

Full Length Research Paper

Antioxidant and antibacterial activity of callus and adventitious root extracts from *Rumex vesicarius* L.

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The present work has been carried out for the *in vitro* production of biologically active constituents from *Rumex vesicarius* L. cultures. Calli and adventitious roots were tested for their antioxidant and antibacterial activities. Results of preliminary phytochemical screening revealed that cultures were rich in flavonoids, alkaloids, tannins, carbohydrates and/or glycosides, coumarins and cardiac glycosides. Sterols and/or triterpenoids, chlorides and sulphates were found in some cultures only. Calli and roots in all treatments were devoid of saponins, anthraquinones, irodoids and sublimable substances. Total phenolic content in roots produced by culture of leaf blade explant on media containing 1 mg/L kinetin (K) + 4 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D) and calli produced on media containing 2 mg/L K + 2mg/L 2,4-D produced high total phenolic content (21.48 ± 0.24 and 18.15 ± 0.238 mg/explants, respectively). Total flavonoid content of calli and roots produced on media containing 2 mg/L K + 2mg/L 2,4-D and 1 mg/L K + 4 mg/L 2,4-D produced high total flavonoid contents (52.82 ± 0.56 and 49.27 ± 0.56 µg/explant, respectively). Results of high performance liquid chromatography (HPLC) analysis of flavonoids of calli obtained on 2 mg/L K + 2mg/L 2,4-D showed high concentration of quercetin (7.35 ± 0.245 µg/g D.W.). High total antioxidant activity was found in the extract of calli obtained on 2 mg/L K + 2 mg/L 2,4-D (617.85 ± 20.11 GAE_s, ppm). Highest 2,2-diphenylpicrylhydrazyl (DPPH) scavenging activity was obtained using adventitious roots and calli extracts ($IC_{50} = 1.57 \pm 0.02$ and 1.76 ± 0.021 mg/ml, respectively). Antibacterial activity of adventitious roots and calli extracts was negative against all 6 human pathogenic isolates investigated.

Key words: Polygonaceae, flavonoids, phenolics, HPLC analysis, callus, *in vitro* culture, plant growth regulators.

INTRODUCTION

Rumex vesicarius L. (Polygonaceae) is a wild edible plant used as a sorrel and collected in spring time, eaten fresh or cooked. The species has many important medicinal uses such as treatment of tumors, hepatic diseases, bad digestion, constipation, calculi, heart troubles, pains, diseases of the spleen, hiccup, flatulence, asthma,

bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea. The plant is also used as cooling, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic, aphrodisiac, antioxidant and antibacterial agents. Leaves and seeds are eaten as antidote for scorpion stings. The roasted

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seeds were eaten for the cure of dysentery. Finally, the plant can be used also to reduce biliary disorders and control cholesterol levels (Mostafa et al., 2011; Ahirrao and Patil, 2012; Sakkir et al., 2012).

The plant contains many bioactive substances such as flavonoids (vitexin, isovitexin, catechin, epicatechin, orientin and isorientin), anthraquinones particularly in roots (emodin and chrysophanol), quinones, carotenoids, vitamins (especially vitamin C, α -tocopherol, β -carotene and β -tocopherol), proteins, essential oils, lipids, carbohydrates, reducing sugars, phenols, tannins, saponins, triterpenoids and organic acids. This plant is also a good source of minerals, such as; K, Na, Ca, Mg, Fe, Mn and Cu. Mineral content in this plant reached 1.49%, while the amount of iron reached 4.01 mg/100 g (Mandle et al., 2012; El-Hawary et al., 2012).

The previously mentioned bioactive phytochemicals found in *R. vesicarius* L. (such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid) have a role as antioxidant and detoxifying agents. The intake of dietary antioxidant phytochemicals like carotenoids, phenolic compounds and flavonoids lead to the protection against noncommunicable diseases in human beings as cancer, cardiovascular diseases and cataract (Rao, 2003; Matkowski, 2008).

Van Berg and Labadie (1981) showed that tissue culture of *Rumex alpinus* produce anthraquinones, dianthrones, -naphthalenes and -benzenes, the production of these substances were affected by changing the hormonal additives in the culture media. This study was followed by few other studies (Jordan and Jones, 1983; Caulafic et al., 1987a, b). *Rumex acetosa* is a perennial species being used as a model plant in molecular genetic studies of sex determination, because of several characteristics, such as dioecy, sex chromosomes and short life cycle. Studies were conducted to determine the regeneration potentials of different organs, such as cotyledon, hypocotyl, leaf, petiole and flowering stem, cultured on media containing different cytokinin and auxin combinations (Shakib et al., 2008). The present study aimed at the study of *in vitro* production of some antioxidant and antibacterial agents (especially phenolics and flavonoids) from the economically and medicinally important plant species *R. vesicarius*.

