Research Article **www.ijcps.com**

International Journal of Chemical and Pharmaceutical Sciences
2014, Dec., Vol. 5 (4)

ISSN: 0976-9390
IJCPS

Comparative antioxidant potentials of exogenous elicitors supplemented *in vitro* propagated *Coleus aromaticus*

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Received: 22 Dec 2014, Revised and Accepted: 29 Dec 2014

ABSTRACT

Plant phytochemicals are important antioxidants in normal diet to reduce the chances of various disorders like diabetes, cancer, atherosclerosis, gastrointestinal diseases and arthritis. This study investigated the antioxidant potential of Murashige and Skoog (MS) exogenous elicitors (L-Phenylalanine (L-Phe), Salicylic acid (SA) and Chitosan (Ch)) supplemented containing *in vitro* propagated *Coleus aromaticus*. The variations of various *in vitro* models as total antioxidant activity (TAA), Phenylalanine ammonia lyase (PAL), Total phenolic contents (TPC) catalase (CAT) and Peroxidase (POX) were analyzed. Of these the higher Phenylalanine ammonia lyase (2.470 µmol/mg), Total phenolic content (2.82µg/g), Total antioxidant activity (0.669µg/g), Catalase (57.18 U/mg) and Peroxidase (7.34U/mg) were determined in 1.0mg/L of BAP+40mg/L of Ch supplemented culture. This study indicates that the supplementation of exogenous elicitors may be an efficient way for improvement of plant secondary metabolites, antioxidant potentials and other therapeutic uses through *in vitro* propagation without any genetic engineering modification.

Keywords: *Coleus aromaticus, in vitro* propagation, Elicitors, Total phenolic content, MS medium.

1. INTRODUCTION

Studies on dietary free radical scavenging molecules have attracted the attention to characterize phenolic compounds and phytochemical as antioxidants [1]. Herbs have also been identified as source of various phytochemicals, many of which possess important antioxidant activity. Flavanoids which are broadly distributed in the leaves, seeds and flowers of plants are a broad class of low molecular weight compounds and highly effective antioxidant and less toxic than synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) and have received superior attention and studied extensively [2]. Synthetic antioxidants such as BHT and BHA should be replaced by natural compounds due to their possible toxicity [3]. Therefore, by considering adverse effects of synthetic antioxidant on human health, alternative natural and safe sources of food antioxidant should be identified [4]. Plant extracts can be natural alternatives to synthetic antioxidants due to the presence of secondary metabolites possess similar or even higher antioxidant activity, so they are strongly of interest in the food industry [5].

Antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide massive scope in correcting the imbalance through regular intake of proper diet. In addition *C.aromaticus* is used in traditional Indian systems as antioxidant, nephroprotectant[6] antimicrobial[7], leishmanial[8], antitumor, antiepileptic activity[9] and additional biological activities. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits [10-13]. Hence, in this view deals comparative antioxidant potentials of L-Phe, SA and Ch supplemented *in vitro* propagated *Coleus aromaticus* was investigated.

2. MATERIALS AND METHODS

2.1. Plant material

Coleus aromaticus (Benth) obatained from ABS botanical garden and maintained in green

house of periyar university, Tamil Nadu, India for throughout the study.

2.2. Micropropagation

2.2.1. Preparation of culture medium

The Basal medium as described by Murashige and Skoog [14] was used. Different concentration of L-Phe (0.5-2.5mg/L), SA (0.2-1.0mg/L) and Ch (20-80mg/L) were supplemented with 1.0mg/L of Benzylaminopurine containing MS medium.

2.2.2. Culturing and propagation

Nodal explants of *C.aromaticus* were inoculated aseptically on 1.0mg/L BAP containing MS medium for shoot bud induction. Further, subculturing was carried out with different concentration of L-Phe, SA and Ch individually. These cultures were incubated at a temperature of 25±2°C under 16/8 h light/dark cycle with relative humidity (60% - 80%). After 45 days of culturing, tissue cultured plants were shifted to soil in glass house for further development.

