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ORIGINAL ARTICLE

IN VITRO ENHANCEMENT OF ANTIOXIDANT ACTIVITY, PHENOLIC CONTENT AND FLAVONOID CONTENT OF *BACOPA MONNIERI* L.

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Abstract:

Bacopa monnieri is a highly vulnerable medicinal plant from the Scorphulariaceae family. It is used as medicinal purpose and main constituent in different pharmaceutical products. The report her for the first time an enhanced Bacopa monnieri shoot for production of some metabolites. Proliferated shoots on MS medium contained some sugar (sucrose, fructose mannitol and sorbitol) and growth retardants (ABA, CCC and PBZ) are using for an enhanced factors for production of antioxidant activity, phenolic content and flavonoid content were studied. Maximum shoots obtained on MS medium supplemented with 1.5mg/l BA where, 1mg/lBA was to be superior to give maximum leaves. Height shoot length was observed on MS medium free hormone. Maximum antioxidant activity enhanced by in vitro proliferated shoots when cultured on MS medium containing 50g/l sucrose; 40g/l mannitol and 200µl/l from extracts (97.81 and 97.43% respectively). The plant extract showed significant antioxidant activitywhen shoots culturedon MS medium supplemented with0.25, 0.50, 0.75 and 0.1PBZ or ascorbic acid (positive control) with 200µl of extract. Maximum phenolic content recorded and enhanced whenshootscultured on MS mediumsupplemented with 50mg/l sucrose (14.35mg GAE/g explant) than other concentrations of fructose, mannitol and sorbitol. Enhanced maximum of total phenolic content of proliferated shoots when cultured on MS medium contained 0.75mg/l PBZ (24.09mg GAE/g explant) than other concentrations of ABA or CCC. Highest flavonoid content was recorded from proliferated shootswere cultured on MS medium supplemented with sucrose at 40 and 50g/l (884.18 and 975.8mgQUE/g explant respectively) without significant difference. The use of MS medium supplemented with 1mg/l PBZ was the provided an identical increase in flavonoid (749.18mgQUE/g explant) than other concentrations of CCC and ABA. The result showed that Bacopa monnieri apart from being a brain tonic, can be using in vitro some treatments for an enhanced antioxidant activity, phenolic content and flavonoid, which will be useful in alleviating several pathological.

KEYWORDS:

Bacopa monnieri, in vitro, antioxidant activity, phenolic content, flavonoid content.

INTRODUCTION

Free radicals are generated in the human body through natural processes. However, stress, disease

and improper lifestyle lead to excessive generation of free radical, which initiates a chain reaction and adversely affects the cellular metabolism. Antioxidants are substances, which scavenge the free radicals and hinder the oxidation process in the body. There is presence of antioxidants in the cell but need of external supplementation of the same in always necessary. Some natural antioxidants are phenolics, flavonoids, carotenoids, Vitamins, anthocyanin etc. *Bacopa monnieri* L. (Family: *Scrophulariaceae*) commonly called as *Brahmi* has been used in traditional and Ayurvedic medicine for many years. Generally formulations used to enhance memory and to cure brain related complications essentially have Brahmi as an important ingredient. The plant is rich in metabolites having therapeutic value. Alkaloids like brahmine, nicotine, herpestine have been identified. Various saponins, sapogenins, bacosides, monnierasides have also been reported (Gohil and Patel, 2010). Antioxidant properties of Bacopa may offer protection from free radical damage in cardiovascular disease and certain types of cancer (Russo *et al*., 2003). Brahmi apart from being brain tonic can also serve as potent antioxidant. Moreover, the range of antimicrobial activity showedby the plant extract presents it as a potential antimicrobial agent having a broad-spectrum activity (Vats and Tiwari, 2014). Advances in the area of tissue culture for the production of secondary metabolites have made it possible to increase the yield of a wide variety of substances with pharmaceutical values such as alkaloids, terpenoids, steroids, phenolics and flavonoids (Rout *et al*., 2000). In vitro technology offered control conditions for culture medium compositions as well as incubation conditions to globalize the production of desired variants in shorter and flexible production cycles. Sugars were reported to have an essential role in general metabolism that regulates many important processes in all stages of the plant life cycle (Smeekens, 2000 and Rolland et al., 2002). In this study, *Bacopa monnieri* has been modified by some sugar, (sucrose, fructose, mannitol and sorbitol) and some growth retardants, (absissic acid, cycocyle and pacloputrazol) to enhance its antioxidant activity, phenolic content, and flavonoid content.

MATERIALS AND METHODS

Plant material and explants preparation:

Bacopa monnieri plant was collected from the garden of the Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GIBRI), University of Sadat City, Egypt. Shoot tips were cut and washed under running tap water for 30 min, after that explants were sterilized as (Showkat *et al*., 2010) and transferred on (Murashige and Skoog, 1962)(MS) medium supplemented with 30g/l sucrose, 1mg/l BA and 7g/l agar for shoot initiation.About 60 ml of the medium was poured into sterile culture jars (350ml). The culture jars with MS medium was autoclaved at 121°C for 20 min. at 15 lbs pressure. Multiplication of shoots was carried out by repeated sub-culturing in MS medium for all experiments.