MATERIALS AND METHODS

Plant

R. vesicarius L. samples were collected at the vegetative stage of growth (March, 2011), 60 km from Ain Sokhna, Quatamia-Ain Sokhna desert road, Egypt. Plant specimens were identified and authenticated by comparing with herbarium specimens (Boulos, 1999). Sample was deposited in the Herbarium of the Botany and Microbiology Department, Faculty of Science, Helwan University, Helwan, Egypt (Number: 1057). All experimental studies on the plant were carried out in Botany Department and Central Services Labs., National Research Centre, Dokki, Giza, Egypt. Samples

were collected from the field, washed with tap water and kept in clean white plastic bags. *In vitro* cultures were initiated on the same day of sample collection.

In vitro culture

Explant sterilization

Different explants (stem segments and leaves blades and petiole sections, 0.5 to 0.7 cm) were surface disinfected in 70% ethanol for 30 s, rinsed with sterile distilled water, then soaked in 20% clorox (5.25% NaOCl) solution for 20 min, followed by 3 rinses in sterile distilled water.

Callus initiation

Preparation of media

To a clean 2 L flask, 60 g sucrose and 8.86 MS powdered medium (Murashige and Skoog, 1962) were added (PhytoTechnology Laboratories, Product No. M26441) and then dissolved in distilled water. The flask was left on magnetic stirrer for few minutes until complete dissolving of the components. The solution was divided into 16 groups. K and/or 2,4-D were added as shown in Table 1. pH of the media was adjusted to 5.8, then agar (10% w/v) was added. Media were then distributed in 250 ml baby food jars (25 ml/jar) after gelling by boiling on heater. All were closed with autoclavable polypropylene caps and autoclaved for 20 min at 121°C and 1.1 kg/cm².

Culture conditions and sample collection

Jars were incubated at 26 ± 2°C under a photoperiod of 16 h light of intensity 2 k lux, provided by cool white fluorescent tubes. Explants consisted of 10 replication jar/explant type; four explants/jar. *In vitro* calli and roots were produced after 8 weeks. Only explants giving callus and/or adventitious roots were weighed and used for chemical analysis (preliminary phytochemical screening, total phenolics and total flavonoids), arranged from 1 to 10 as follows: (1) Culture of stem segments on media containing 1 mg/L K + 0 mg/L 2,4-D; (2) stem segments on media containing 4 mg/L K + 2 mg/L 2,4-D; (3) stem segments on media containing 2 mg/L K + 4 mg/L 2,4-D; (4) leaf blade segments on media containing 1 mg/L K + 0 mg/L 2,4-D; (5) leaf blade segments on media containing 1 mg/L K + 4 mg/L 2,4-D; (6) leaf blade segments on media containing 2 mg/L K + 2 mg/L 2,4-D; (7) leaf blade segments on media containing 1 mg/L K + 1 mg/L 2,4-D; (8) leaf blade segments on media containing 4 mg/L K + 1 mg/L 2,4-D; (9) leaf petiole segments on media containing 1 mg/L K + 1 mg/L 2,4-D and (10) leaf petiole segments on media containing 0 mg/L K + 1 mg/L 2,4-D.

Preliminary phytochemical screening of calli and adventitious roots

The following substances were investigated for their presence/amount in 8 weeks old calli and adventitious roots: Flavonoids (Mabry et al., 1970); anthraquinones (Farnsworth et al., 1969); tannins (Trease and Evans, 1978); alkaloids (Shellard, 1957); saponins (Hungund and Pathak, 1971); carbohydrates and/or glycosides (Stank et al., 1963); irodoids (Weiffering, 1966); coumarins (Feigl, 1960); chlorides and sulphates (Islam et al., 1993); sterols and/or triterpenes (Claus, 1967); cardiac glycosides (Balbaa et al., 1981) and sublimable substances (Affi, 1972).

Table 1. Concentrations of K and/or 2, 4-D used for the *in vitro* culture of *R. vesicarius* seedling explants.

