Ethanolic extract of sweet basil callus cultures as a source of antioxidant and sun-protective agents

Mona M. Ibrahim, Nancy Danial, Mohamed K. El-Bahr

Department of Plant Biotechnology, Biotechnology Research Institute, National Research Center, Cairo, Egypt

Correspondence to Nancy Danial, Department of Plant Biotechnology, National Research Center, Cairo 12622, Egypt.
Tel: + 20 122 267 3398;
e-mail: nancydanial333@yahoo.com

Received: 5 September 2022
Revised: 9 December 2022
Accepted: 9 December 2022
Published: 8 March 2023

Egyptian Pharmaceutical Journal 2023, 22:78–86

Background
Basil (*Ocimum basilicum* L.) is a medicinal plant largely used in medicine, cosmetics, and cooking.
Objective
The current work aimed to improve the production of both phenolic and flavonoid compounds in the callus cultures of sweet basil (*O. basilicum* L.), which can be used in cosmetics as antioxidant and sun-protection agents.
Materials and methods
Different combinations of growth regulators have been used to induce calli. Phenylalanine and salicylic acid have been used to enhance phenolics and flavonoids production. Quantitative analyses including total phenolics (TPC), total flavonoids, 2,2′-diphenyl 1-Picryl-hydrazyl radical scavenging activity, half-maximal inhibitory concentration (IC_{50}), correlation coefficient (R2) between antioxidant activity and both TPC and TFC, and sun-protective factor have been performed for both treatments and control.
Results and conclusions
Results reported that 1-naphthaleneacetic acid (NAA)+6-benzylaminopurine (BAP) was the best combination to induce calli tissue with good texture. The addition of 1.0 g/l phenylalanine for 2 weeks and 0.5 mm salicylic acid for 4 weeks were the best treatments to increase the production of phenolic and flavonoid components, and it showed the maximum % radical scavenging capacity. Higher correlation coefficient was found between % radical scavenging capacity and TPC compounds (0.83). The treatment of 1.0 g/l phenylalanine for 2 weeks indicated the lowest and best $IC_{50,}$ and it showed the maximum sun-protective factor value (36.50±0.003).

Keywords:
antioxidant activity, *Ocimum basilicum* L, phenolics and flavonoids, sun-protective factor

Egypt Pharmaceut J 22:78–86

1687-4315

Introduction

Sweet basil (*Ocimum basilicum* L.), family Lamiaceae, is one of the most important aromatic herb native to tropical and subtropical Asia. It is cultivated economically worldwide for its use in pharmaceuticals, cosmetics, ornamental use, and in food industry [1]. Sweet basil contains more than 200 phytochemicals, with phenolics and flavonoids being the main phytochemical compounds [2].

The main phenolic compounds in basil are rosmarinic acid, vanillic acid, lithospermic acid, hydroxybenzoic acid, coumarinic acid, syringic acid, ferulic acid, protocatechuic acid, and caffeic acid [3,4].

Secondary metabolites could be enhanced and produced through plant tissue culture by many ways such as organ and callus cultures, cell suspensions, precursors and elicitors addition to the growth media, bioreactors, and immobilization of the plant cells [5,6].

In *vitro* cultures of sweet basil were subjected to study for different objectives, including micropropagation [7], physiological investigations [8], and production of some important secondary metabolic compounds such as essential oils [9], rosmarinic acid [10], anthocyanins [11], and triterpenes [12]. The elicitation of cultures is required to achieve higher metabolite production, including chemical, biological, physical, and chemical elicitation. The addition of precursors was the most widely used [13]. Plant growth regulators can also be used to induce chemical elicitation, leading to a significantly higher production of phenolic compounds [14].

Free radicals cause damage of most body tissues, leading to the occurrence of many diseases. The main role of antioxidants is to neutralize the effect of free radicals and thus prevent diseases [15]. Currently, discovery of plant natural compounds that

have antioxidant properties are safe is the great interest to many studies. Among the most essential effects of antioxidant compounds is their use as sunscreens. The use of phenolics and flavonoids as natural substances extracted from plants to absorb harmful sunlight to prevent ultraviolet (UV)-mediated diseases was reported by Chandrakasan and Neelamegam [16]. The current study aimed to evaluate the ethanolic extract produced from sweet basil callus cultures as an antioxidant and sun-protective agent. In this context, the main components accountable for these effects (phenolics and flavonoids) were estimated.

Materials and methods

Plant material

Seeds of sweet basil (*O. basilicum* L.) were supplied by Medicinal and Aromatic Plants Farm, Faculty of Pharmacy, Cairo University. The plant samples were identified and authenticated by a plant taxonomist Prof. Dr. Abdel-Haleem Abdel-Megaly, Department of Flora Research and Plant Taxonomy, Horticultural research Institute, Agriculture Research Center.

The seeds were immersed in 70% ethanol for 2–3 min and then rinsed in sterile distilled water. The seeds were then sterilized for 20 min in 20% commercial Clorox (5% NaOCl) containing 0.5% Tween 20, followed by rinsing three times with sterile distilled water. Under aseptic condition, the seeds were cultured on MS-medium [17] containing 3% (w/v) sucrose and solidified with 0.2% (w/v) Gelzan (Gelrite). Culture medium was adjusted to pH 5.8. The cultures were incubated in a culture room at 26±2°C and kept under 16-h photoperiod of fluorescent, 45 μmol cool white light tubes and 8-h dark.

