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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *IN VITRO* INDUCED CALLUS OF *FARSETIA AEGYPTIA*

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ABSTRACT

A comparative study was carried out on the total phenolic contents and the antioxidant activities of the 70% methanolic extracts of the *in vitro* induced callus of *Farsetia aegyptia*, and the aerial parts and roots of the conventional plant. The effect of using different explant types and phytohormone combinations on the induction of callus, its content and activity was studied. MS medium supplemented with 2,4 dichlorophenoxyacetic acid : benzyl aminopurine : naphthalene acetic acid, 1 : 2 : 0.1 mg/L gave the peak callusing capacity (80 %) using cotyledon explants. Extracts of the calli induced on this medium using leaf and root explants showed comparable phenolic contents (7.32 ± 0.01 and 6.33 ± 0.10 mg GAE/g, respectively) to those of the aerial part and root extracts of the conventional plant (9.21 ± 0.06 and 6.2 ± 0.17 mg GAE/g, respectively). Consequently, the callus extract of the leaf explant showed the highest antioxidant activity (55.03 ± 0.09 % inhibition of DPPH free radical) at a concentration of 50 mg/ml. However, extracts of the aerial parts and roots of the conventional plant displayed higher activities (70.47 ± 0.96 and 79.04 ± 0.05 %, respectively) at a much lower concentration (10 mg/ml).

KEYWORDS: *Farsetia*, callus, phenolic content, antioxidant activity.

INTRODUCTION

Family Crucifereae is one of the largest families in the plant kingdom. It is known for its richness in medicinal plants containing flavonoids and glucosinolates [1]. *Farsetia* is one of the cruciferous genera [1,2]. It consists of 25 species, distributed from North Africa to North West India [2]. The most common one being *Farsetia aegyptia* Turra growing in Egypt. It is a herb used by native Bedouins in the Egyptian traditional medicine as an anti-diabetic and antispasmodic. Moreover, it is used for the relief of rheumatic pains and taken internally as cooling medicine after pounding [3].

In vitro cultures have the potential to form secondary metabolites and to exhibit bioactivities comparable to the original plants [4,5]. Phenolic compounds have been reported to have multiple biological effects, including anticancer and antioxidant activities [6-8]. They exhibit considerable free-radical scavenging activities, which is determined by their reactivity as hydrogen- or electron- donating agents, as well as the stability of the resulting antioxidant-derived radicals [9,10]. Flavonol di-*O*-glycosides were previously reported in the 80% methanolic extract of the herb of *F. aegyptia* [11, 12]. Glucosinolate hydrolysis products were produced in *F. aegyptia* suspension cultures using various elicitors [13].

In the present work, the effect of explant type and phytohormone combinations on the *in vitro* calli production of *F. aegyptia* was investigated, comparing their phenolic contents and potential as antioxidant agents to those of the aerial parts and roots of the conventional plant.

MATERIALS AND METHODS

Plant material

Samples of *F. aegyptia* were collected in the fruiting stage in May 2010 from El-Katameya El-Ain El-Soukhna road, Egypt and were authenticated by Dr. Mohamed El-Gebali, senior botanist at the National Research Centre, Cairo, Egypt.

Chemicals

Benzyl aminopurine (BAP), 2,4 dichlorophenoxyacetic acid (2,4-D), kinetin (KIN), naphthalene acetic acid (NAA), 2,2- Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent and gallic acid were obtained from Sigma-Aldrich, Germany. Sucrose, agar and ascorbic acid were obtained from ADWIC, Egypt, BD Bacto, USA and Fluka, Switzerland, respectively. All chemical reagents and extraction solvents were of analytical grade and all analysis solvents were of HPLC grade.