Effect of cytokinin at different concentrations on shoot proliferation:

In order to achieve shoot proliferation of *Bacopa monnieri* (shoots of about 2cm long with 2-5 leaves) obtained from the starting stage were cultured in jars (350 ml) containing 60 ml MS medium supplemented with different concentrations of benzylaminopurine (BA), kinetin (Kin) and thidiazuron (TDZ) (0.0, 0.25, 0.50 and 1 mg/l) each alone. Each treatment was 10 replicates; each contained a jar with three shoots. The pH of all media was adjusted to 5.8 before autoclaving. The cultures were incubated at 25ºC day and night (16 and 8). White fluorescent tubes giving light intensity 2000 lux. The shoots proliferation was evaluated after six weeks and the number of shoots, shoot number and shoot length (cm)/culture (jar) were recorded.

Effect of some sugar on antioxidant activity, phenolic content and flavonoid content:

Explants obtained from shoot proliferation cultured on MS medium supplemented with 1.0mg/l BA with different sugar, (sucrose, fructose, mannitol and sorbitol) at 20, 30, 40 and 50g/l for enhanced antioxidant activity, phenolic content and flavonoid content. After six weeksfrom cultured the proliferated shoots extracted for determination of antioxidant activity, total phenolic content and flavonoid content.

Effect of some growth retardants on antioxidant activity, phenolic content and flavonoid content:

Shoots from proliferation were cultured on MS medium supplemented with 0.0, 0.25, 0.5, 0.75, 1.0 or 1.5 mg/l abscisic acid (ABA), cycocyl (CCC) or paclobutrazol (PBZ). The medium of each treatment was also supplemented with 30g/l sucrose and 7g/l agar. After six weeks from cultured the proliferated

shoots extracted for determination of antioxidant activity, phenolic content and flavonoid content.

Determination of antioxidant activity:

Plant materials

Samples in this experiment were chosen from in vitro proliferated shoots to measured antioxidant activity, phenolic content and flavonoid content. Shoots produced on MS medium contained different sugar and some growth retardants were using for enhancement of secondary metabolites.

Extraction and preparation of aqueous extracts:

The extracts were prepared by 2g of plants in 20ml-distilled water and boiling for 15 min. The mixtures were filtered through a filter paper, which then stored at -18^{o}C until used. All analyses were performed in three replicates.

Estimation of antioxidant activity (DPPH radical scavenging activity):

The free radical scavenging activity was estimated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay using Blois method (Blois, 1958) with some modifications (Nand *et al*., 2012). The reaction mixture contained 50, 100 and 200µl of test extracts and 2 ml of methanolic solution of 0.1M DPPH radical. The mixture was then vigorously shaken and incubated at 37ºC for 30 min. The absorbance was measured at 517 nm. For the baseline control, 200µl of distilled water was used. Ascorbic acid (50-500 µg/ml) was used as positive control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity that was calculated using the following equation: DPPH scavenging effect (%) = 100 x (Ao- A1)/(Ao); Where (Ao) is the absorbance of the control reaction and (A1) is the absorbance of reaction mixture containing DPPH and extract at 517nm spectrophotometer.

Estimation of total phenolic content (TPC):

The total phenolic content of *Bacopa monnieri* was determined by the Folin–Ciocalteu (FC) method using spectrophotometer (Singleton and Rossi, 1965) with some modification made by (Nand *et al.,* 2012) and expressed as grams of gallic acid equivalents per 2g plant extract. Distilled water (3.16 ml) was mixed with the 40µl of sample, and then 200µl of Folin Ciocalteu reagent was added. After 5 min, 600µl of 20% sodium carbonate solution was added and solutions were mixed again. The solution was left at room temperature for 2 hrs. The color intensities were measured at wavelength 765nm spectrophotometer. TPC expressed as grams of Gallic acid equivalents using the equation (y=0.017 x 0.076: x = y-0.076/0.017) obtained from the galic acid (5-50 µg/ml) calibration curve and the phenolic content was expressed as mg of galic acid equivalents (GAE) per 2g explant.

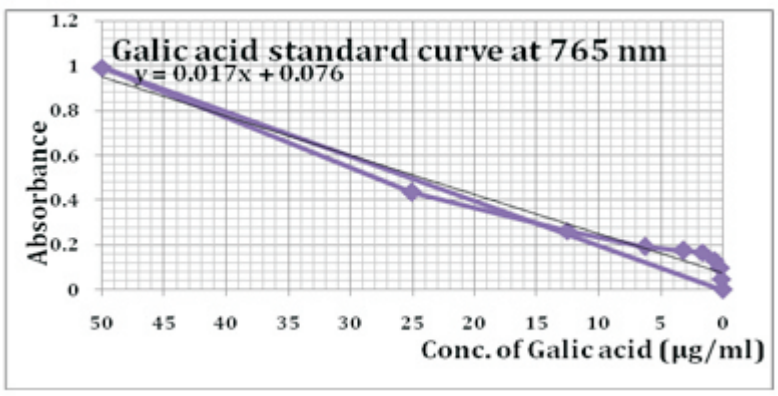

Fig. 1: Total phenolic content of Gallic acid extract at different concentrations.

