academicJournals

Vol. 7(29), pp. 2158-2164, 3 August, 2013
DOI: 10.5897/JMPR2013.4444
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Antibacterial and antioxidant activities of seedlings of *Rumex vesicarius* L. (Polygonaceae)

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Accepted 26 June, 2013

The present work has been carried out to investigate both the antibacterial and antioxidant activities of seedlings grown *in vitro* and *in vivo*. Only ethanol extract of *in vitro* germinated seedlings on solidified Murashige and Skoog (MS) hormone free medium, had antibacterial activity. Activity was high in case of *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pneumoniae* (inhibition zones = 55.00 ± 0.00, 55.00 ± 0.00 and 26.25 ± 1.58 mm; activity index = 4.15, 1.67 and 0.48, respectively). The effect was moderate in case of *Staphylococcus aureus* (inhibition zone = 15.75 ± 1.58 mm, activity index = 1.67). The extract had no effect against *Klebsiella pneumoniae* and *Streptococcus pyogenes.* Total antioxidant activity studies (GAE_s in ppm) showed that 20 days old *in vivo* grown seedlings were highest in total antioxidants (1810.00 ± 60.12), followed by 30 days old *in vivo* grown seedlings (1133.13 ± 55.35). Results of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity showed that the least IC_{50} in mg/ml was obtained using 30 days old *in vivo* grown seedlings extract (IC_{50} = 1.16 ± 0.02), followed by 20 days old *in vivo* grown seedlings extract (IC_{50} = 1.26 ± 0.02). The study shows the possibility of producing biological activity from early developmental growth stages of *Rumex vesicarius.*

Key words: Biological activity, *in vitro* seedlings, *in vivo* seedlings, anthraquinones, phenolics, flavonoids.

INTRODUCTION

Genus *Rumex* (family: Polygonaceae) includes many edible plants which attracted the attention of many investigators because of their medicinal importance for the treatment of several diseases. *Rumex vesicarius* L. is a wild edible plant used as a sorrel and is collected in spring time and eaten fresh (Batanouny, 1999), or cooked (Al-Quran, 2009). It was considered a dietary complementary plant, since it is a rich source of β carotenes (Bélanger et al., 2010). The plant has many important medicinal uses. It is a stimulant, tonic, and acts as an aphrodisiac agent (Gopal et al., 2008). The plant also contains many bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin). It is rich in anthraquinones, particularly in roots (emodin and chrysophanol), contains carotenoids, vitamins (especially vitamin C), proteins, lipids and organic acids. This plant is a good source of minerals such as K, Na, Ca, Mg, Fe, Mn and Cu (Saleh et al., 1993; Al–Rumaih et al., 2002; Alfawaz, 2006; Filho et al., 2008). The bioactive phytochemicals (such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid) are known to have a role as antioxidant and detoxifying agents, their intake leads to protection against non-communicable diseases that is, cancer, cardiovascular diseases and cataract. Phenolics and flavonoids are very important biologically active constituents, since they are considered to be anticancer, antioxidant and antimicrobial agents (Rao, 2003; Alberto et al., 2006; Matkowski, 2008; Abd

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Ghafar et al., 2010; Imran et al., 2011).

The phytochemical composition, in particular secondary metabolite content, varies with the developmental stage of the plant (Mostafa et al., 2011, 2012; El-Bakry et al., 2011, 2012; Alam, 2012). *In vitro* grown seedlings can be considered rich sources of many biologically active constituents, especially flavonoids and phenolics, the formation of these substances varied with seedling ages (El-Bakry et al., 2011).

The aim of this study was to evaluate antibacterial and antioxidant activities of *in vitro* and *in vivo* grown seedlings of *R. vesicarius* L. at different seedling growth stages.

MATERIALS AND METHODS

Material

Seeds were collected during August, 2010 (at the ripening fruiting stage), 60 km from Ain Sokhna, Quatamia- Ain Sokhna desert road, Egypt. Plant specimens were identified according to Boulos (1999). Sample was deposited in the Herbarium of the Botany and Microbiology Department, Faculty of Science, Helwan University, Helwan, Egypt (Number: 1057).

Seed germination

In vitro germination

Seeds were immersed in 70% ethanol for 30 to 60 s, soaked in 20% of commercial clorox (5.25% NaOCl) for 15 to 20 min and washed 3 times with sterile double distilled water. Sterile seeds were then aseptically transferred to hormone free MS medium (Murashige and Skoog, 1962) supplemented with 1% agar (5 seeds/jar, 10 jars/treatment). Incubation was at 25± 2°C in 16 h light.