Media	Growth regulators mg/L	
	K	2, 4-D
1	0	0
2	0	1
3	0	2
4	0	4
5	1	0
6	1	1
7	1	2
8	1	4
9	2	0
10	2	1
11	2	2
12	2	4
13	4	0
14	4	1
15	4	2
16	4	4

Assay for total phenolics

Total phenolics were estimated following the method of Gursoy et al. (2009) involving Folin–Ciocalteu reagent and gallic acid as standard with 1 ml of each extract of different calli and roots (samples from 1 to 10, produced by *in vitro* culturing of stem, leaf blades and petiole segments) containing 66.7 mg F.W. Concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph. The calibration curve of reference standard (gallic acid) was made using four different concentrations (25, 50, 100 and 200 ppm).

$$\text{Absorbance} = 0.0167 \text{ gallic acid (ug)} + 0.017 \text{ (R}^2\text{: 0.99)}$$

Assay for total flavonoids

Total flavonoids were determined using the method of Gursoy et al. (2009) with 1 ml of each extract of different calli and roots (samples from 1 to 10, produced by culturing of stem, leaf blades and petiole segments) containing 66.7 mg F.W. Concentrations of flavonoids were calculated according to the following equation obtained from the standard quercetin graph. The calibration curve of reference standard (quercetin) was made using four different concentrations (6.25, 12.5, 25 and 50 ppm).

$$\text{Absorbance} = 0.0228 \text{ quercetin (ug)} - 0.0045 \text{ (R}^2\text{: 0.9979)}$$

HPLC analysis of flavonoids

Callus obtained by the culture of leaf blade segments on 2 mg/L K + 2 mg/L 2,4-D, the highest sample containing total flavonoids, was used. Extraction was carried out according to Taskeen et al. (2009), using HPLC-grade chemicals. HPLC analysis was carried out using the modified method of Olszewska (2008), as follows: Filtration through membrane filter (0.4 µm); the filtrate was subjected to

separation by HPLC instruments under the following conditions (conditions in case of standard; were the same with that used for all samples): Mobile phase, acetonitrile (86 %): methanol (100 %), 75:25 (v/v); Buffer 14% (Pot. Dihydrogen Phosphate: Phosphoric acid, 2:1 v/v); flow rate: 1 ml/min; Agilent 1100 series (Waldborn, Germany); Quaternary pump (G1311A); Degasser (G1322A); Thermostated Autosampler (G1329A); variable wave lengths detector (G1314A); Column Zorabax 3005 B C₁₈ column; 25 × 4.6 mm, 5µ (Agilent Technologies, USA). Concentration of quercetin (Sigma) in each sample was 6 mg/ml, while concentration of callus sample was 50 mg/ml. Injection volume was 20 µl. Each sample was measured in two replicates. Wave length was adjusted at 370 nm for separation of different compounds.

Antioxidant bioassay

Total antioxidant activity of adventitious roots produced by culture of leaf blade segments on media containing 1 mg/L K + 4 mg/L 2,4-D and calli produced by culture of leaf blade segments on media containing 2 mg/L K + 2 mg/L 2,4-D (the highest containing samples regarding total phenolics and flavonoids) was performed using phosphomolybdenum reagent solution method of Prieto et al. (1999) and adopted by Kumar et al. (2008). The antioxidant capacity was expressed as gallic acid equivalent (GAE) by using the standard gallic acid graph. The calibration curve of reference standard (gallic acid) was made from four different concentrations (R² = 0.870). DPPH (1.1 diphenyl-2 picryl hydrazyl) scavenging activity of adventitious roots produced by culture of leaf blade segments on media containing 1 mg/L K + 4 mg/L 2,4-D calli produced by culture of leaf blade segments on media containing 2 mg/L K + 2 mg/L 2,4-D (the highest containing samples regarding total phenolics and flavonoids) was carried out using the method of Gursoy et al. (2009).

Antibacterial activity

Tested microorganisms

Antibacterial activity of roots produced by culture of leaf blade segments on media containing 1 mg/L K + 4 mg/L 2,4-D calli produced by culture of leaf blade segments on media containing 2 mg/L K + 2 mg/L 2,4-D (the highest containing samples regarding total phenolics and flavonoids) was investigated against six human pathogenic bacteria isolates, obtained from Clinical Pathology Department, Faculty of Medicine (Kasr El- Eini), Cairo University, Egypt. These included three gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603), three gram-positive bacteria: *Streptococcus pneumoniae* (ATCC 49619), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615). The purity and viability of cultures were checked by culturing on nutrient agar slants, incubated at 37°C for 24 h. Cultures were subcultured every week and stored at 4°C (Yaecob and Tolba, 2006; Arya et al., 2010).

Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37°C for 4 h. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1 to 2 × 10⁸ colony-forming units per milliliter (cfu/ml), the suspension was then used for further testing (Arya et al., 2010).