Estimation of total flavonoid content (TFC)

The total flavonoid of test extracts was determined using determined according to the aluminum

chloride colorimetric method described by (Barku *et al*., 2013) with some modifications. 100µl of the extracts was mixed with 500µl of distilled water and 650µl of 5% $NaNO_2$. The mixture was kept at room temperature for 5 min followed by addition of 150µl of 10% $AlCl_3$, 0.5 ml of 1M NaOH and 0.275 distilled water, shaken and left to stand for 15 min before determination using the sample solution without coloration as reference solution. The absorbance of the reaction mixture was measured at 510 nm with spectrophotometer. The concentration of the flavonoid content was calculated using the equation (y=0.0014 x +0.0798: x=y-0.0798/0.0014) obtained from the quercetin (1000-6000µg/ml) calibration curve and the flavonoid content was expressed as mg of quercetin equivalents (QUE)/2g fresh weight.

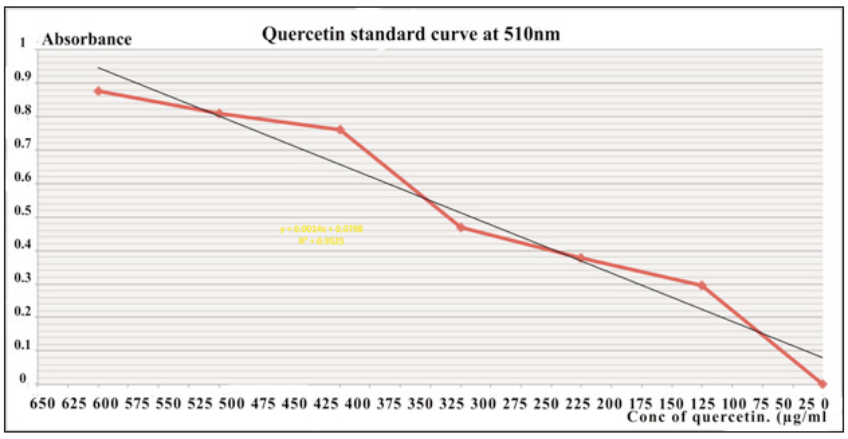

Fig. 2: Standard curve of Quercetin. The curve used to determine the equivalent that expresses flavonoid as grams of Quercetin equivalents (QUE) per 2g explant.

Statistical analysis:

All the samples were run in triplicate and then mean values were used for result analysis (Snedecor and Cochran, 1989). The statistical significance between antioxidant activity, phenolic content and flavonoid content of extracts and standards were evaluated using MSTATC software version 2.10. 22.

RESULTS AND DISSCUSION

Effect of cytokinin at different concentrations on shoot proliferation:

The use of plant growth regulators for shoot proliferation in vitro is important for production and improvement of shoots. Shoots taken from six weeks of aseptic culture on MS medium supplemented with 0.0 to 1.5mg/l BA, Kin and TDZ for increased of shoot proliferation. Maximum number of shoots and produced healthy shoots obtained on MS medium supplemented with BA (216.75) than Kin and TDZ (Figs. 3 and 4). The shoots production was increased by increasing BA from1 to1.5mg/l (102.77 and 108.9 shoots, respectively) without significant. Interaction between cytokinin type and its concentrations produced maximum number of shoots on MS medium supplemented with 1.5mg/l BA (310.66) than concentrations of Kin and TDZ. The effect of Kin and TDZ was tested for in vitro shoot multiplication, the multiplication rate declined on MS medium supplemented with Kin and TDZ. Maximum shoots length showed on MS medium free hormone. Highest leaves number recorded at MS supplemented with BA (194.41) than Kin and TDZ. Increased of leaves number when cultured on MS medium contained1mg/lBA than other treatments. Interaction between cytokinin and its concentrations found that highest leaves number obtained at 0.5 and 1mg/l BA without significant differences. cytokinin plays an important role on in vitro shoot development of *Bacopa monnieri*. Best result of axillary bud of *Bacopa monnieri* induction and observed on medium containing 1.0 mg /l BAP (Kaur et al., 3013). The best performance for shoot multiplication was showed in MS medium supplemented with 1.5 mg/l BAP+ 0.5mg/l IAA (Kapil and Sharma, 2014).