In vivo germination

Seeds were cleaned and planted in 12 cm plastic pots containing sand and peat moss (3:1). Incubation was in a growth chamber under the same growth conditions of *in vitro* germination. Replicates for determination of germination percentage = 500 seeds (10 seeds/pot, 50 pots/treatment). Thirty seedlings were used for the determination of growth parameters.

Biological activity studies

The choice of 10 and 30 days old for *in vitro* grown on MS medium and 20 and 30 days old for *in vivo* germinated seedlings was based on results of the chemical investigation of the different growth stages of *in vitro* and *in vivo* germinated seedlings (El-Bakry et al., 2011).

Antibacterial activity

Tested microorganisms: Antibacterial activity of different extracts was investigated against six human pathogenic bacterial isolates (ATCC collection), obtained from the Clinical Pathology Department, Faculty of Medicine (Kasr El- Eini), Cairo University, Egypt. These included 3 Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603), 3 Gram-positive bacteria: *Streptococcus pneumoniae* (ATCC 49619), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615). The purity and viability of cultures was checked by culturing on nutrient agar slants, incubated at 37°C for 24 h. Cultures were sub-cultured weekly 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 was adjusted to match the turbidity standard of 0.5 McFarland units (Arya et al., 2010).

Antibacterial bioassay: The antibacterial bioassay was carried out following disc diffusion method (Arya et al., 2010). The concentration of each extract per disc was 50 mg/disc in case of *in vitro* and *in vivo* grown seedling extracts. Positive controls were cefotaxime, cephradine, amoxycilin + flucloxacilin and quercetin and emodin (25, 50, 100 µg/disc). Negative controls were ethanol, water and empty discs. Petri dish contained four disks, r value of each disk = 5 mm, one layer, Whatman number 1 filter paper. Each sample was carried out in triplicate.

Determination of activity and proportion indexes: Activity index was calculated according to Singh et al. (2002), while proportion index was calculated according to Borgio et al. (2008).

Antioxidant bioassay: Total antioxidant activity was performed using phosphomolybdenum reagent solution method of Prieto et al. (1999), adopted by Kumar et al. (2008). This method can be summarized as: An aliquot of 0.1 ml of sample solution containing a reducing species, 1 ml of ethanol extract of *in vitro* and *in vivo* grown seedlings (contains 66.7 mg plant material) was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Tubes were capped and incubated in a thermal block at 95°C for 90 min. Each sample was carried out in triplicate. After all samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank (by using UV 2401 Pc, UV-VIS recording spectrophotometer, Shimadzu, Japan).

A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples. 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 using four different concentrations ($R^2 = 0.870$).

DPPH (1,1-diphenyl-2 picryl hydrazyl) scavenging activity was carried out by using the method of Gursoy et al. (2009). 1 ml ethanol extract of *in vitro* and *in vivo* grown seedlings at various concentrations (0.5, 1, 1.5 and 2 mg/ml) were, respectively added to 1 ml of DPPH methanol solution (0.1 Mm). Mixtures were shaken vigorously and allowed to stand in the dark for 30 min. Each sample was done in triplicate. The absorbance of these mixtures was measured using a spectrophotometer at 517 nm. Inhibition of free radical DPPH in percent (1%) was calculated as follows:

$$1\% = 100 \times (A_{control} - A_{sample}) / A_{control}$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the tested sample. Positive controls in these experiments were Quercetin and Emodin (0.05, 0.10, 0.15 and 0.20 mg/ml).

