal + 25µM CoCl2	5.31	4.38	+ 2.27	+ 1.34
Basal + 50µM CoCl2	3.86	5.15	+0.82	+2.11
Basal + 75µM CoCl2	4.40	3.58	+ 1.36	+ 0.54
Basal + 100 µM CoCl2	3.40	2.91	+ 0.36	-0.94
Basal	4.05	4.10	+1.01	+1.06
Control *in situ*		3.04		

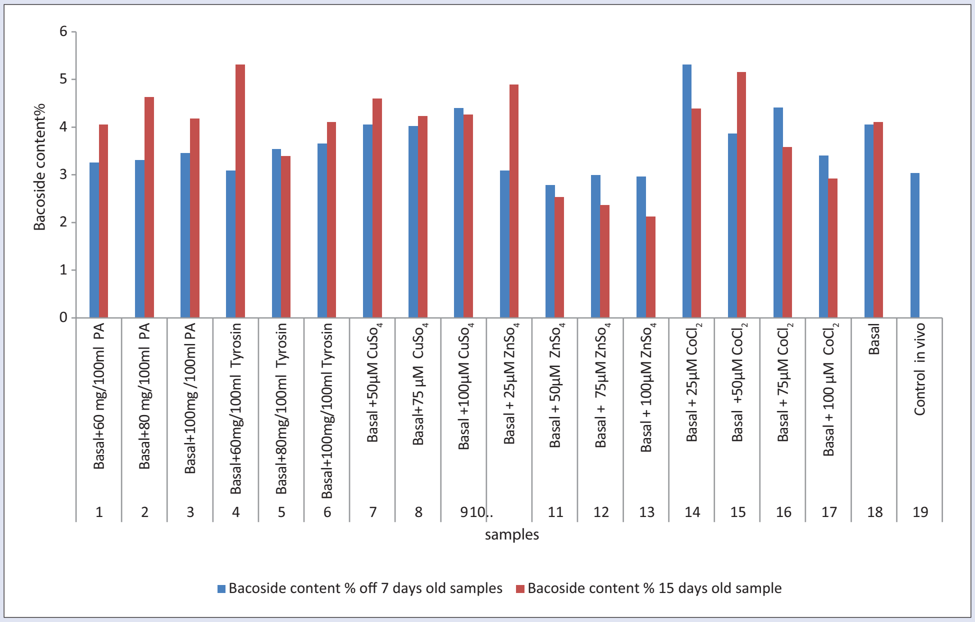

Figure 3: Graphical presentation of enhancement of Bacoside in *Bacopa monnieri* using metallic stress and precursors compounds

Table 2: The effect of various metallic and precursor compounds on biosynthesis of bacoside in *Bacopa monnieri*

Media composition	Bacoside content percentage of 7 days old samples	Bacoside content percentage of 15 days old sample	Bacpside content percentage increase/decrease with control (+/-)	
			After 7 days	After 15 days
Basal +100 mg/100ml PA	3.46	4.17	+0.42	+1.13
Basal +60 mg/100ml PA	3.26	4.05	+0.22	+1.01
Basal +80 mg/100ml PA	3.31	4.63	+0.27	+1.59
Basal +60 mg/100ml Ty	3.09	5.31	+0.04	+2.27
Basal +80 mg/100ml Ty	3.54	3.39	+0.5	+0.35
Basal +100 mg/100ml Ty	3.65	4.10	+0.061	+1.06
Basal +50 µM $CuSo_4$	4.04	4.59	+1	+1.55
Basal +75 µM $CuSo_4$	4.02	4.23	+0.98	+1.19
Basal +100 µM $CuSo_4$	4.39	4.25	+1.35	+1.21
Basal +25 µM $ZnSo_4$	3.09	4.89	+0.05	+1.85
Basal +50 µM $ZnSo_4$	2.78	2.53	−0.26	−0.51
Basal +75 µM $ZnSo_4$	2.99	2.36	−0.05	−0.68
Basal +100 µM $ZnSo_4$	2.96	2.11	−0.08	−0.93
Basal +25 µM $CoCl_2$	5.31	4.38	+2.27	+1.34
Basal +50 µM $CoCl_2$	3.86	5.15	+0.82	+2.11
Basal +75 µM $CoCl_2$	4.40	3.58	+1.36	+0.54
Basal +100 µM $CoCl_2$	3.40	2.91	+0.36	−0.94
Basal	4.05	4.10	+1.01	+1.06
Control *in situ*		3.04		