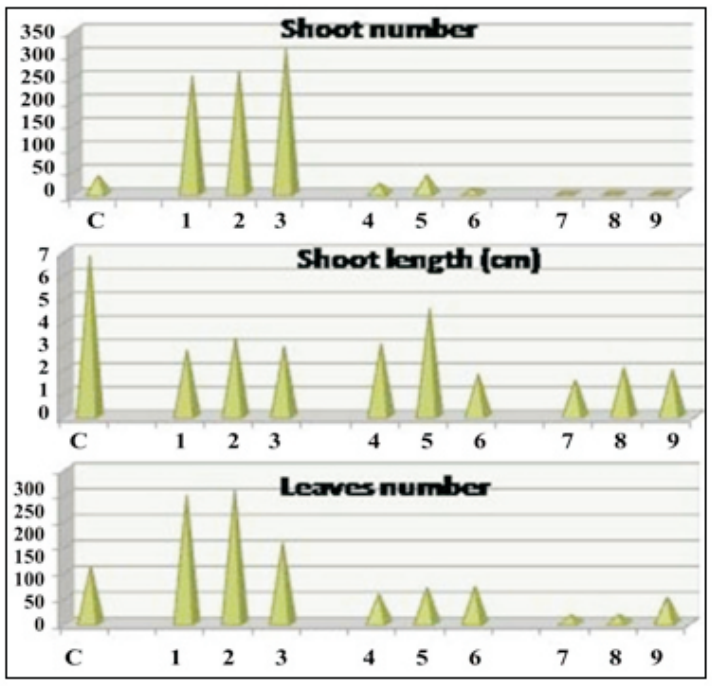

Fig. 3: In vitro development shoots proliferation of *Bacopa monnieri* on MS medium supplemented with BA, Kin and TDZ.

Where: (C) free hormone, (1) 0.5mg/l BA, (2) 1mg/l BA, (3) 1.5mg/l BA, (4) 0.5mg/l Kin, (5) 1mg/l Kin, (6) 1.5mg/l Kin, (7) 0.5mg/l TDZ, (8) 1mg/l TDZ, (9) 1.5mg/l TDZ.

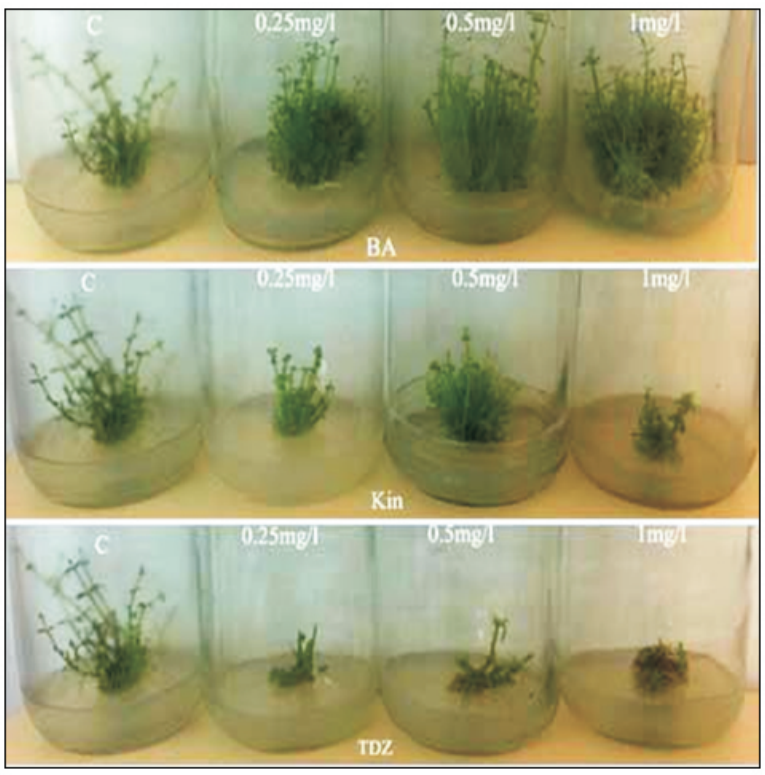

Fig. 4: *In vitro* shoot proliferation of *Bacopa monnieri*.

Effect of some sugars on antioxidant activity:

Antioxidant activity (DPPH radical scavenging activity)

Bacopa monnieri aqueous extracts were shown to be active in DPPH radical scavenging activity. The control (standard ascorbic acid) and the plant extracts showed their enhanced maximum activity at: control (96.28%), 40 and 50g/l sucrose and 40g/l mannitol (96.50, 97.43 and 96.04% respectively). While the lowest percentage was obtained with 20g/l sucrose 59.74%. However the stronger scavenging activity was significantly higher with 200µl of extract (91.879%) than 50 and 100µl of extracts. DPPH radical scavenging activity assay assesses the capacity of the extract to donate hydrogen or to scavenge free radicals. Interaction between sugar and its concentrations, the highest radical scavenging activity exhibited on MS medium supplemented with (50g/l sucrose, 40g/l mannitol) and 200µl extract of proliferated shoots were (97.81 and 97.43% respectively) without significant (Fig. 5). These results help to understand that, further the using sucrose improved antioxidant activity thought *in vitro* culture of *Bacopa monnieri* than other sugars. Sucrose responsible for induced of antioxidant activity and it is suitable precursor to increase the activity. This result is in agreement with that reported by Thimann *et al*. (1950) who stated that sugars are one of the most important externally substances for the phenolic biosynthetic pathway, it is also proofed by Solfanelli *et al*. (2006) who found that the induction of flavonoid/anthocyanin synthesis genes is sugar specific.