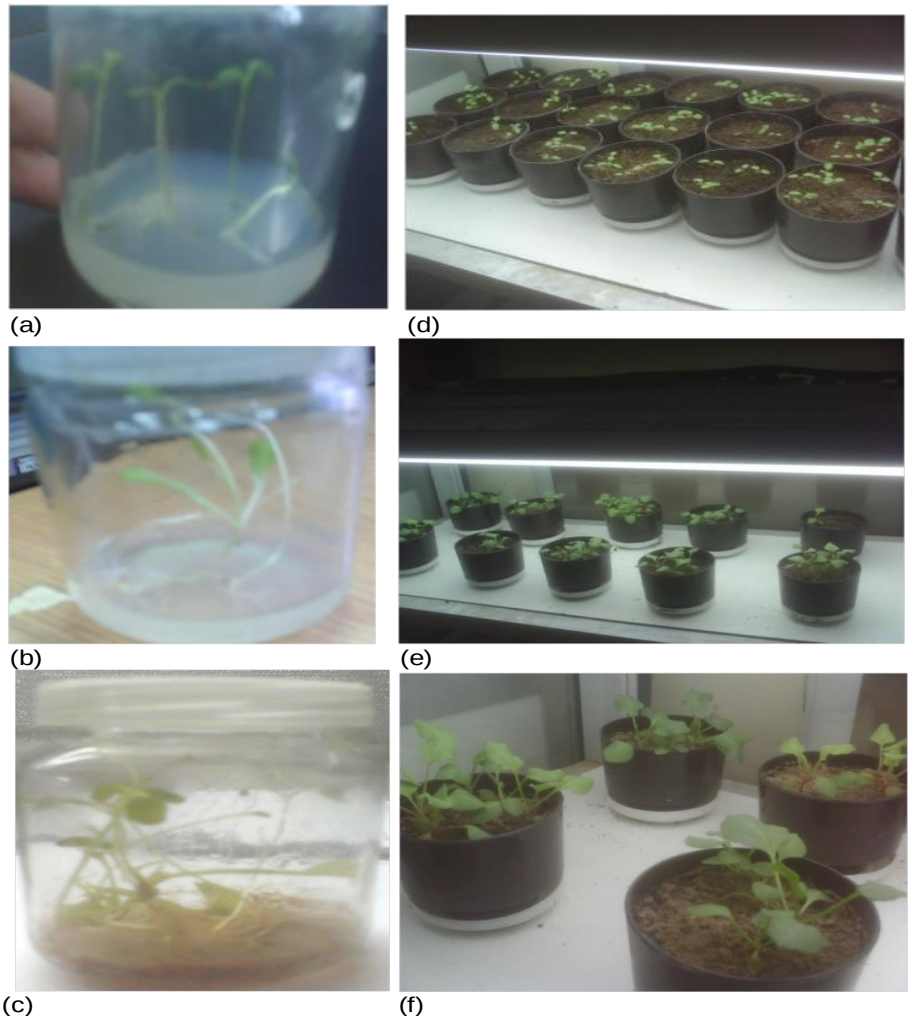

Figure 1. a, b and c = 10, 20 and 30 days old *in vitro* grown seedlings on MS media; d, e and f = 10, 20 and 30 days old *in vivo* grown seedlings.

RESULTS

Antibacterial activity

Studies on seedlings extracts

Results of antibacterial activity studies on *in vitro* and *in vivo* grown seedlings (Figure 1) revealed that only ethanolic extract of *in vitro* grown seedlings on MS media (30 days old) have antibacterial effects against pathogenic bacterial isolates under investigation. Antibacterial activity of ethanol extract of these seedlings (Figure 2) was high in case of *P. aeruginosa*, *E. coli* and *S. pneumoniae* (inhibition zones = 55.00 ± 0.00, 55.00 ± 0.00 mm and 26.25 ± 1.58 mm, activity indexes = 4.15, 1.67 and 0.48, respectively). The effect was moderate in case of *S. aureus* (inhibition zone =15.75 ± 1.58 mm, activity index = 1.67). This extract had no effect against *K. pneumoniae* and *S. pyogenes*. The proportion index of ethanol extract of these seedlings = 0.667.

Different antibacterial agents (positive controls)

Positive controls in these experiments (Table 1 and Figure 3) were quercetin and emodin (natural products) and it was found that quercetin is a potent antibacterial agent, while emodin has lesser effect at the used concentrations. In addition to three synthetic drugs, cefotaxime was the most effective one, followed by amoxycilin, flucloxacilin, while cephradine was the least effective one. Proportion index reached its highest value (1) in case of cephradine.

Antioxidant activity

Results of total antioxidant (GAE_s in ppm) activity studies (Table 2) on the *in vitro* grown seedlings and *in vivo* grown seedlings revealed that *in vivo* grown seedlings, 20 days old, was found to have the highest amount of total antioxidants (1810.00 ± 60.12), followed by *in vivo*