Callus initiation

Leaf explants (about 0.5 cm) were taken from 30-day-old *in vitro* growing seedlings and cultured on MS-solid medium containing different combinations of 2,4-dichlorophenoxy acetic acid (2,4-D) or naphthalene acetic acid (NAA) as auxin, whereas kinetin (kin) or benzylaminopurine (BAP) were used as cytokinins. The following culture media were subjected for callus induction:

MS+1.0 mg/l 2,4-D+0.5 mg/l kin (B1).

MS+1.0 mg/l 2,4-D+1.0 mg/l kin (B2).

MS+1.0 mg/l NAA + 0.5 mg/l BAP (B3).

MS+1.0 mg/l NAA + 1.0 mg/l BAP (B4).

MS+2.0 mg/l NAA + 1.0 mg/l BAP (B5).

After 30 days of culturing, callus initiation frequency percentages were recorded and calculated based on the following equation:

$$\text{Callus initiation frequency}\% = (\text{Number of initiated calli})/(\text{Number of inoculated explants}) \times 100.$$

Addition of phenylalanine and salicylic acid on the culture medium

The culture medium (1.0 mg/l NAA+1.0 mg/l BAP) which showed maximum % callus initiation frequency in the previous experiment was selected in callus production. After two subcultures onto the same culture medium, two concentrations of both phenylalanine (0.5 and 1.0 g/l), and salicylic acid (0.5 and 1.0 mm) were added to the culture medium. The callus tissues were harvested after 2 and 4 weeks to perform the extraction procedure and different determination analysis.

Extraction method and determination analysis

Extract preparation

Callus tissues were lyophilized using Labconco freeze dryer (United states, Kansas city), Console, 12 l, −50°C, Stoppering Tray Dryer, FreeZone, 240 V, Catalog No. 7754030, Serial No. 100931482 D, USA. The drying was performed on −50°C, 0.1 mbar and 48 h. Overall, 1.0 g of each sample was grinded, then 20 ml of ethanol 80% was added, left it on an orbital shaker overnight, then filtered, evaporated below 40°C, and stored at 4°C until further use.

Determination of total phenolics and total flavonoids contents

The concentration of total phenolic (TPC) compounds was determined by the spectrophotometric method using the Folin–Ciocalteu reagent [18]. A calibration curve of gallic acid, Merk 97.5% (20, 40, 40, 60, 80, and 100 μg/ml), was prepared, and the absorbance for tests and standard solutions was determined against the reagent blank at 550 nm. TPC content was expressed as milligrams of gallic acid equivalent/g of dry weight (DW) plant material.

TFC content was measured using a modified colorimetric method of Vabkova and Neugebauerova [19]. The standard curve was prepared using different

concentrations of quercetin (Sigma-Aldrich, 95%). The flavonoid content was expressed as milligrams of quercetin equivalents per gram of plant material DW.

2,2′-diphenyl 1-Picryl-hydrazyl radical scavenging capacity (%)

Radical scavenging activity of plant extracts against stable 2,2′-diphenyl 1-Picryl-hydrazyl (DPPH) was determined by a slightly modified method of Brand-Williams *et al.* [20]. Overall, 500 µl of each extract was added to 2.5 ml of methanolic solution of DPPH (0.3 mM). After 30 min at room temperature, the absorbance values were measured at 517 nm.

Radical scavenging capacity (%) was calculated by the following formula:

$$\mathrm{RSC}\% = [A_{\mathrm{DPPH}} A_{\mathrm{S}} / A_{\mathrm{DPPH}}] \times 100.$$

Where A_{DPPH} is the absorbance of the DPPH solution and A_{S} is the absorbance of the sample.

IC_{50} was performed by using different concentrations of plant extracts (200, 400, 600, 800, and 1000 µg/ml). A calibration curves were established between concentration of plant extract and RSC%, and then IC_{50} was calculated from the resulting equation (Y= 0.032X+21.81).

Determination of sun-protective factor

The determination of sun-protective factor (SPF) is performed according to the method described by More *et al.* [21]. From the dried extract, 100 µg/ml stock solution was made of aqueous ethanol extracts. Spectrophotometric readings of these solutions were taken in wavelength ranging from 290 to 320nm at 5-nm interval, and the readings were noted down. All the readings were taken in triplicate at each point. The Mansur equation [22] was used to determine the SPF values of the formulations:

$$\mathrm{SPF} = \mathrm{CF} \times \sum_{320}^{290} \mathrm{EE}(\lambda) \times \mathrm{I}(\lambda) \times \mathrm{Abs}(\lambda).$$

In this equation, CF=10 (correction factor), EE (λ)= erythemogenic effect of radiation at wavelength λ, I (λ)=intensity of solar light at wavelength λ, and Abs (λ)=absorbance of wavelength (λ) by a solution of the preparation. The obtained absorbance values were multiplied by the EE (λ) values; their summation was taken and multiplied by the correction factor 10.

Statistics analysis

Means and SE were obtained from analysis of each treatment by using computer program Microsoft Excel 2010. Data were presented as means±SE.

Results

Callus initiation

This experiment aimed to obtain callus tissues from leaf explant. After one month of culturing on MS-medium contained different combinations of 2,4-D+kin and NAA+BAP data was recorded. According to the data in Fig. 1, the NAA+BAP combination achieved a higher callus initiation frequency (%) than 2,4-D +kin.