Sterilization and seed germination

Seeds were disinfected by washing thoroughly with running tap water for 5 min, transferred into aseptic conditions and immersed in 70 % alcohol (v/v) for 30 seconds. Finally the seeds were washed twice (3 min each) with sterilized distilled water. For the seeds enclosed in their pericarps, the closed fruits were washed thoroughly with tap water, followed by 70 % alcohol (v/v) (5 min). The fruits were transferred into aseptic conditions and washed twice with sterile distilled water (5 min each). The fruits were then opened and the seeds were washed twice with sterilized distilled water (2 min each).

Sterilized seeds were germinated aseptically on MS medium containing 0.8 % agar and 2 % sucrose. PH was adjusted to 5.6-5.8. The jars were kept in the dark for 2 days then incubated under fluorescent lamps giving light intensity 3500 Lux with a 16 hrs photoperiod. The *in vitro* seedlings were used as the source of the explants in the induction of callus. The germination capacity, which is the percentage of the germinated seeds to the total number of seeds cultured, was recorded.

Induction of callus

Uniformly sized explants (0.2 - 0.4 cm in length) were aseptically dissected from different organs of the seedlings to produce three types of explants which were the leaf, node and root, in addition to the cotyledon explants from the sterilized seeds. The different explants were cultured on MS media supplemented with different concentrations of various phytohormones (mg/L) giving medium I: KIN (0.1),(2, 4-D) (1), ascorbic acid (10); medium II: KIN (0.1), 2, 4-D (2), ascorbic acid (10); medium III: 2, 4-D (1) + BAP (2) + NAA (0.1).The pH was adjusted to 5.8 ± 1 and 20 jars were prepared each containing 6 explants. The cultures were incubated at 25 ± 2 ˚C under continuous light. Subcultures were made by cutting the callus produced after 6 weeks into small pieces and transferring them on a fresh medium. The behavior of the induced *in vitro* callus was monitored as for the callusing capacity and the morphological characters for the four types of explants on the different media.

Preparation of the extracts

The air-dried powdered aerial parts and roots (5 g each) of *F. aegyptia* were extracted with 50×4 ml of 70 % methanol by sonication. The methanolic extracts were evaporated under reduced pressure at a temperature not exceeding 50 °C. Both extracts were then lyophilized to obtain dry residues of 390 mg and 320 mg of the aerial parts and root, respectively.

Five calli were chosen for extraction and determination of their phenolic contents due to their higher yields. The air-dried powdered calli (120 mg) were similarly extracted with 70 % methanol and evaporated under reduced pressure. The extracts were then lyophilized to give 47, 23, 46, 36 and 45 mg dry residue of the calli of the leaf explant (medium III), the root explant (medium III), the nodal explant (medium III), the nodal explant (medium II) and the cotyledon explant (medium I), respectively.

Quantitative estimation of the total phenolic content

Spectrophotometric determination of the total phenolic content was carried out using the Folin-Ciocalteu colorimetric method [14]. A standard calibration curve was established using serial dilutions of gallic acid, ranging from 0.25 to 5 µg/ml, prepared by dissolving 25 mg of gallic acid in 100 ml distilled water and diluting with 50 % methanol. The absorbance was measured at 760 nm using a spectrophotometer (Jenway double beam, UK) against a blank (50 % methanol instead of the test solution). All sample manipulations were performed protected from light. Three replicates were carried out for each concentration.

The residues of the 70 % methanolic extracts were dissolved in 50% methanol to give a final concentration of 1 mg/ml for both the aerial parts and the root, and 9 mg/ml for the calli. Total phenolic contents were expressed as mg of gallic acid equivalents (mg GAE)/g of the dry extract ± SD (n=3), using the pre-established standard calibration curve and calculated according to the following equation:

Phenolic content (mg GAE/g extract) = conc/1000 × 10/0.8 × 1000/x

where; conc = Concentration from equation of the standard calibration curve.

x = Concentration of the extract (mg/ml).