Effect of some growth retardants on antioxidant activity:

The antioxidant activity of three extract concentrations from proliferated shoots of Bacopa monnieri when cultured on MS medium supplemented with ABA, CCC and PBZ expressed in terms of percentage of inhibition percent (Fig. 6). The examination of antioxidant activities of proliferated shoots from different concentrations of its extract showed different values. The obtained values varied from 500 and 200µl were 82.712 to 94.467% respectively. The largest capacity to neutralize DPPH radicals was found with ascorbic acid (positive control) and extract of shoot proliferated which obtained from MS medium supplemented with 1.25mg/l PBZ (95.06 %). While the lowest activity was found with extract derived from proliferated shoots cultured on MS medium contained 1.25mg/l CCC and 0.75mg/l PBZ. Data showed that the extract that perform the highest antioxidant activity obtained from 0.25, 0.50, 0.75 or 1.25 mg/l PBZ with 200µl from extract than other treatments (Fig. 6). From the results of this investigation, it is clear that, PBZ increased the antioxidant activity than ABA and CCC.

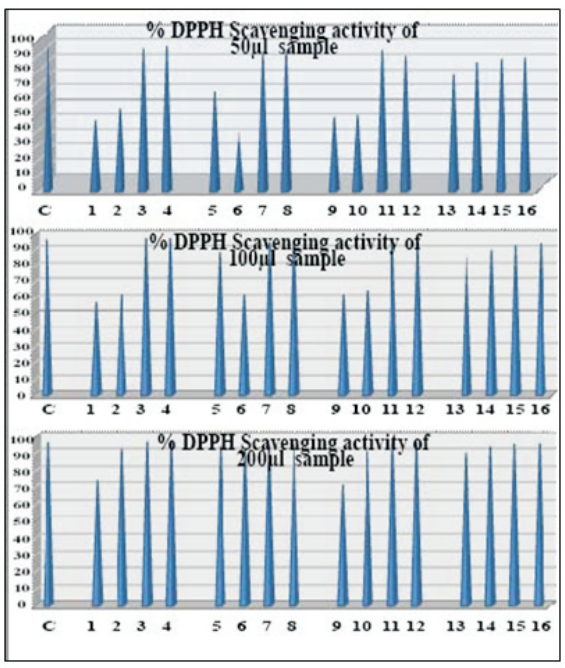

Fig. 5: Percentage inhibition in DPPH free radical scavenging assay of *Bacopa monnieri* aqueous extract at different sugar concentrations.

Where: (C) Ascorbic acid (positive control), (1) 20g/l sucrose, (2) 30g/l sucrose, (3) 40g/l sucrose, (4) 50g/l sucrose, (5) 20g/l fructose, (6) 30g/l fructose, (7) 40g/l fructose, (8) 50g/l fructose, (9) 20g/l mannitol, (10) 20g/l mannitol, (11) 30g/l mannitol, (12) 40g/l mannitol, (13) 20g/l sorbitol, (14) 30g/l sorbitol, (15) 40g/l sorbitol, (16) 50g/l sorbitol.