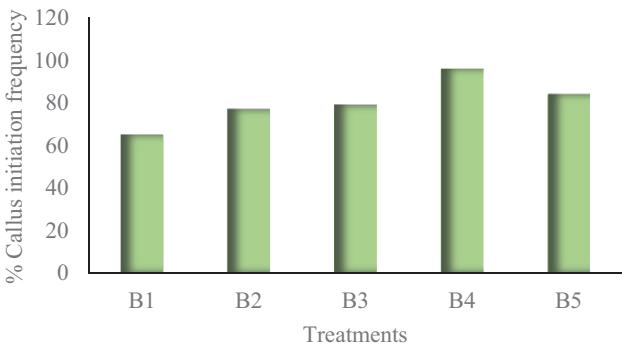
Among the different five treatments, maximum callus initiation frequency (%) was recorded with 1.0 mg/l NAA+1.0 mg/l BAP, which represents 96% (Fig. 1), whereas the minimum value (65%) was recorded with 1.0 mg/l 2,4-D+0.5 mg/l kin treatment.

It is obvious from Fig. 2 that callus tissues were varied because of the different treatments. With B1 and B2 combinations, noticed callus tissues tend to be creamy with some brown color, whereas with B4 and B5 combinations, callus tissues were creamy green in color. It is different from the treatment B3, which formed more white root hairs than callus formation. Therefore, callus tissue formed on MS-medium contained 1.0 mg/l of each NAA and BAP (B4 treatment) was subjected in the followed experiment.

Total phenolics and flavonoids

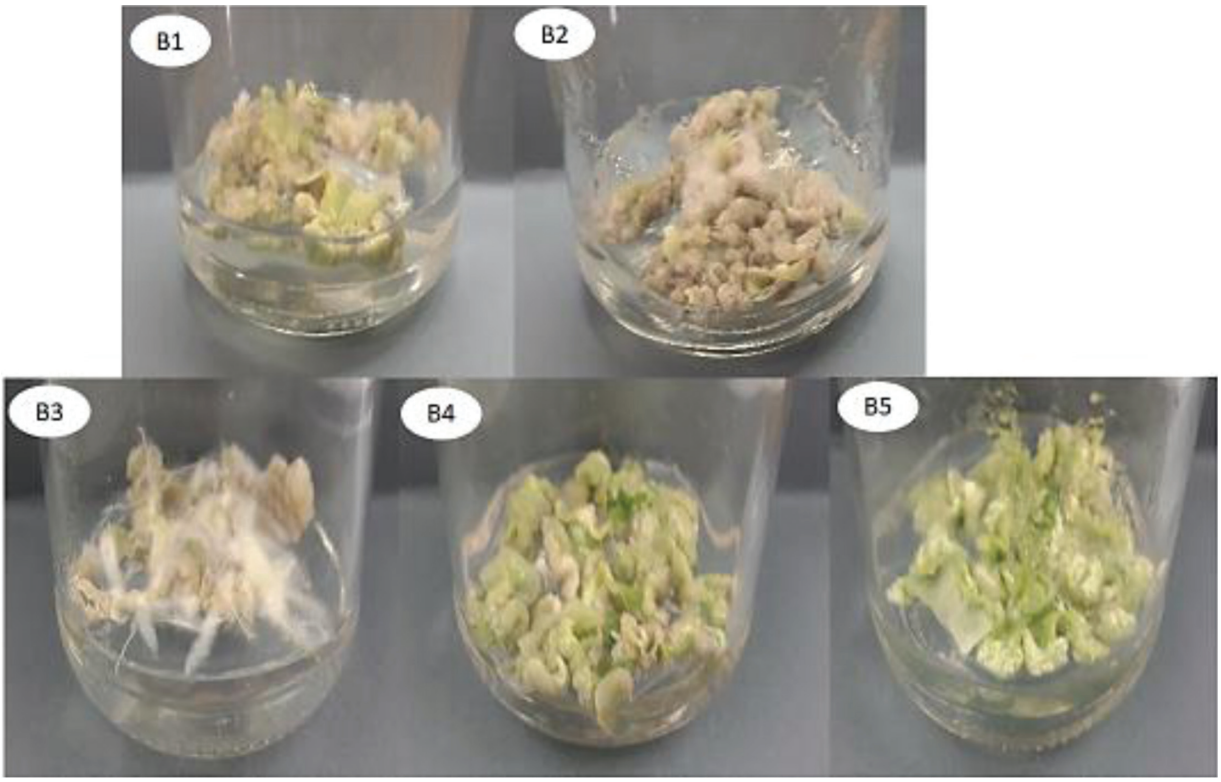
Phenolics and flavonoids are the common important secondary metabolites formed in plants that act as antioxidants. Adding precursors and elicitors in the culture medium is one of the most serious strategies that increase desired components to approximate the percentage that is found in the mother plant within a shorter time. In this experiment, the effects of phenylalanine and salicylic acid have been examined

Figure 1



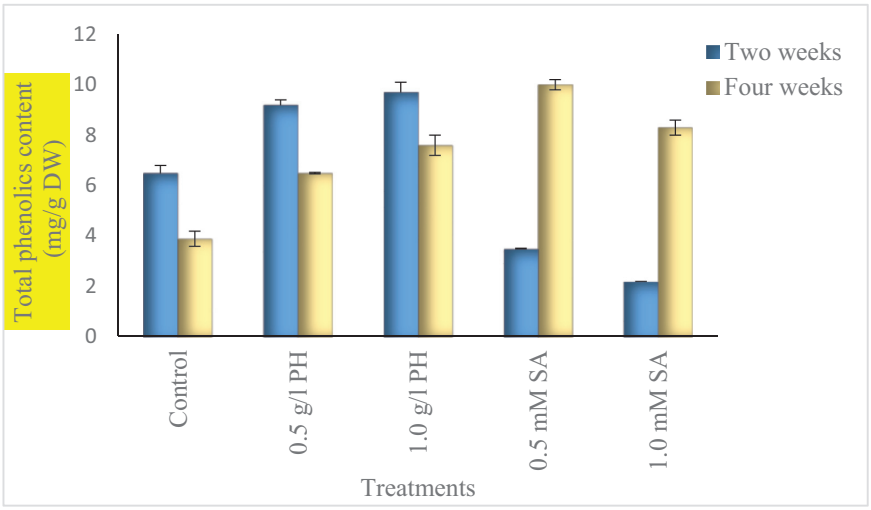
Callus initiation frequency (%) after one month of culturing from leaf explant on different treatments, B1 (1.0 mg/l 2,4-D+0.5 mg/l kin); B2 (1.0 mg/l 2,4-D+1.0 mg/l kin); B3 (1.0 mg/l NAA + 0.5 mg/l BAP); B4 (1.0 mg/l NAA + 1.0 mg/l BAP)); B5 (2.0 mg/l NAA + 1.0 mg/l BAP).