Determination of the antioxidant activity

The antioxidant activity was assessed using a modified quantitative 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay [15]. The DPPH was prepared at a concentration of 0.004 % in methanol. The lyophilized 70 % methanolic extracts were dissolved in 50 % methanol giving concentrations of 2, 3, 4, 6, 8, 10 mg/ml of the aerial parts and the root, and 9, 15, 25 and 50 mg/ml of the chosen calli. Blank samples were run using 99.9 % methanol. The absorbance was recorded at 517 nm against the blank. Gallic acid was used as a positive control at a concentration of 0.005, 0.01, 0.02, 0.04, 0.06 mg/ml. The antioxidant activity was expressed in terms of the percentage of inhibition of the DPPH free radical (I %) ± SD (n=3) which was calculated according to the formula: $I\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$, where A_{blank} is the

absorbance of the blank, and A_{sample} is the absorbance of the extract. The concentration in mg/ml causing 50 % inhibition of the free radical [IC_{50} ± SD (n=3)] was also determined.

RESULTS AND DISCUSSION

Induction of callus from the *in vitro* germinated seedlings

The germination capacity of seeds using MS medium reached 65 % after 3 weeks. The obtained 6 - 11 weeks old *in vitro* seedlings were used as the source of explants in the induction of callus (Fig. 1). The highest callusing capacity was recorded for the leaf explant (57.1 %) followed by the cotyledon and node explants (53.5 % and 53 %, respectively) while the lowest callusing capacity was recorded for the root explant (50.8 %), regardless of the combination of phytohormones used (Table 1). Hypocotyl and leaf explants were the best explants in callus percent formation in *Hyoscyamus muticus* [16].

On the other hand, medium I induced the highest percentage of callus formation (67.5 %) followed by medium III (59.48 %) then medium II (33.87 %), regardless of the type of explant used. Media containing high auxin and low cytokinin concentrations were reported to promote cell proliferation resulting in callus formation [6,16]. Comparing both media I and II, it was clear that increasing the concentration of 2, 4-D from 1 to 2 mg/L lowered the callusing capacity to nearly half of its value. Also replacing the combination 0.l mg/L KIN and 10 mg/L ascorbic acid in medium I with 2 mg BAP and 0.1 mg/L NAA in medium III resulted in relatively the same callusing capacity for the leaf, root and cotyledon explants while it tremendously lowered the callusing capacity for the node explant (Table 1).

Table 1: Callusing capacity* of different plant explants of *Farsetia aegyptia*.

Explants / Media	Leaf	Node	Root	Cotyledon	Mean
Medium I	72	75	62.5	60.5	**67.5**
Medium II	25	70.5	20	20	**33.87**
Medium III	74.4	13.5	70	80	**59.48**
Mean	**57.1**	**53**	**50.8**	**53.5**	

***** Callusing capacity (%) = number of calli formed/number of explants cultured × 100. **medium I**, KIN (0.1 mg/L) + 2, 4-D (1 mg/L) + ascorbic acid (10 mg/L); **medium II**, KIN (0.1 mg/L) + 2, 4-D (2 mg/L) + ascorbic acid (10 mg/L); **medium III**, 2, 4-D (1 mg/L) + BAP (2 mg/L) + NAA (0.1 mg/L).