Antibacterial bioassay

The antibacterial bioassay was carried out following disc diffusion method according to Arya et al. (2010). The concentration of each extract per disc equals 50 mg/disc in case of roots produced by culture of leaf blade segments on media containing 1 mg/L K + 4 mg/L 2,4-D calli produced by culture of leaf blade segments on media containing 2 mg/L K + 2 mg/L 2,4-D and positive controls (synthetic drugs; Cefotaxime, Cephadrine and Amoxycilin, Flucloxacilin), while in case of quercetin and emodin (natural compounds used as positive controls) lower concentrations were used (25, 50, 100 µg/disc). Negative controls were methanol, ethanol and empty discs. The diameter of inhibition zone (mm) as indicated by clear area in the Petri dish which was devoid of bacterial cells growth was measured. Each Petri dish contained four centered disks, r value of each disk = 5 mm, one layer, Whatman number 1 filter paper.

Determination of activity and proportion indexes

Calculations were carried out following the methods of Singh et al. (2002) and Borgio et al. (2008).

Statistical analysis

Statistical analysis was done using Fisher analysis of variance methodology. A least significant difference test was applied at 5 and 1% probability level to determine differences among treatment means (Steel and Torrie, 1984). The CO-STAT computerized package program was subjected to the regular statistical analysis of variance (Nissen et al., 1985) using two designs: (1) Anova-1 completely randomized design (CRD) and (2) factorial implemented in completely randomized design. Each reading = mean of three replicates ± standard error (SE) for all experiments. For *in vitro* studies, replicates were ten.

RESULTS

Fresh and dry weights of calli and/or roots

Fresh and dry weights of calli or adventitious roots obtained by culture of stem or leaf blade and petiole segments on media (after 8 weeks) containing different concentrations of Kinetin and/or 2, 4-D is represented in Table 2. Results showed variations in both fresh and dry weights of calli or adventitious roots obtained by culture of stem or leaf blade or leaf petiole segments within differences in concentrations of kinetin and/or 2,4-D. Only explants giving callus and/or adventitious roots were weighed.

Phytochemical studies

Preliminary phytochemical screening of calli and/or adventitious roots

Results of preliminary phytochemical screening of calli and adventitious roots are represented in Table 3. There were variations in the presence and/or amount of these active ingredients within calli and roots. Calli and adventi-

tious roots in all treatments were rich in flavonoids, alkaloids, tannins, carbohydrates and/or glycosides, coumarins, cardiac glycosides. Sterols and/or triterpenoids, chlorides and sulphates were detected in some treatments only.

Calli or roots in all treatments were devoid of saponins, anthraquinones, irodoids and sublimable substances.

Total phenolics and total flavonoids of calli and/or adventitious roots

Results of total phenolics expressed as mg GAEs/g F.W. (Figure 1a) showed that the maximum amount was found in calli obtained from leaf petiole segments, cultured on medium containing 0 mg/L K + 1 mg/L 2,4-D (8.46 ± 0.434), followed by calli obtained by the culture of leaf blade segments on 2 mg/L K + 2 mg/L 2,4-D (3.38 ± 0.256). Calli obtained by the culture of leaf blade segments on 4 mg/L K + 1 mg/L 2,4-D was found to contain the least amount of phenolics (1.00 ± 0.055). Results of total phenolics, expressed as mg GAEs/explant F.W. (Figure 1b) was different, since the maximum amount of total phenolics was found to be in adventitious roots obtained by the culture of leaf blade segments on 1 mg/L K + 4 mg/L 2, 4-D (21.48 ± 0.24), followed by calli obtained by the culture of leaf blade segments on 2 mg/L K + 2 mg/L 2,4-D (18.15 ± 0.238). Calli obtained by the culture of leaf blade segments on 4 mg/L K + 1 mg/L 2, 4-D was found to contain the least amount of phenolics (1.95 ± 0.03). Variations in the amount of total phenolics between all samples were non significant.

Results of total flavonoids, expressed as µg/g F.W. (Figure 2a) showed that maximum amount of total flavonoids was found in calli obtained from leaf petiole segments cultured on medium containing 0 mg/L K + 1 mg/L 2,4-D (21.61 ± 0.646) followed by calli obtained by the culture of leaf blade segments on 2 mg/L K + 2 mg/L 2,4-D (9.84 ± 0.543). Calli obtained by the culture of stem segments on 2 mg/L K + 4 mg/L 2,4-D was found to contain the least amount of flavonoids (3.16 ± 0.258).