2.2.3. Analysis of variations in antioxidant potentials

The leaves from each elicitors (L-Phe, SA and Ch) supplemented *in vitro* raised plants were collected and the variations in antioxidant potentials were determined.

2.2.4. Preparation of plant extract

Enzyme extraction was carried out at 4 ºC according to the reported procedures Percira et al. [15]. Plant tissues were homogenized in an ice cold mortar and pestle in 100mM potassium phosphate buffer (pH 7.8) containing 1mM EDTA, 5% (w/v) soluble PVB in the ratio of 1:3 (Grinding buffer), then the homogenate was centrifuged (8000rpm) at 4ºC for 12mins. The supernatant was collected and stored in -80 ℃ for enzyme assays.

2.2.5. Total antioxidant activity

Total antioxidant activity was measured from 50mg of samples by the Phosphomolybdenum method based on the reduction of Mo (VI) to Mo (V) according to the procedure described by Prieto et al [16]. The antioxidant activity is expressed as the number of gram equivalents of ascorbic acid.

2.2.6. Total phenolic contents

The amount of TPC was determined from 50mg of samples by the Folin-Ciocaltaeu's reagent method by MC Donald [17]. Total phenolic content was expressed in terms of Gallic acid equivalent (mg g-1 of FW), which is a common reference for phenolic compounds.

2.2.7. Phenylalanine ammonia lyase

PAL activity was determined from 200mg of samples based on the rate of cinnamic acid production, according to Wang et al [18]. The unit of enzyme activity expressed as one unit of enzyme activity was defined as the amount of PAL that produced 1µmol of cinnamic acid within 1min and was expressed as µ mol cinnamic acid/min.

2.2.8. Peroxidase

Peroxidase (EC 1.11.1.7; POX) activity was measured as increase in optical density due to the oxidation of guaiacol to tetra- guaiuacol as by Castillo et al [19]. Assay was performed from 20mg of samples. A unit of peroxidase activity was expressed as the change in absorbance per min at the range of 290nm and the enzyme activity was calculated using the extinction coefficient of 25.5 mM-1 cm-1. One unit of POX is the amount required to decompose 1µmol of substrate U/g FW under assay condition.

2.2.9. Catalase

Catalase (EC.1.11.1.6; CAT) is one of the most potent catalysts known. The catalase assay was performed from 20mg of samples based on the absorbance of H_2O_2 at the UV range of 240nm. A decrease in the absorbance was recorded over a time period as described by Aebi [20]. The activity was measured using the extinction coefficient of 39.4mM^{-1} one unit CAT activity was defined as the amount of enzyme required to decompose 1µmol of hydrogen peroxide U/g FW under assay condition.

2.2.10. Statistical analysis

The each experiment had three replicates. The analysis of variance (ANOVA) was performed using graph pad prism 5.01.

3. RESULTS AND DISCUSSION

In vitro plant regeneration was carried out with three elicitors (L-Phe, SA and Chitosan) supplementation. Comparatively the better propagation was obtained in chitosan supplemented culture (Figure 1a-c). The variations in antioxidant potential of *in vitro* regenerated *C.aromaticus* were determined (Table 1, Figure 2a-e). The higher PAL activity (2.470µmol cinnamic acid/mg protein/min) was determined in 1.0mg/L BAP and 40 mg/L Chitosan supplemented culture. Phenylalanine amonia-lyase (PAL) is one of the most important enzymes that play a key role in regulation of phenylpropanoid production in plants. It catalyzes the first step of the phenylpropanoid pathway in which L-phenylalanine is deaminated to trans-cinnamic acid. This step is significant for metabolic engineering and hyper-expression of the major phenylpropanoid and methyl chavicol. Similarly, many researchers were reported in

Table - 1: Antioxidant potentials of elicitors (L-Phe, SA and Ch) supplemented *in vitro* propagated *C.aromaticus*