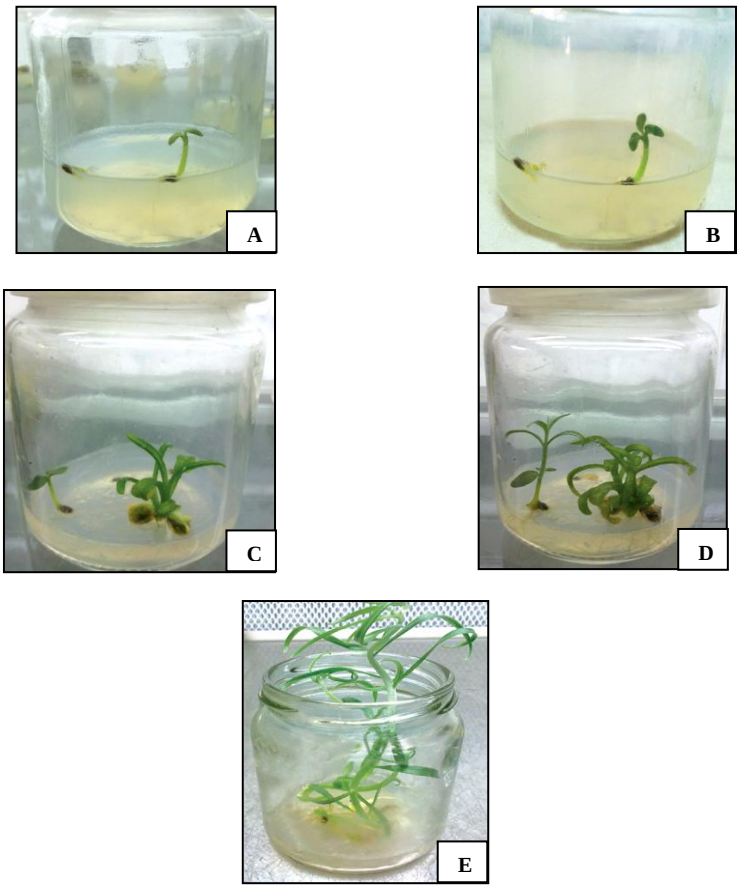

Fig. 1: Different stages of the *in vitro* seed germination.

A, two weeks old seedling; B, three weeks old seedling; C, six weeks old seedlings; D, eight weeks old seedlings; E, eleven weeks old seedlings.

Medium III induced relatively high callusing capacities (80 and 74.4%) using cotyledon and leaf explants, respectively, followed by medium I using nodal explants (75 %). The lowest callusing capacity was recorded when culturing the nodal explant on medium III (13.5 %). Induced calli were mostly compact and yellowish brown in colour except for the calli of the cotyledon explant which were yellowish green in colour (Table 2, Fig. 2).

Table 2: Morphological characters of the calli from different plant explants of *Farsetia aegyptia*.

Media \ Explants	Leaf	Node	Root	Cotyledon
Medium I	Yellowish brown, compact	Greenish brown, compact	Yellowish brown, compact	Green then yellow, compact
Medium II	Yellowish brown, compact	Yellowish brown, friable	Yellowish brown, compact	Yellowish green, compact
Medium III	Dark yellowish brown, compact	Yellowish brown, friable	Yellowish brown to greenish brown, friable	Yellowish green, compact

Medium I, KIN (0.1 mg/L) + 2, 4-D (1 mg/L) + ascorbic acid (10 mg/L); **medium II**, KIN (0.1 mg/L) + 2, 4-D (2 mg/L) + ascorbic acid (10 mg/L); **medium III**, 2, 4-D (1 mg/L) + BAP (2 mg/L) + NAA (0.1 mg/L).

The total phenolic content and antioxidant activity

The 70% methanolic extracts of five of the high yield induced calli and the aerial parts and roots of *F. aegyptia* were standardized to their content of total phenolic compounds using the standard calibration curve (Fig. 3). There was a significant variation in the phenolic content of the calli of different explants ranging from 7.32 ± 0.01 mg GAE/g for the callus of the leaf explant (medium III) to 1.09 ± 0.1 mg GAE/g for the callus of the nodal explant (medium II). Also the medium composition displayed a significant influence on the phenolic content, as medium III induced relatively high phenolic contents using the leaf, root and nodal explants (7.32 ± 0.01, 6.33 ± 0.1 and 5.56 ± 0.02 mg GAE/g, respectively).