Results of total flavonoids, expressed as µg/explant F.W. (Figure 2b) showed the maximum amount of total flavonoids in calli obtained by the culture of leaf blade segments on 2 mg/L K + 2 mg/L 2,4-D (52.82 ± 0.56), followed by adventitious roots obtained by the culture of leaf blade segments on 1 mg/L K + 4 mg/L 2,4-D (49.27 ± 0.56). Calli obtained from leaf petiole segments cultured on medium containing 0 mg/L K + 1 mg/L 2,4-D contained the least amount of flavonoids (5.40 ± 0.199). Variations in the amount of total flavonoids between all samples were non significant.

HPLC analysis of flavonoids of callus

Results of HPLC analysis of flavonoids (using quercetin

Table 2. Types of morphogenesis, fresh and dry weights of calli and adventitious roots obtained by culture of different explants (1, 2 and 3 = Stem segments, leaf blade segments and leaf petiole segments respectively) on different concentrations of K and 2,4-D.

Concentration (mg/L)		Type of morphogenesis			Fresh weight (mg)			Dry weight (mg)		
K	2,4-D	1	2	3	1	2	3	1	2	3
0	0	-	-	-	-	-	-	-	-	-
0	1	-	-	Callus	-	-	250±5.20	-	-	42±0.27
0	2	-	-	-	-	-	-	-	-	-
0	4	-	-	-	-	-	-	-	-	-
1	0	Callus+ Root	Root	-	3300±58.89	4330±51.38	-	550±11.02	722±13.28	-
1	1	-	Root	Callus	-	7600±68.71	1120±20.79	-	1267±45.03	187±4.04
1	2	-	-	-	-	-	-	-	-	-
1	4	-	Root	-	-	13910±109.12	-	-	2318±56	-
2	0	-	-	-	-	-	-	-	-	-
2	1	-	-	-	-	-	-	-	-	-
2	2	-	Callus	-	-	5370±56	-	-	895±9.82	-
2	4	Callus	-	-	2000±20.79	-	-	330±7.23	-	-
4	0	-	-	-	-	-	-	-	-	-
4	1	-	Callus	-	-	1950±32.33	-	-	325±10.97	-
4	2	Callus	-	-	5400±56.58	-	-	900±11.02	-	-
4	4	-	-	-	-	-	-	-	-	-
		L.S.D. (0.05)			2.37	2.39	2.36	2.37	2.39	2.36
		L.S.D. (0.01)			3.17	3.21	3.16	3.17	3.21	3.16

as standard) of callus obtained by the *in vitro* culture of leaf blade segments on 2 mg/L K + 2 mg/L 2,4-D (the highest sample containing total flavonoids) revealed that this sample contains high amounts of quercetin ($7.350 \pm 0.245 \mu\text{g/g D.W.}$).

Biological studies on calli and/or adventitious roots of different explants

Antioxidant activity

Results of total antioxidant activity studies (Figure

3) on the selected samples of adventitious roots produced by culture of leaf blade segments on media containing 1 mg/L K + 4 mg/L 2,4-D and calli produced by culture of leaf blade segments on media containing 2 mg/L K + 2 mg/L 2,4-D were found to have 175.625 ± 6.323 and $617.850 \pm 20.109 \text{ GAE}_s \text{ ppm}$, respectively. Results of DPPH scavenging activity (Figure 4) revealed that adventitious roots produced by culture of leaf blade segments on media containing 1 mg/L K + 4 mg/L 2,4-D calli produced by culture of leaf blade segments on media containing 2 mg/L K + 2 mg/L 2,4-D were found to have $\text{IC}_{50} = 1.574 \pm 0.02$ and $1.755 \pm 0.021 \text{ mg/ml}$, respectively. Positive

controls in these experiments were quercetin and emodin; quercetin was a potent antioxidant agent ($\text{IC}_{50} = 0.801 \pm 0.26 \text{ mg/ml}$) while emodin had no effect at the used concentrations.

Antibacterial activity

Results of antibacterial activity using ethanol and methanol extracts of adventitious roots produced by culture of leaf blade segments on media containing 1 mg/L K + 4 mg/L 2,4-D and callus produced by culture of leaf blade segments on media containing 2 mg/L K + 2 mg/L 2,4-D

Table 3. Preliminary phytochemical screen of calli and/or adventitious roots obtained by culture of different explants on media (1 to 10 materials and methods, sample collection).