Figure 2



Callus formation from leaf explant after one month of culturing on the different treatments.

Figure 3



Total phenolics content as mg gallic acid per gram DW of sweet basil calli treated with phenylalanine and salicylic acid after 2 and 4 weeks of culturing. DW, dry weight; PH, phenylalanine; SA, salicylic acid.

on TPC and TFC in calli cultures after 2 and 4 weeks. Data shown in Fig. 3 reveal that both phenylalanine and salicylic acid have stimulating effect on phenolic compounds production in sweet basil calli compared with untreated callus tissues. Phenylalanine addition generates a positive effect after 2 and 4 weeks, whereas salicylic acid was only influential after 4 weeks, and had a negative effect after 2 weeks compared with the control. When comparing these values, it turns out that, the maximum value of TPC was recorded by adding salicylic acid (0.5 mm) after 4 weeks, representing 10 mg/g DW, followed by phenylalanine (1.0 g/l) after 2 weeks, recording 9.7 mg/g DW, compared with untreated calli, which

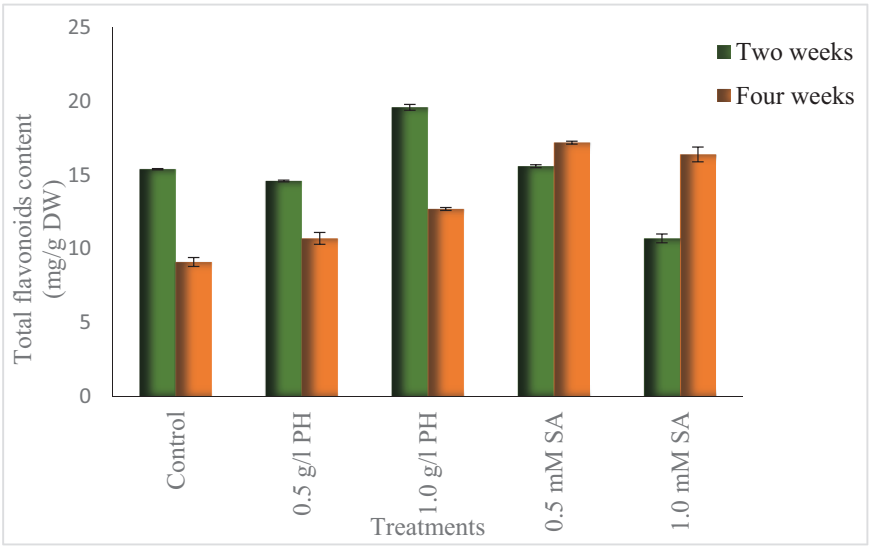
recorded 6.5 and 3.9 mg/g DW after 2 and 4 weeks, respectively.

Regarding the TFC content (Fig. 4), the same direction in the results was also found. Phenylalanine had a positive effect in both 2 and 4 weeks, counter to salicylic acid, which was more effective after 4 weeks. The maximum TFC content recorded with phenylalanine (1.0 g/l) after 2 weeks represents 19.6 mg/g DW, followed by salicylic acid (0.5 mm) after 4 weeks, which recorded 17.2 mg/g DW, compared with untreated calli, which recorded 15.4 and 9.1 mg/g DW after 2 and 4 weeks, respectively. From these observations, it can be deduced that, the addition of 1.0 g/l phenylalanine for 2 weeks or 0.5 mm salicylic acid for 4 weeks had a functional role in increasing the production of both TPC and TFC compounds.

Antioxidant activity of basil extracts

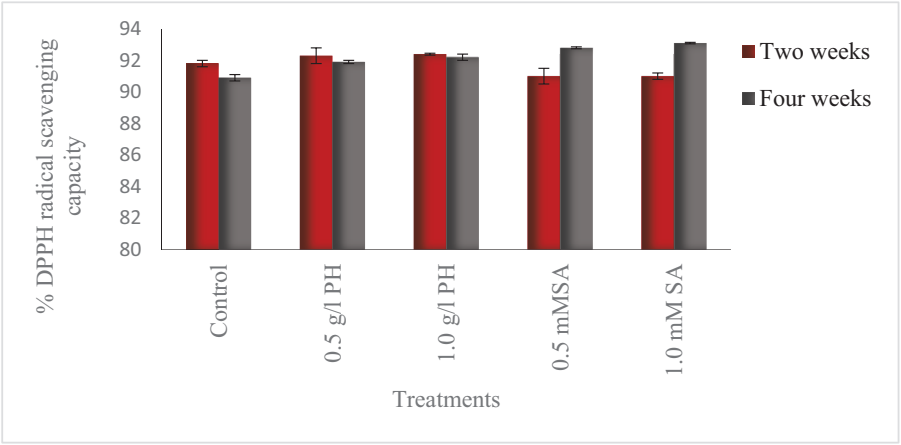
Antioxidant activity of different sweet basil extracts was measured by the DPPH assay (Fig. 5). The results of the current experiment support the pervious findings of TPC and TFC in response to different treatments. Radical scavenging capacity (%) increased with phenylalanine addition after both 2 and 4 weeks, whereas increased only after 4 weeks with salicylic acid addition compared with the control. Maximum

Figure 4



Total flavonoids content as mg quercetin per gram DW of sweet basil calli treated with phenylalanine and salicylic acid after 2 and 4 weeks of culturing. DW, dry weight; PH, phenylalanine; SA, salicylic acid.