Compositions with MS media	Conc. mg/L	Conc. of efficient Propagation	Antioxidant potential				
			PAL µmol	CAT U/mg	POX U/mg	TAA µg/g	TPC µg/g
1.0mg/L BAP +L-Phe	L-Phe 0.5-2.5	1.0	1.396±1.07	48.33±3.46	7.01±0.98	0.373±0.12	2.20±0.59
1.0mg/L BAP+SA	SA 0.2-1.0	0.4	1.919±1.01	57.18±2.44	7.34±0.71	0.412±0.63	2.51±0.17
1.0mg/L BAP+Ch	Ch 20-80	40	2.470±1.09	44.11±1.37	6.34±0.97	0.669±0.49	2.82±0.60

increased PAL activity of tissue cultured plants such as *Glycyrrhiza uralensis*[21] and *Luffa cylindrical* cotyledons[22] under Cd and Pb treatments.

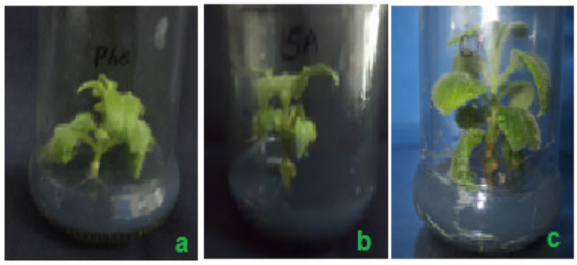

Figure - 1: Exogenous elicitors (a) L-Phenylalanine (b) Salicylic acid (c) Chitosan supplemented *in vitro* culturing *C.aromaticus.*

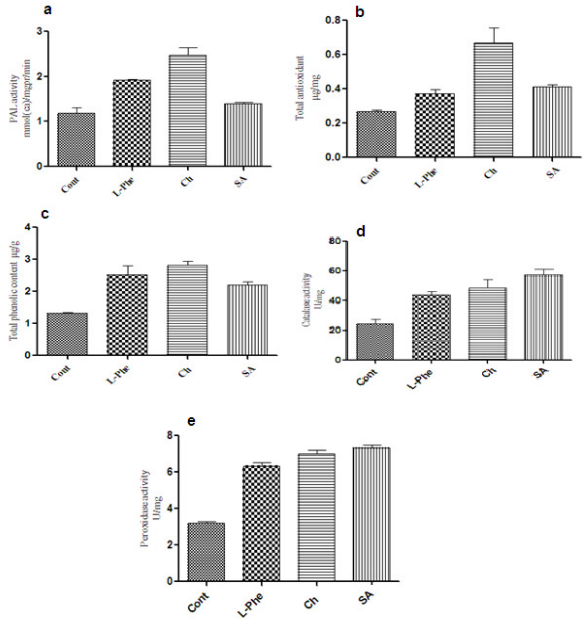

Figure - 2: Antioxidant potentials of *in vitro* propagated *C.aromaticus* (a) PAL activity (b) Total antioxidants (c) Total phenolic content (d) Catalse e) Peroxidase.

Comparatively the higher antioxidant activity (0.669µg/mg) and total phenolic content (2.82µg/g) was determined in Ch supplemented plants. Few reports were suggested that antioxidant activity of plant extracts is not limited to phenolics may also come from the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [23]. Similarly, the increased level of antioxidant activity and secondary metabolites were determined in abiotic elicitors (jasmonic acid, salicylic acid) treated adventitious shoot cultures of *H. perforatum*[24].

Compared to L- Phe and SA supplemented *in vitro* raised plant, low level of catalase (44.11 ±1.37U/mg) and Peroxidase (6.34±0.97U/mg) activities were determined in chitosan supplemented culture. Comparatively significant increase in catalase (57.18 ±2.44U/mg) FW and peroxidase (7.34±0.71U/mg) was recorded in SA supplied culture. To better understand the influence of the elicitors on CAT and POD directly related to the secondary metabolites accumulation and antioxidant activity. The variation in the phenolic levels seemed to be related to the POD activity [25]. According to the result, further research is needed to reveal the possible synergistic or antagonistic relations between the individual components and assess their impact on the antioxidant responses of *C.aromaticus* shoot culture.

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