S/no	Phytochemical screen	1	2	3	4	5	6	7	8	9	10
1	Carbohydrates and/or glycosides	+	+	+	+	+	+	+	+	+	+
2	Saponins	-	-	-	-	-	-	-	-	-	-
3	Tannins	+	+	+	++	++	++	++	+	+	++
4	Sterols and/or triterpenoids	-	-	+	+	+	+	+	-	-	+
5	Alkaloids	++	++	++	++	++	++	++	++	++	++
6	Cardiac glycosides	++	+	+	++	+	++	++	++	+	++
7	Flavonoids	++	+	+	++	++	++	++	++	+	++
8	Anthraquinones	-	-	-	-	-	-	-	-	-	-
9	Coumarins	+	+	+	+	+	+	+	+	+	+
10	Irodoids	-	-	-	-	-	-	-	-	-	-
11	a-Chlorides	-	+	+	-	-	+	-	+	+	++
	b-Sulphates	+	+	+	-	-	+	+	+	+	++
12	Sublimation	-	-	-	-	-	-	-	-	-	-

- = absent; + =mild reaction; ++ = strong reaction.

showed no antibacterial activity against all human pathogenic bacterial isolates under investigation (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. pneumonia*, *S. aureus* and *S. pyogenes*).

DISCUSSION

Total flavonoids of calli and adventitious roots in the present study were higher than that of whole plant parts of wild samples. Mostafa et al. (2011) found that whole plant parts collected from the desert during the flowering stage contain $11.223 \pm 1.850 \mu\text{g/g}$ F.W. Results of phytochemical screening study on calli and adventitious roots agreed with El-Bakry et al. (2011), since they found that there were differences in growth and chemical compositions of *in vitro* grown seedlings of *R. vesicarius* L. at 10, 20 and 30 days old on either solidified MS medium or agar. Phytochemical screening of 10, 20 and 30 days old seedlings showed variations in the presence and/or amount of some biologically active constituents under investigation such as; flavonoids, saponins, alkaloids, tannins, chlorides and sulphates. Total phenolics and total flavonoids and HPLC analysis of seedlings grown on MS medium or agar varied in their concentrations.

Results of HPLC analysis of callus agreed with Mostafa et al. (2011), since they found that different plant parts at reproductive stages (flowering and fruiting) contained flavonoids (using quercetin as standard). Antioxidant activity of callus and adventitious roots agreed with results of Mostafa et al. (2011) and Tavares et al. (2010), where *R. vesicarius* and *R. maderensis* exhibited

antioxidant activity. Arya et al. (2008) found that 6 weeks old callus tissues derived from leaf explants of *Pluchea lanceolata* Oliver and Hiern. produced high amounts of quercetin (0.23 mg/g dry weight of tissues).

Babu et al. (2008) found that cell suspension cultures (60 days old) of *Azadirachta indica* (A. Juss), grown on liquid MS medium containing 5% sucrose, 1% mannitol and 0.1 mg/L IBA, produced high amounts of secondary metabolites including three terpenoid compounds (azadirachtin, mimbin and salamin) and two flavonoids (quercetin and kaempferol). Hairy root cultures of *Fagopyrum esculentum* established by infection with *Agrobacterium rhizogenes* strain 15834 produced five flavanols obtained: (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-O-gallate, procyanidin B2 and procyanidin BZ3'-O-gallate, also present in normal root cultures with a common prominence of epicatechin-3-O-gallate synthesis and inversion in the contents rates of (+)-catechin and (-)-epicatechin. The highest content of procyanidin B2-3'-O-gallate was obtained in hairy root cultures which can be considered as a better production source for flavanols, especially of the two galloylated derivatives (Trotin et al., 1993).

Matkowski (2008) found that antioxidants can be produced *in vitro* from different plants. Anthocyanins, rosmarinic acid, flavonoids, stilbene and alkaloids were produced from *Ajuga reptans*, *Anchusa officinalis*, *Artemisia judaica*, *Arachis hypogaea* and *Withania somnifera*, respectively. Results of the present study confirm the *in vitro* production of biologically active constituents such as flavonoids, coumarins, cardiac glycosides, sterols and/or triterpenoids. It also shows similarities and differences, as well as variations in the