Figure 5



DPPH radical scavenging capacity (%) of sweet basil calli treated with phenylalanine and salicylic acid after 2 and 4 weeks. DPPH, 2,2′-diphenyl 1-Picryl-hydrazyl; PH, phenylalanine; SA, salicylic acid.

radical scavenging capacity (%) recorded by salicylic acid addition after 4 weeks two concentrations 0.5 and 1.0 mM represents 93.1 and 92.8%, respectively, followed by phenylalanine addition after 2 weeks concentration 1.0 g/l, which represents 92.4%.

IC_{50} values were calculated from the resulting equation (Y= 0.032X+21.81) and estimated for the sweet basil extracts that own the highest phenolics and flavonoids content as well as the highest antioxidant activity compared with control. Three extracts were selected based on this criterion (2 weeks control, 1.0 g/l 2 weeks phenylalanine, and 0.5 mM 4 weeks salicylic acid). Data shown in Table 1 clarify that, the lowest IC_{50} was detected in 0.5 mM 4-week salicylic acid (0.88 mg/ml) followed by 1.0 g/l 2-week phenylalanine (0.91 mg/ml), whereas the highest value was detected in 2-week control (1.01 mg/ml). Based on these results, the treated calli with 0.5 mM salicylic acid for 4 weeks is considered the best extract, inducing the maximum radical scavenging capacity.

The correlation between the total phenolics, flavonoids content, and the antioxidant activity

Many studies have confirmed the existence of a positive correlation between antioxidant activity and phenolic and flavonoid yields. In the current study, a higher correlation coefficient was detected between TPC content and antioxidant activity (0.83) compared with TFC, which recorded a relatively lower value (0.42); this is shown in Fig. 6a and b. A high correlation coefficient was found between TPC content and antioxidant capacity of different varieties of *Lantana camara* leaves extracted with methanol [23]. This was also confirmed by another report [24] on Plomis species.

Table 1 IC_{50} value of sweet basil calli extract treated with phenylalanine and salicylic acid by 2,2′-diphenyl 1-Picryl-hydrazyl assay

Extracts	IC_{50} (mg extract/ml)
Two weeks control	1.01
1.0 g/l 2 weeks phenylalanine	0.91
0.5 mm 4 weeks salicylic acid	0.88

Sun-protective factor of basil extracts

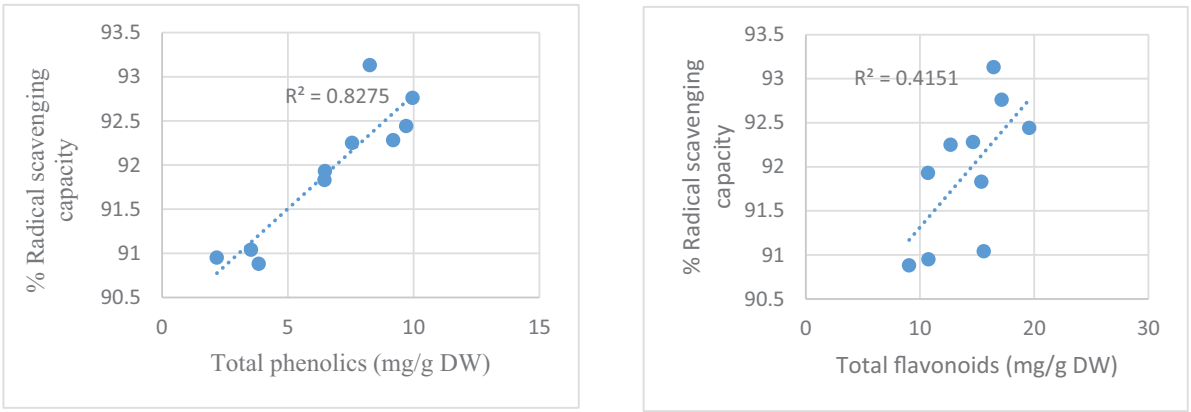
SPF values of three basil extracts (2-week control, 2-week 1.0 g/l phenylalanine, and 4-week 0.5 mM salicylic acid) were assessed by measuring the absorbance using the UV spectrophotometric method in the range of 290–320 nm, every 5 nm. Overall, 100 µg extract/ml of each sample was used.

Table 2 Sun-protective factor of sweet basil calli extracts treated with phenylalanine and salicylic acid

λ	EExI	Two weeks control	Two weeks 1.0 g/l phenylalanine	Four weeks 0.5 mM salicylic acid
290	0.0150	0.0112 ±0.001	0.0321±0.006	0.0111±0.001
295	0.0817	0.0912 ±0.002	0.2609±0.004	0.0866±0.099
300	0.2874	0.3209 ±0.009	1.0088±0.002	0.2989±0.002
305	0.3278	0.3651 ±0.003	1.2052±0.010	0.3507±0.004
310	0.1864	0.1994 ±0.001	0.7270±0.001	0.2081±0.005
315	0.0837	0.0928± ±0.001	0.3437±0.007	0.2081±0.007
320	0.0180	0.0204 ±0.002	0.0725±0.001	0.0218±0.002
Total SPF	1	11.01 ±0.003	36.50±0.003	11.85±0.017
SPF range*		10–14	30–49	10–14
Class of protection		Low	High	Low

SPF, sun-protective factor. *Ionescu and Gougerot [25].