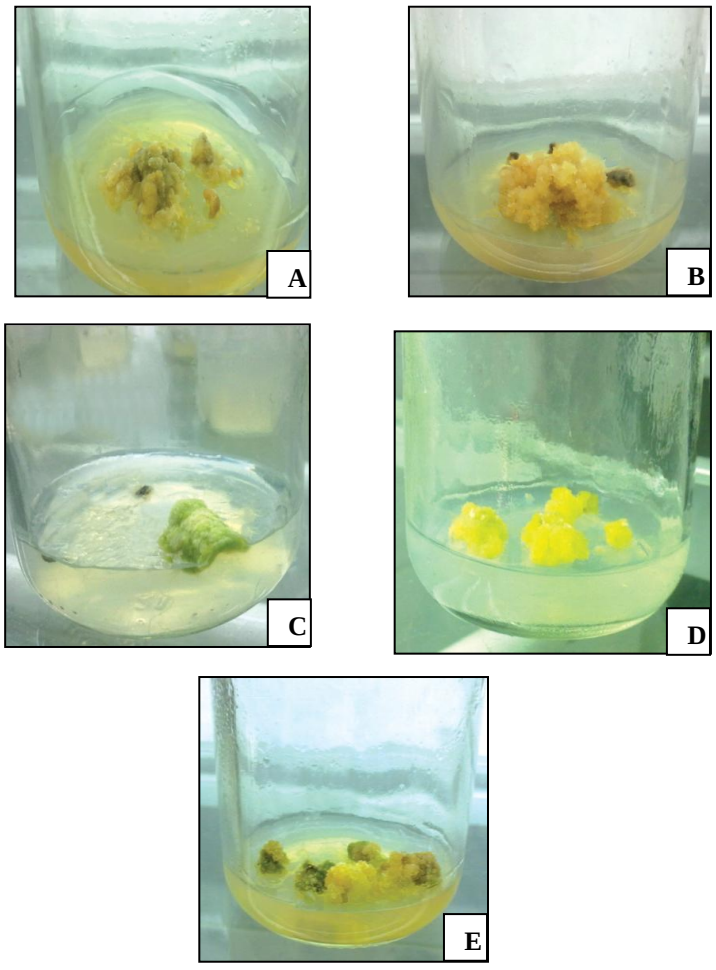

Fig. 2: Calli of some explants on different media.

A, callus of leaf explant cultured on medium III; B, callus of node explant cultured on medium II; C, callus of root explant cultured on medium III; D, callus of cotyledon explant cultured on medium I; E, callus of cotyledon explant cultured on medium I.

The calli of the leaf and root explants cultured on medium III showed comparable phenolic contents (7.32 ± 0.01 and 6.33 ± 0.1 mg GAE/g, respectively) to the aerial parts and root of the conventional plant (9.21 ± 0.06 and 6.2 ± 0.17 mg GAE/g, respectively) (Table 3). The standardized extracts were assessed for their capacity to scavenge DDPH free radical along with gallic acid as a positive control. The antioxidant activities of the different calli were directly correlated with their phenolic content where the callus of the leaf explant (medium III) containing highest phenolic content showed the highest antioxidant activity (55.03 ± 0.09 %) at 50 mg/ml.

The aerial parts and root extracts of the conventional plant displayed much higher antioxidant activities (IC_{50} values of 6.15 ± 0.05and 6.35± 0.01 mg/ml, respectively) compared to the extracts of the calli of the leaf and root explants cultured on medium III (IC_{50} values of 45.08 ± 0.04 and 57.54 ± 0.05 mg/ml, respectively) (Tables 4-6). So the antioxidant activity of *F. aegyptia* could be attributed to the presence of active constituents, other than the phenolic compounds, which are present in the conventional plant and not in the induced callus. Such compounds as glucosinolates previously reported to be present in *F. aegyptia* and known for their chemoprevention against cancer and their antioxidant activities [17]. Previous studies on three Callistemon species failed to produce high phenolic content in the *in vitro* cell cultures, on MS media fortified with KIN (0.9 mg/L) and NAA (1.1 mg/L), compared to the leafs of the conventional plant [6]. However the 80% ethanolic extracts of the cell cultures showed comparable antioxidant activities to the leaf extracts of the conventional plant [6].