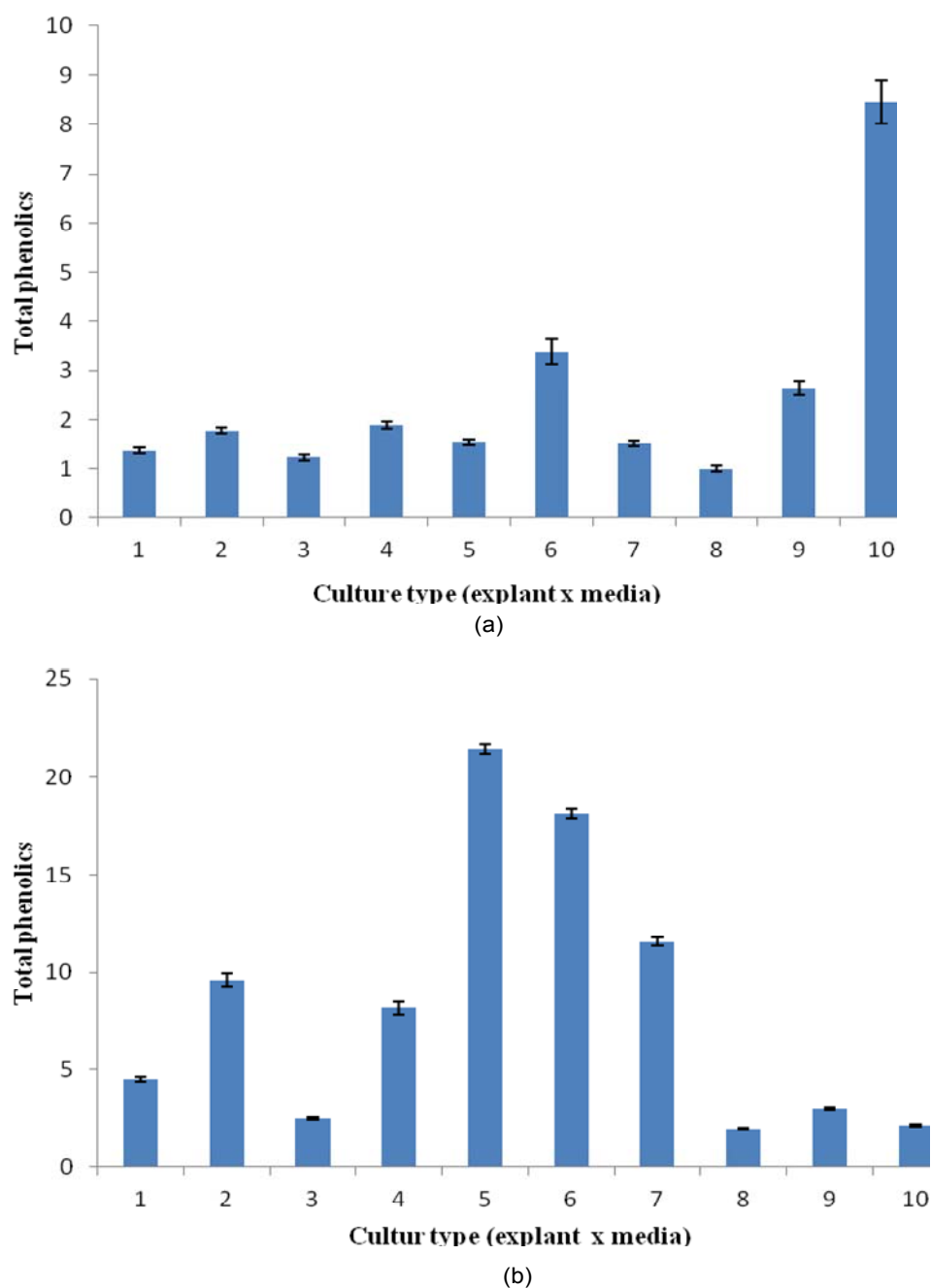


Figure 1. Total phenolics of calli and/or adventitious roots obtained by culture of different explants 1 to 10 on different media expressed as: (a) mgGAEs/g F.W., (b) mgGAEs/explant F.W.

production of such compounds *in vitro* than those produced *in vivo*.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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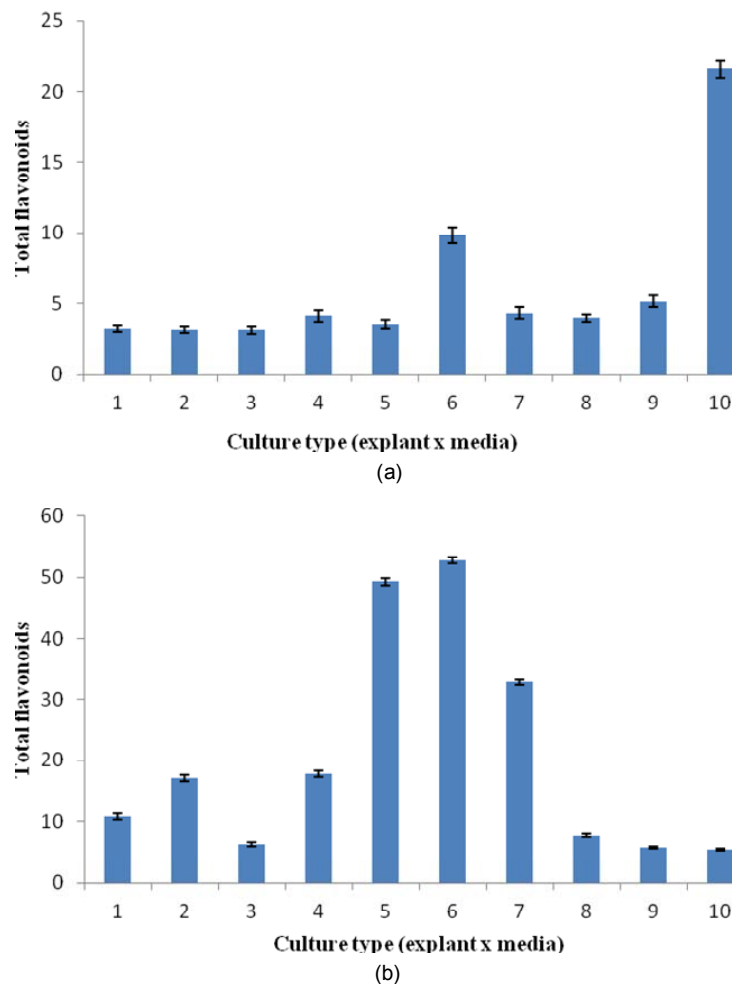