Figure 6



Correlation coefficient between total phenolics (a), total flavonoids (b), and % radical scavenging capacity.

SPF values were calculated and are presented in Table 2. The maximum SPF value was recorded with 2-week 1.0 g/l phenylalanine, representing 36.50±0.003, followed by 4-week 0.5 mM salicylic acid and 2-week control, which recorded 11.85 ±0.017 and 11.01±0.003, respectively. COLIPA (European Cosmetics Trade Association) recommends categories of photoprotection, updated in 2007, applied on skin of volunteers or plastic screen, and it classifies the class of protection as low, medium, high, and very high [25]. Based on the study's findings, ethanolic extract of sweet basil calli that has been exposed to phenylalanine for 2 weeks exhibits a high level of sunscreen protection. This means that it can be used in lotions that are designed for sun protection.

Discussions

As mentioned by Priyanka and Vikas [26], callus cultures play an important role in large-scale production of plant materials that produce secondary metabolites and bioactive compounds.

In the preceding study, friable callus of *O. basilicum* L. was obtained from leaves with 0.2 mg/l NAA in combination with high or low levels of BA [27]. Likewise in another study, good texture of callus tissues was obtained from *O. basilicum* L. leaves by using NAA+BAP combination [28]. The combination of BAP : NAA (5 : 1 mg/l) yields the maximum biomass accumulation of Purple Basil (*O. basilicum* L. var purpurascens) [14]. Unlike these findings, callus tissues of leaves of *O. basilicum* L. were obtained using 2,4-D, maximum callus fresh weight recorded with 0.5 mg/l [29]. Callus induction is regulated by various conditions; the most fundamental factor is the balance between cytokinins and auxins in the culture medium [30].

Sweet basil contains appreciable levels of phenolic and flavonoid compounds as reported by different previous studies. For example, TPC and TFC in the methanol extract of sweet basil seeds obtained 7.15 and 3.28 mg/g extract, respectively [31]. TPC and TFC in sweet basil leaves were evaluated in ethanol and aqueous extracts; higher levels of TPC and TFC were obtained in the ethanol compared with the aqueous extract, with values of 29.60 and 19.85 mg/g DW, respectively [32].

When referring to the previous studies at the level of callus culture, the content of phenolics and flavonoids increased after 1 week of sweet basil culture, recording 6.54 mg gallic acid/g FW and 7.38 mg rutin/g FW, respectively, using 0.5 mg/l 2,4-D [29].

The maximum TPC and TFC were obtained in sweet basil calli exposed to UV for 50 min, recording 18.4 and 13.4 mg/g DW, respectively, compared with other treatments (melatonin addition and other UV durations time) [14]. The maximum radical scavenging activity with IC_{50} value of 1.29 mg/ml was detected in sweet basil leaves collected from Minia than from other regions [33]. Additionally, maximum radical scavenging capacity with IC_{50} of 85.17 µg/ml was reported in the ethanol extract of sweet basil leaves compared with the aqueous extract, which presented a lower value [32].

Salicylic acid and phenylalanine have been applied widely in previous studies to increase phenolic and flavonoids [34,35]. In the current study, 10 mg/g DW of phenolics was achieved and recorded the maximum value when salicylic acid (0.5 mM) was used for 4 weeks. The maximum TFC was recorded using 1.0 g/l phenylalanine after 2 weeks, representing 19.6 mg/g DW.

Phenylalanine is a precursor for different classes of phenolic biosynthesis, including phenolic acids, flavonoids, coumarins, lignins, and tannins. However, salicylic acid works as a signal molecule, activates plant receptors, and induces second messenger molecules that lead to increase in the expression of secondary metabolite-related genes [36].

Antioxidant activity was previously evaluated for sweet basil methanolic extract using the DPPH assay [4]. The authors mentioned that among different phenolic acids that have been detected, rosmarinic acid was the major component accountable for antioxidant activity. Antioxidant activity of five green basil cultivars was also studied using ethanol 80% as an extraction solvent [37]. The results indicated an intense correlation between TPC and antioxidant activity, representing 0.93; this is also strongly consistent with our results. A significant antioxidant activity of sweet basil callus tissue extract grown on the medium containing 0.5 mg/l 2,4-D was previously reported [29].

Phenylalanine has been used extensively in the culture medium to increase phenolic compounds as well as antioxidant activity, for example, in *Ginkgo biloba* cell cultures [35] and in culture medium of carrot [38]. Salicylic acid plays a vital role in plant development and promotes stress tolerance [39]. *Rosmarinus officinallis* L. plants treated with 100–300 ppm salicylic acid

showed a higher antioxidant activity than untreated plants, including boost in enzymes activity of ascorbate peroxidase, catalase, and superoxide dismutase [40].

Phenolics and flavonoids are the major components accountable for antioxidant activity as a result of their ability to catch free radicals. This is due to the nature of their chemical composition, which enable them to interact with free radicals with several mechanisms; the most common mechanism is donating hydrogen atoms to the free radicals [41].