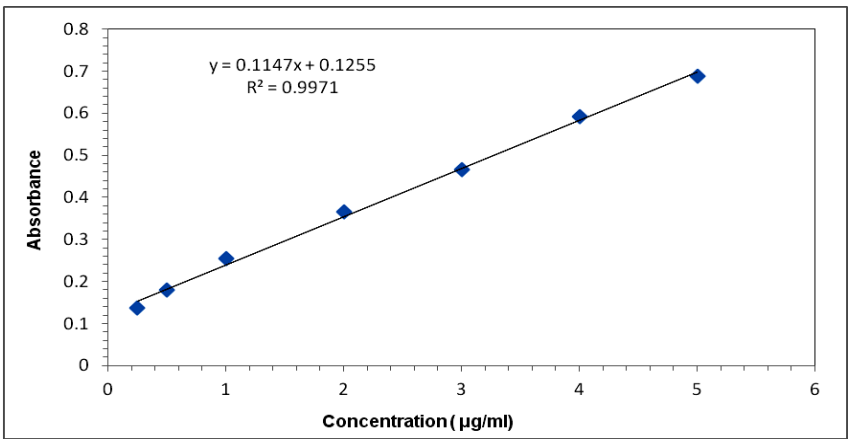

Fig. 3: Standard calibration curve for gallic acid standard.

Table 3: Total phenolic content of the 70 % methanolic extracts of the aerial parts, root and the calli from different explants of *Farsetia aegyptia*.

Extract	Phenolic content GAE (mg/g extract) ± SD (n=3)
Aerial parts	9.21 ± 0.06
Root	6.2 ± 0.17
Leaf callus (medium III)	7.32 ± 0.01
Root callus (medium III)	6.33 ± 0.10
Nodal callus (medium III)	5.56 ± 0.02
Nodal callus (medium II)	1.09 ± 0.10
Cotyledon callus (medium I)	3.52 ± 0.03

Table 4: Antioxidant activity of the 70 % methanolic extracts of the aerial parts and the root of *Farsetia aegyptia* assayed by the DPPH assay.

Methanolic extract	% Inhibition ± SD (n=3)					
	2 mg/ml	3 mg/ml	4 mg/ml	6 mg/ml	8 mg/ml	10 mg/ml
Aerial parts	24.13 ± 0.56	32.01 ± 0.23	37.79 ± 0.11	51.02 ± 0.40	61.89 ± 0.10	70.47 ± 0.96
Root	16.50 ± 0.06	23.86 ± 0.10	30.13 ± 0.06	41.72 ± 0.15	67.62 ± 0.06	79.04 ± 0.05

Table 5: IC_{50} values of the antioxidant activities of the 70 % methanolic extracts of the aerial parts, root and the calli of *Farsetia aegyptia* assayed by the DPPH assay.

Methanolic extract	IC_{50} (mg/ml) ± SD (n=3)
Aerial parts	6.15 ± 0.05
Root	6.35 ± 0.01
Gallic Acid	0.03 ± 0.01
Leaf callus (medium III)	45.08 ± 0.04
Root callus (medium III)	57.54 ± 0.05
Nodal callus (medium III)	67.91 ± 0.01
Nodal callus (medium II)	135.34 ± 0.03
Cotyledon callus (medium I)	84.39 ± 0.05

Table 6: Antioxidant activity of the 70 % methanolic extracts of the calli of different explants of *Farsetia aegyptia* assayed by the DPPH assay.