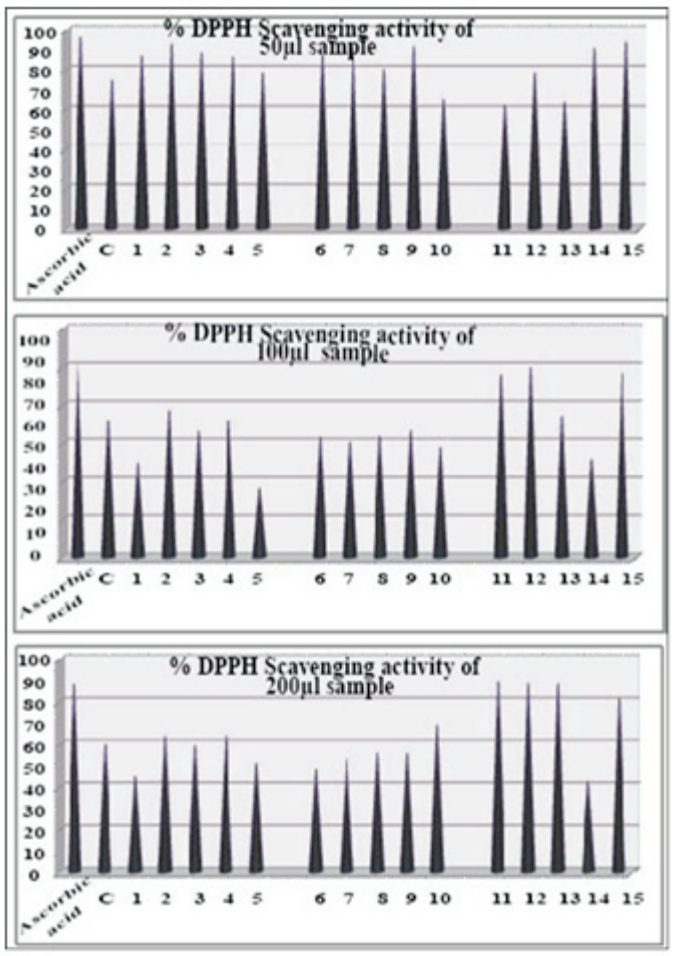

Fig. 6: Percentage inhibition in DPPH free radical scavenging assay of *Bacopa monnieri* aqueous extract at different concentrations and different growth retardants concentrations.

Where: ascorbic acid (positive control), (C) untreated shoots (1) 0.25mg/l ABA, (2) 0.50mg/l ABA, (3) 0.75mg/l ABA, (4) 1.0mg/l ABA, (5) 1.25mg/l ABA, (6) 0.25mg/l CCC, (7) 0.50mg/l CCC, (8) 0.75mg/l CCC, (9) 1.0mg/l CCC, (10) 1.25mg/l CCC, (11) 0.25mg/l PBZ, (12) 0.50mg/l PBZ, (13) 0.75mg/l PBZ, (14) 1.0mg/l PBZ, (15) 1.25mg/l PBZ.

The antioxidant potential of the plant extracts are influenced by various factors and largely depends on both the composition of the extract and the analytical test system. These results are in agreement with Divya Nair *et al*. (2009). Tupe *et al*. (2013) found that the plants extracts offer promising sources of natural antioxidants. Increase in ascorbic acid content was reported in the paclobutrazol treated citrus fruits (Jain *et al*., 2002). A decrease in ascorbic acid was reported in the ABA treatment in plants (Zhang *et al*., 2006). Paclobutrazol enhanced the free radical scavenging capacity of treated plants including the levels of ascorbate and APX in wheat seedlings (Berova *et al.,* 2002). PBZ treatments increased the ascorbic acid content in the root tissue of O. sanctum (Divya Nair *et al*., 2009).

Effect of some sugar on phenolic content:

The highest phenolic content in the examined extract obtained from sucrose (8.11mg GAE/g explant) than fructose, mannitol and sorbitol. The highest concentration of phenolic content was measured on MS medium supplemented with 50g/l (10.78 mg GAE/g explant) than other concentrations of sugar.

Maximum phenolic content recorded and enhanced on MS medium supplemented with 50mg/l sucrose (14.35mg GAE/g explant). While the lowest phenolic content measured on MS medium supplemented with 20 g/l sucrose and mannitol as shown in (Fig. 7). The phenolic content in plant extract of *Bacopa monnieri* depends on the type and concentration of sugar. Phenolics or polyphenols have received considerable attention because of their physiological function, including antioxidant, antimutagenic and anti-tumor activities (Othman *et al*., 2007).

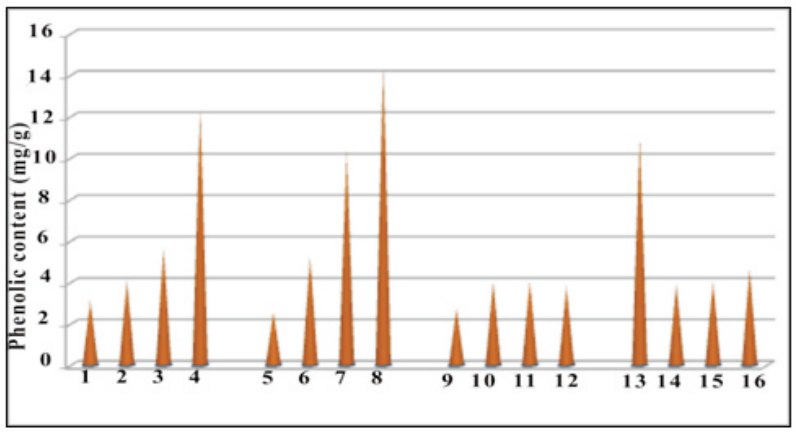

Fig. 7: Phenolic content (GAE mg/g explant) of *Bacopa monnieri* which enhanced by some sugars.

Where: (1) 20g/l sucrose, (2) 30g/l sucrose, (3) 40g/l sucrose, (4) 50g/l sucrose, (5) 20g/l fructose, (6) 30g/l fructose, (7) 40g/l fructose, (8) 50g/l fructose, (9) 20g/l mannitol, (10) 20g/l mannitol, (11) 30g/l mannitol, (12) 40g/l mannitol, (13) 20g/l sorbitol, (14) 30g/l sorbitol, (15) 40g/l sorbitol, (16) 50g/l sorbitol.