The efficacy of sunscreen is commonly measured by SPF, which is the ratio of UV energy required to produce a minimal erythemal dose in protected skin to unprotected skin [42]. The absorbance of the product is measured between 290 and 320 nm at every 5-nm intervals. There is limited application for using some sunscreens in cosmetics, due to their possibility to induce toxicity when used on the skin; plant extracts may be an alternative way to achieve the same function without inducing any harm on the skin [42]. SPF values of leaf extracts of different plant (*Datura metel*, *Loranthus longiflorus,* and *Eucalyptus*) were previously evaluated using extraction by water and methanol, and the maximum value was recorded with *Eucalyptus* in both water and methanol extracts, representing 17.9 and 23.5, respectively, at 150 µg/ml [43]. Another study examined SPF value of different plant extracts [44], where they found *Punica granatum* (Linn) extract was the most effective, recording 6.06 at concentration of 40 µg/ml.

Scientists are trying to examine the effect of various plant extracts to UV absorption spectra for the possibility of their use and application in sunscreens [45]. 4-nerolidylcathecol is a plant-derived compound that showed promise as a sunscreen, in addition to polyphenolic compound isolated from *Pothomorphe umbellata* roots [46].

Conclusion

Plant tissue cultures, especially callus cultures, can be effective for gaining plant extracts for serious industrial applications. In this study, ethanolic extract was prepared from basil callus cultures and treated with phenylalanine and salicylic acid for 2 and 4 weeks, and then evaluated in terms of active compounds (phenolics and flavonoids), antioxidative activity, and sun-protection effect. The findings indicated a potential for increasing the active ingredients, which in turn increased antioxidative activity and a sun-protective agents.

Acknowledgements

This research is quite compatible with the research plan of National Research Centre. The authors are greatly indebted to the National Research Centre for the generous financing support that enabled us to achieve these results.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Makri O, Kintzios S. Ocimum sp. (Basil): botany, cultivation, pharmaceutical properties, and biotechnology. J Herbs Spices Med Plants 2008; 13:123–150.

2 Ghasemzadeh A, Ashkani S, Baghdadi A, Pazoki A, Jaafar HZ, Rahmat A. Improvement in flavonoids and phenolic acids production and pharmaceutical quality of sweet basil (*Ocimum basilicum* L.) by ultraviolet-B irradiation. Molecules 2016; 21:1203.

3 Javanmardi J, Khalighi A, Kashi A, Bais HP, Vivanco JM. Chemical characterization of basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. J Agric Food Chem 2002; 50:5878–588.

4 Jayasingne C, Gotoh N, Aoki T, Wada S. Phenolic composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). J Agric Food Chem 2003; 51:4442–4449.

5 Abdel Rahman RAI, El-Wakil H, Abdelsalam NR, Elsaadany RMA. *In vitro* production of rosmarinic acid from basil (*Ocimum basilicum* L.) and lemon balm (*Melissa officinalis* L.). Middle East J Appl Sci 2015; 5:47–51.

6 Sharma V, Wani SR, Chaudhary P. Precursor mediated enhanced eugenol production in *Ocimum sanctum* L. through tissue culture methodologies and quantitative estimation through HPLC. J Essent Oil-Bear Plants 2016; 19:241–246.

7 Shahzad A, Faisal M, Ahmad N, Anis M, Alatar A, Hend AA. An efficient system for in vitro multiplication of *Ocimum basilicum* through node culture. Afr J Biotechnol 2012; 11: 6055–6059.

8 Dalton CC. The effect of sugar supply rate on photosynthetic development of *Ocimum basilicum* (sweet basil) cells in continuous culture. J Exp Bot 1984; 35:505–516.

9 Dambolena JS, Zunino MP, López AG, Rubinstein HR, Zygadlo JA, Mwangi JW, *et al.* Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by *Fusarium verticillioides*. Innov Food Sci Emerg Technol 2010; 11:410–414.

10 Kiferle C, Lucchesini M, Mensuali-Sodi A, Maggini R, Raffaelli A, Pardossi A. Rosmarinic acid content in basil plants grown *in vitro* and in hydroponics. Cent Eur J Biol 2011; 6: 946–957.

11 Strazzer P, Guzzo F, Levi M. Correlated accumulation of anthocyanins and rosmarinic acid in mechanically stressed red cell suspensions of basil *(Ocimum basilicum)*. J Plant Physiol 2011; 168:288–293.

12 Marzouk AM. Hepatoprotective triterpenes from hairy root cultures of *Ocimum basilicum* L. Z Naturforsch C J Biosci 2009; 64:201–209.

13 Dias MI, Sousa MJ, Alves RC, Ferreira ICF. Exploring plant tissue culture to improve the production of phenolic compounds a review. Ind Crops Prod 2016; 82: 9–22.

14 Nazir M, Ullah MA, Mumtaz S, Siddiquah A, Shah M, Drouet S, *et al.* Interactive effect of melatonin and UV-C on phenylpropanoid metabolite production and antioxidant potential in callus cultures of purple basil (*Ocimum basilicum* L. var *purpurascens*). Molecules 2020; 25:1072.

15 Gupta VK, Sharma SK. Plants as natural antioxidants. Nat Prod Radiance 2006; 5:326–334.

16 Chandrakasan L, Neelamegam R. In vitro studies on antioxidants and free radical scavenging activities in the extracts of Loranthus longiflorus Desr. Bark samples obtained from two host trees. J Phytol 2011; 3:22–30.

17 Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 1962; 15:473–497.

18 Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299:152–178.

19 Vabkova J, Neugebauerova J. Determination of total phenolic content, total flavonoid content and frap in culinary herbs in relation to harvest time. Acta Univ Agric Silvic Mendel Brun 2012; 20:167–172.

20 Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995; 28:25–30.

21 More BH, Sakharwade SN, Tembhurne SV, Sakarkar DM. Evaluation of sunscreen activity of cream containing leaves extract of *Butea monosperma* for topical application. Int J Cosmet Sci 2013; 3:1–6.