Callus	% Inhibition ± SD (n=3)			
	9 mg/ml	15 mg/ml	25 mg/ml	50 mg/ml
Leaf (medium III)	11.75 ± 0.24	18.49 ± 0.13	29.18 ± 0.22	55.03 ± 0.09
Root (medium III)	6.06 ± 0.09	12.64 ± 0.09	20.57 ± 0.09	43.22 ± 0.29
Nodal (medium III)	20.71 ± 0.09	24.56 ± 0.10	28.21 ± 0.26	41.24 ± 0.15
Nodal (medium II)	1.81 ± 0.05	3.83 ± 0.89	8.22 ± 0.34	17.33 ± 0.04
Cotyledon (medium I)	20.36 ± 0.15	25.08 ± 0.13	28.93 ± 0.35	36.09 ± 0.09

CONCLUSION

Establishment of a protocol for the *in vitro* callus production of *Farsetia agyptia* was successfully achieved using four types of explants on three different media. The medium composition had a significant effect on the phenolic content of the different calli. The antioxidant activities of the different calli were directly correlated to their phenolic contents. Future studies will include investigation of the use of different elicitors to increase the antioxidant activities of the induced calli, correlating it with other produced secondary metabolites.

REFERENCES

1. Täckholm V. Students' Flora of Egypt. Beirut; Cairo University and Cooperative Printing Co.: 1974.
2. Boulos L, Flora of Egypt. Cairo; Al-Hadara Publishing: 1999.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed., vol II, Dehradun, India; Bishen Singh Mahendra Pal Singh: 1975, pp. 894-95.
4. Ahuja MR, Evens DA, Sharp WR, Am-mirato PJ. Handbook of Plant Cell Culture. New York; Macmillan:1986, pp. 626-51.
5. Parsaeimehr A, Sargsyan E, Javidnia K. A comparative study of the antibacterial, antifungal and anti-oxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of ephedra. Molecules, 2010; 15: 1668-78.
6. Abdelhady MI, Abdel Motaal A, Beerhues L. Total phenolic content and antioxidant activity of standardized extracts from leaves and cell cultures of three Callistemon species. AJPS, 2011; 2: 847-50.

7. Abdel Motaal A, Shaker S. Anticancer and antioxidant activities of standardized whole fruit, pulp, and peel extracts of Egyptian pomegranate. TOPROCJ, 2011; 2: 41-5.
8. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K et al. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem, 1999; 47: 3954–62.
9. Ang-Lee MK, Moss SJ, Yuan CS. Herbal Medicines and Preoperative Care. J Amer Med Assoc, 2001; 286: 208-16.
10. Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry, 2007; 105: 940- 49.
11. Cuyckens F, Shahat AA, Pieters L, Claeys M. Direct stereochemical assignment of hexose and pentose residues in flavonoid O-glycosides by fast atom bombardment and electrospray ionization mass spectrometry. J Mass Spectrom, 2002; 37: 1272–79.
12. Shahat A A, Cuyckens F, Wang W, Abdel-Shafeek KA, Husseiny HA et al. Structural characterization of flavonol di-O-glycosides from *Farsetia aegyptia* by electrospray ionization and collision-induced dissociation mass spectrometry. Rapid Commun Mass Spectrom, 2005;19: 2172–78.
13. Al-Gendy AA, Lockwood GB. Production of glucosinolate hydrolysis products in *Farsetia aegyptia* suspension cultures following elicitation. Fitoterapia, 2005; 76: 288–95.
14. Druckerei CH. European Pharmacopoeia. 4th ed., Nördlingen; Beck: 2002, p.187.
15. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem, 1992; 40: 945-48.
16. Ibrahim AI, Abd El-Kawi M, Nower A, Abdel Motaal A, Abd El-Aal A. Alkaloid production and organogenesis from callus of *Hyoscyamus muticus* L. *in vitro*. JASR, 2009; 5: 82-92.
17. Al-Gendy AA, Lockwood GB. GC–MS analysis of volatile hydrolysis products from glucosinolates in *Farsetia aegyptia* var. ovalis. Flavour Fragr J, 2003; 18: 148-52.