Effect of some growth retardants on phenolic content:

Total phenolic content of the plants was measured using the Folin-Ciocalteu method and the results are presented in (Fig. 8). There was highest total phenolic content recordedfrom proliferated shoots when cultured on MS medium supplemented with PBZ as the value (15.55mg GAE/g explant) than other growth retardant. *Bacopa monniera* extract, results indicated that higher total phenolic content was obtained on MS medium supplemented with 0.0 and 0.75 mg/l (13.47 and 12.83mg GAE/g explant, respectively) than other concentrations of plant growth retardants. Whereas, lowest contents of phenolic were observed shoots proliferated on MS medium supplemented with 1.25mg/1ABA (4.915mg GAE/g explant). Interaction between plant growth retardants and its concentration stimulated and enhancedthe total phenolic content and gave highest value with 0.75mg/l PBZ (24.09mg GAE/g explant) than other concentrations of growth retardants. The antioxidant activity of the extracted shoots mainly due to good phenolic and flavonoid content. These phytochemicals have been known to possessantioxidant potential are in use to treat manydisorders and diseases (Ross and Kasum, 2002).

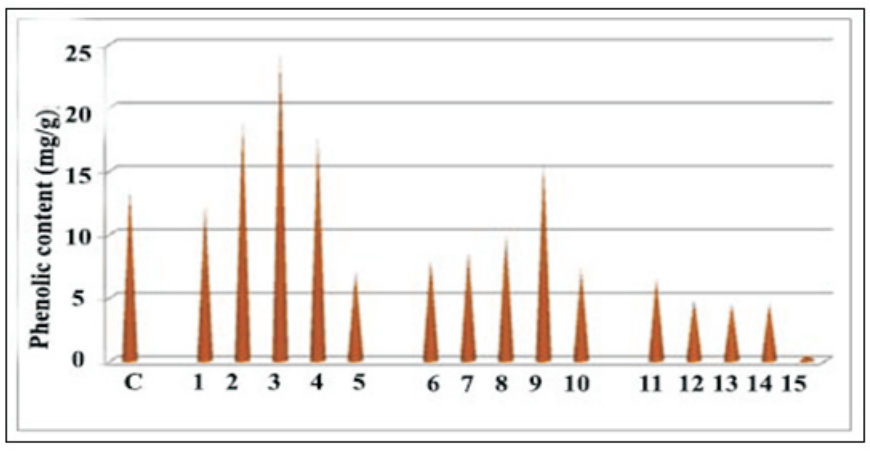

Fig. 8: Phenolic content (GAE mg/g explant) of *Bacopa monnieri,* which enhanced by some growth retardants.

Where: (C) Untreated shoots (1) 0.25mg/l ABA, (2) 0.50mg/l ABA, (3) 0.75mg/l ABA, (4) 1.0mg/l ABA, (5) 1.25mg/l ABA, (6) 0.25mg/l CCC, (7) 0.50mg/l CCC, (8) 0.75mg/l CCC, (9) 1.0mg/l CCC, (10) 1.25mg/l CCC, (11) 0.25mg/l PBZ, (12) 0.50mg/l PBZ, (13) 0.75mg/l PBZ, (14) 1.0mg/l PBZ, (15) 1.25mg/l PBZ.

Effect of some sugars on flavonoid content:

Table (4) indicated that among tested sugar type, sucrose has the highest total flavonoid content followed by fructose. Maximum flavonoid content observed form proliferated shoots when cultured on MS medium supplemented with 40g/l sucrose than other concentrations of sugar (Fig. 9). Interaction between sugar type and its concentration recorded highest flavonoid content at 50g/l with sucrose (975.853mg QUE/g explant) than other sugar type and its concentrations. Sugars can play a great role for enhanced the production of the secondary metabolites in *Bacopa monnieri*. Results from this study support the previous findings as (Sato et al., 1996) found that the role of sugar in stimulating the production metabolites is differ some suggest that it is an osmotic agent. Brahmi, a known drug for memory enhancing and for sedation also possesses anti-lipid peroxidative property in general. It might serve as a medicine for aging and several nervous disorders because free radicals are involved in these pathologies (Dabrowiecki et al., 1985). Solfanelli et al. (2006) found that the induction of flavonoid/anthocyanin synthesis genes is sugar specific.

Effect of some growth retardants on flavonoid content:

Flavonoid is a class of secondary plant metabolites with significant antioxidant and chelating properties. The concentration of flavonoid in shoots extract of the *Bacopa monnieri* was determined using spectrophotometric method with aluminum chloride. Extract of shoots proliferated on MS medium contained PBZ has highest flavonoid content (560.139 mg QUE/g explant) than CCC and ABA as shown in (Fig. 10). Maximum flavonoid content observed at1.0 mg/l (615.616 mg QUE/g explant) than other concentrations.