22 Mansur JS, Breder MNR, Mansur MCA, Azuiay RD. Determinação do fator de proteção solar por espectrofotometria. An Bras Dermatol Rio de Janeiro 1986; 61:121–124.

23 Kumar S, Sandhir R, Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. BMC Res Notes 2014; 7:560–568.

24 Zhang Y, Wang Z. Phenolic composition and antioxidant activities of two Plomis species: a correlation study. CR Biologies 2009; 332:816–826.

25 Ionescu MA, Gougerot A. Sun protection and sunscreen labeling – an update. Acta Dermatovenerol Croat 2007; 15:92–95.

26 Priyanka C, Vikas S. Impact of 2,4 dichlorophenoxyacetic acid (2, 4-D) plant growth regulator on *in vitro* callus induction of *Ocimum gratissimum*. Int J Adv Res Sci Eng 2017; 6:125–131.

27 Kintzios S, Makri O, Panagiotopoulos E, Scapeti M. In vitro rosmarinic acid accumulation in sweet basil (Ocimum basilicum L.). Biotechnol Lett 2003; 25:405–408.

28 Trettel JR, Nascimento AB, Barbosa LN, Magalhães HM. In vitro organogenesis and growth of *Ocimum basilicum* 'Genovese' (basil) cultivated with growth regulators. Aust J Crop Sci 2019; 13:1131–1140.

29 Wongsen W, Bodhipadma K, Noichinda S, Leung DWM. Influence of different 2, 4–D concentrations on antioxidant contents and activities in sweet basil leaf–derived callus during proliferation. Int J Food Sci 2015; 22:638–643.

30 Ikeuchi M, Sugimoto K, Iwase A. Plant callus: mechanisms of induction and repression. Plant Cell 2013; 25:3159–3173.

31 Parikh NH, Kothari CS. Phytochemical analysis and total phenolic and flavonoid contents determination of methanolic extract of *Ocimum basilicum* L. seed. Int J Pharmtech Res 2016; 9:215–219.

32 Nguyen VT, Nguyen NQ, Thi NQN, Thi CQN, Truc TT, Nghi PTB. Studies on chemical, polyphenol content, flavonoid content, and antioxidant activity of sweet basil leaves (Ocimum basilicum L.). IOP Conf Ser: Mater Sci Eng 2021; 1092:12083–12089.

33 Ahmed AF, Attia FAK, Liu Z, Li C, Wei J, Kang W. Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. Food Sci Human Wellness 2019; 8:299–305.

34 Díaz-Rivas JO, González-Laredo RF, Chávez-Simental JA, Montoya-Ayón JB, Moreno-Jiménez MR, Gallegos-Infante JA, Rocha-Guzmán NE. Comprehensive characterization of extractable phenolic compounds by UPLC-PDA-ESI-QqQ of *Buddleja scordioides* plants elicited with salicylic acid. J Chem 2018; 2:1–10.

35 Szewczyk A, Kwiecie Ì n I, Grabowski M, Rajek K, Cavò E, Taviano MF, Miceli N. Phenylalanine increases the production of antioxidant phenolic acids in *Ginkgo biloba* cell cultures. Molecules 2021; 26:4965.

36 Ramirez-Estrada K, Vidal-Limon H, Hidalgo D, Moyano E, Golenioswki M, Cusidó RM, Palazon J. Elicitation, an effective strategy for the biotechnological production of bioactive high-added value compounds in plant cell factories. Molecules 2016; 21:182.

37 Juliani HR, Simon JE. Antioxidant Activity of Basil. In: Janick J, Whipkey A. Ed, Trends in new crops and new uses. Alexandria, VA: ASHS Press 2002. p. 575–579.

38 Arafa N, Ibrahim MM, Ali U. Evaluation of total phenolic contents and antioxidant activity of carrot callus extracts as affected by phenylalanine precursor. Plant Tissue Cult Biotec 2015; 25:207–221.

39 Li T, Hu Y, Du X, Tang H, Shen C, Wu J. Salicylic acid alleviates the adverse effects of salt stress in Torreya grandis cv. merrillii seedlings by activating photosynthesis and enhancing antioxidant systems. PLoS One 2014; 9: e109492.

40 El-Esawi MA, Elansary HO, El-Shanhorey NA, Abdel-Hamid AME, Ali HA, Elshikh MS. Salicylic acid-regulated antioxidant mechanisms and gene expression enhance rosemary performance under saline conditions. Front Physiol 2017; 8:716.

41 Zin ZM, Hamid AA, Osman A, Saari N. Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.). Food Chem 2006; 94:169–178.

42 Malsawmtluangi C, Nath DK, Jamatia I, Ralte L, Zarzoliana E, Pachuau L. Determination of sun factor (SPF) number of some aqueous herbal extracts. J App Pharm Sci 2013; 3:150–151.

43 Priyanka S, Inala MSR, Nandini HS, Kutty AVM, Kiranmayee P. A pilot study on sun protection factor of plant extracts: an observational study. Asian J Pharm Clin Res 2018; 11:67–71.

44 Sutar MP, Chaudhari SR. Screening of in vitro sun protection factor of some medicinal plant extracts by ultraviolet spectroscopy method. J App Biol Biotechnol 2020; 8:48–53.

45 Hu G, Wang X. Research on a natural sunscreen from Chinese herbs. Int J Cosmet Sci 1998; 20:175–181.

46 Da Silva VV, Ropke CD, De Almeida RL, Miranda DV, Era CZ, Rivelli DP, *et al.* Chemical stability and SPF determination of *Pothomorphe umbellata* extract gel and photostability of 4-nerolidylcathecol. Int J Pharm 2005; 303:125–131.