PA: Phenyl alanine; Ty: Tyrosine

Standard Solution a Preparation (Bacoside A3)

Sonicate a weighed quantity of USP Bacoside A3 RS in methanol to obtain a solution with a concentration of about 0.5 mg/mL, filter it through 0.2 µm membrane filter, and inject standard 20 µl.

Sample solution preparation

Transfer 50 mg of powder Bacopa extract, equivalent to about 25 mg triterpene glycosides, to a 25 mL volumetric flask, and add 15 mL of methanol. Sonicate and heat gently for 15–20 min, dilute with methanol to volume, and mix. Before injection, pass through a membrane filter of 0.2 µm or fine pore size, discarding the first 5 mL of the filtrate and inject 20 µl 20 min, dilute with methanol to volume, and mix. Before injection, pass through a membrane filter of 0.2 µm or fine pore size, discarding the first 5 mL of the filtrate, and inject 20 µl.

RESULTS AND DISCUSSIONS

BM is a Medhya Rasayana plant, and it is used for the treatment of several diseases since time immemorial including neurological disorders. It is a well-known fact that Ty and PA serve as intermediate compounds in biosynthetic pathway of saponin (bacoside). PA produced by shikimic acid pathway would use in the formation of acetyl COA, which further use in the production of saponin through mevalonate pathway.[24] Hence, Ty and PA have a role in the biosynthesis of saponin. To keep this fact in the present investigation, we have used Ty and PA for treatment to maximize the bacoside production *in situ* condition [Figure 1].

As it is shown in Figure 2, stress produces reactive oxygen species (ROS) which in turn effect the expression of phenylalanine lyase enzyme gene and further effect the production of saponin. We have selected Zn, Co, and Cu stress that we have given to growing plant system in artificial production media [Table]. Hence, in the present report, we have used different elicitors (PA and Ty [60, 80, and 100 mg/100 ml]) $CuSo_4$, $CoCl_2$, and $ZnSo_4$ (25, 50, 75, and 100 μM) separately to enhance bacoside content. As results depict that the maximum content of bacoside recorded in 7-day-old plant treated with 25 μM $CoCl_2$ (5.313%) separately followed by 15-day-old plants treated with 60 mg/100 ml Ty (5.15%) and 15-day-old plants treated with 50 μM of $CoCl_2$ (5.15%). Maximum bacoside content (%) was observed in sample treated with 25 μM $CoCl_2$ (2.27%) and sample treated with 60 mg/100 ml Ty (2.27%) that followed by 15-day-old sample treated with 50 μM $CoCl_2$ in basal media (2.11%) [Table 2 and Figure 3]. Cobalt chloride exerts an effect on plant in such a way that it developed ROS that in turn favors the production of bacoside, whereas in case of Ty treatment that works being a precursor compound in bacoside biosynthesis and finally contribute in the production of bacoside.

CONCLUSION

After analyzing the results, we can conclude that Ty incorporation is the best enhancer treatment than PA in bacoside production. $CoCl_2$ is effective stress-generating compound that favors the bacoside biosynthesis in BM [Table 2]. Ty is comparable cheap compound than cobalt chloride that gives the same results in 7 days. This study is useful for industries. This report is not well documented till date.

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Conflicts of Interest

There are no conflicts of interest.

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