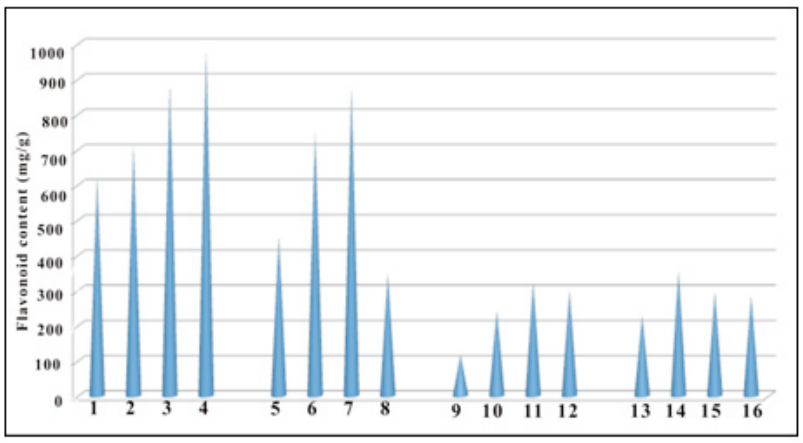

Fig. 9: Flavonoid content (QUEmg/g explant) from extracted proliferated shoots of *Bacopa monnieri* on MS medium supplemented with some sugar.

Where: (1) 20g/l sucrose, (2) 30g/l sucrose, (3) 40g/l sucrose, (4) 50g/l sucrose, (5) 20g/l fructose, (6) 30g/l fructose, (7) 40g/l fructose, (8) 50g/l fructose, (9) 20g/l mannitol, (10) 20g/l mannitol, (11) 30g/l mannitol, (12) 40g/l mannitol, (13) 20g/l sorbitol, (14) 30g/l sorbitol, (15) 40g/l sorbitol, (16) 50g/l sorbitol.

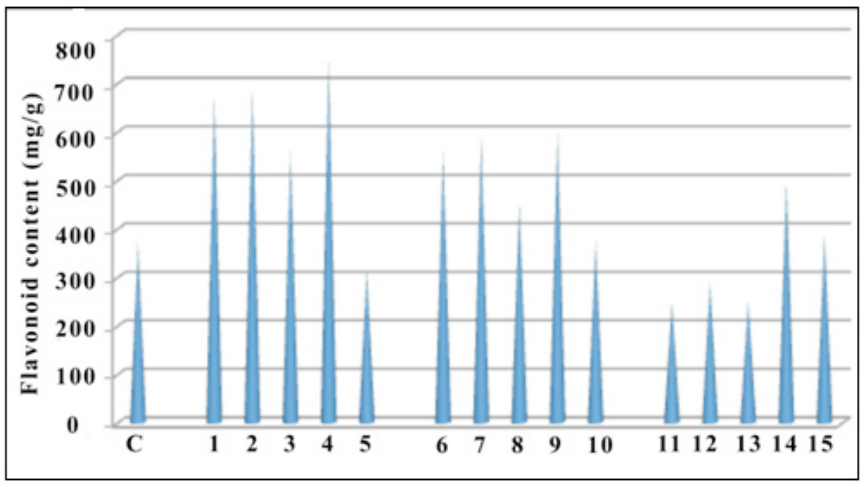

Fig. 10: Flavonoid content (QUE mg/g explant) from extracted proliferated shoots of *Bacopa monnieri* on MS medium supplemented with some growth retardants.

Where: (C) Untreated shoots (1) 0.25mg/l ABA, (2) 0.50mg/l ABA, (3) 0.75mg/l ABA, (4) 1.0mg/l ABA, (5) 1.25mg/l ABA, (6) 0.25mg/l CCC, (7) 0.50mg/l CCC, (8) 0.75mg/l CCC, (9) 1.0mg/l CCC, (10) 1.25mg/l CCC, (11) 0.25mg/l PBZ, (12) 0.50mg/l PBZ, (13) 0.75mg/l PBZ, (14) 1.0mg/l PBZ, (15) 1.25mg/l PBZ.

Interaction between growth retardants and its concentrations gave highest flavonoids content on MS medium supplemented with 1.0 mg/lPBZ (749.188 mg QUE/g explant) than other treatments. In vitro culture accumulated flavonoid in higher levels, which reflect the effect and enhancement of growth retardants supplemented on MS medium. In addition to their antioxidant properties, flavonoids have anti-proliferative, anti-tumor and activities being used as medicinal plant, the relationship of growth retardants on total flavonoids contents may be of great economic value. This finding is in accordance with Sharififar et al. (2008) who showed that flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups. The total phenolic content and total flavonoid content was found to be 1.59% ± 0.24 GAE and 0.4% ± 0.1, respectively (Vatsand Tiwari, 2014).

CONCLUSION

The results clearly indicate that aqueous extracts of *Bacopa monnieri* possesses antioxidants activity, phenolic content and flavonoid content. Presence of higher concentration of secondary metabolites in these test extract makes them strong free radical. In vitro enhanced metabolites of *Bacopa monnieri* proliferated shoots with some sugar and growth retardants can be stimulated and a good source of natural antioxidants to prevent free radical mediated oxidative stress. However other antioxidant activity substances compounds still need to be identified.

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