Figure 2. Total flavonoids of calli and/or adventitious roots obtained by culture of different explants 1 to 10 on different media expressed as: (a) µg/g F.W., (b) µg/explant F.W.

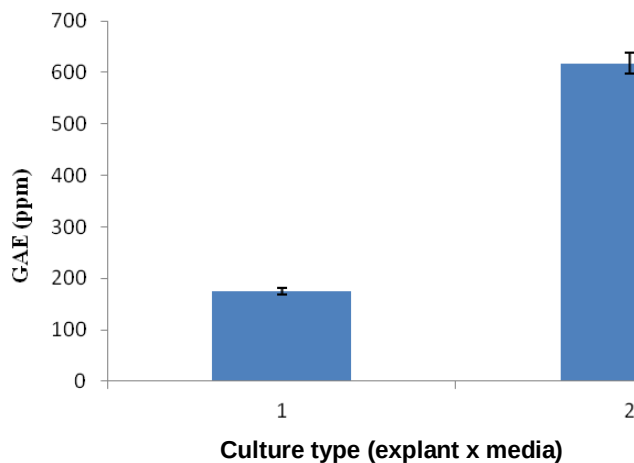


Figure 3. Total antioxidant activity (GAE_s, ppm) of: 1- Adventitious roots obtained by culture of leaf blade segments on 1 mg/L K + 4 mg/L 2,4-D; 2 - Callus obtained by culture of leaf blade segments on 2 mg/L K + 2 mg/L 2,4-D.

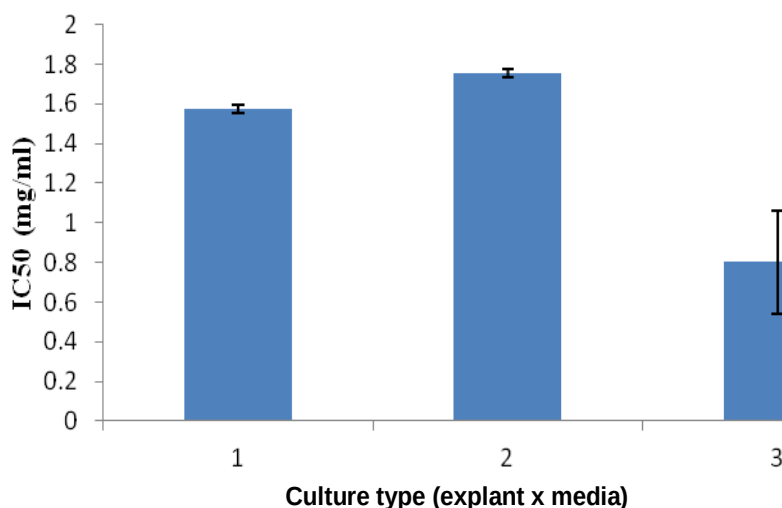


Figure 4. Antioxidant activity DPPH scavenging activity method (IC₅₀ in mg/ml) for: 1- Adventitious roots obtained by culture of leaf blade segments on 1 mg/l K + 4 mg/L 2,4-D; 2- Callus obtained by culture of leaf blade segments on 2 mg/L K + 2 mg/L 2,4-D; 3- Quercetin.

ABBREVIATIONS

GA, Gallic acid; **HPLC**, high performance liquid chromatography; **K**, kinetin; **MS**, Murashige and Skoog medium (1962); **2,4-D**, 2,4-Dichlorophenoxyacetic acid.

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