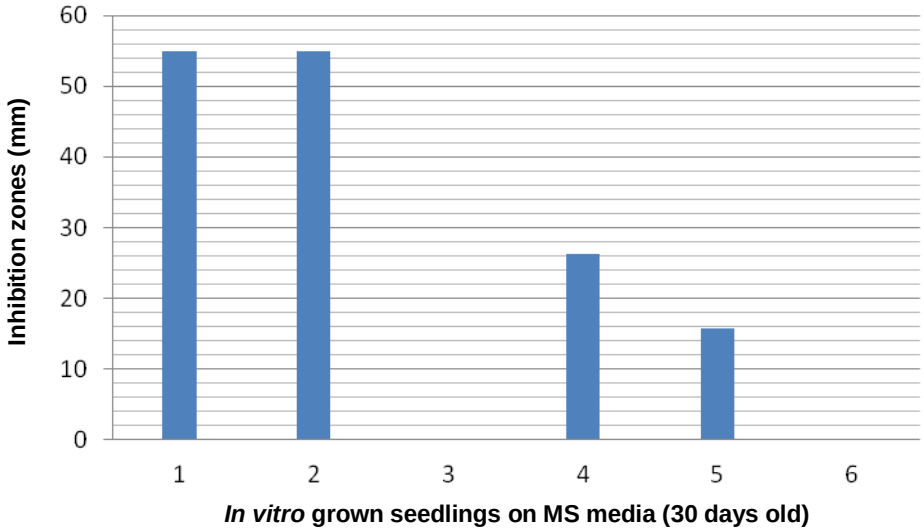

Figure 2. Antibacterial activity of 30 days old *in vitro* grown seedlings on MS media, (inhibition zones in millimeters). 1. *Escherichia coli* (ATCC 25922). 2. *Pseudomonas aeruginosa* (ATCC 27853). 3. *Klebsiella pneumoniae* (ATCC 700603). 4. *Streptococcus pneumoniae* (ATCC 49619). 5. *Staphylococcus aureus* (ATCC 25923). 6. *Streptococcus pyogenes* (ATCC 49623).

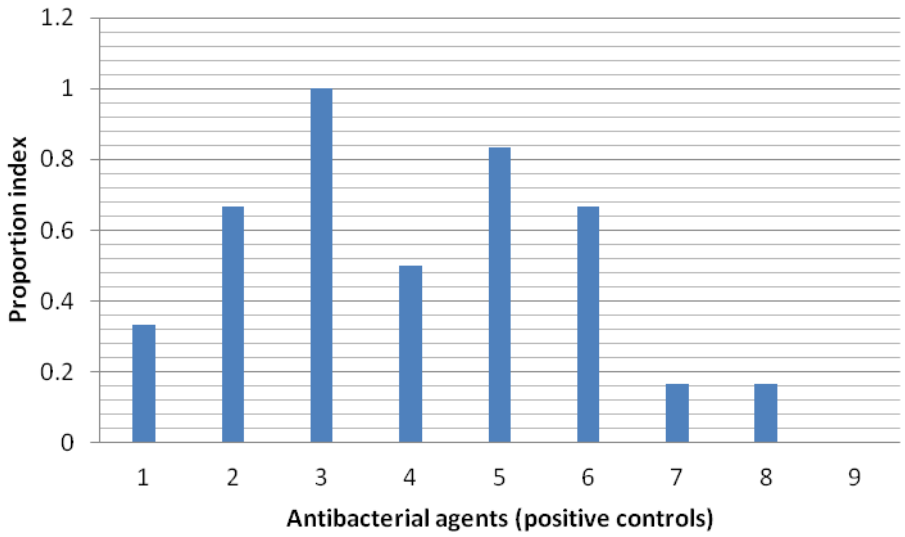

Figure 3. Proportion index of antibacterial activity of different antibacterial agents (Positive controls), 1, 2, 3 = Cephradine, Amoxycillin, Flucloxacillin and Cephotaxime, respectively (50 mg/disc), 4-6 = Quercetin and 7-9 Emodin (25, 50, 100 µg/disc), respectively.

grown seedlings, 30 days old (1133.13 ± 55.35). DPPH scavenging activity results (Table 2) revealed that the least IC_{50} in mg/ml, the highest the effectiveness, was obtained using *in vivo* grown seedlings extract, 30 days old (IC_{50} = 1.16 ± 0.02), followed by *in vivo* grown seedlings extract, 20 days old (IC_{50} = 1.26 ± 0.02). Positive controls in these experiments were quercetin and emodin, it was found that, quercetin is a potent antioxidant agent (IC_{50} = 0.80 ± 0.26), while emodin had no effect at the used concentrations.

DISCUSSION

Studying the different phytochemical properties of the plant species at the different developmental stages of its life cycle allows the understanding and possible control of target biologically active compound(s) of medicinal and

Table 1. Antibacterial activity of different antibacterial agents (positive controls) on pathogenic bacterial isolates under investigation.

Bacteria	Cephotaxime (50 mg/disc)	Amoxycillin, flucloxacillin (50 mg/disc)	Cephradine (50 mg/disc)	Quercetin (µg/disc)			Emodin (µg/disc)		
				25	50	100	25	50	100
Escherichia coli (ATCC 25922)	0.00	0.00	31.25±0.00	6.38±0.07	8.25±0.14	11.50±0.14	0.00	0.00	0.00
Pseudomonas aeruginosa (ATCC 27853)	0.00	0.00	13.25±7.65	0.00	0.00	0.00	0.00	13.38±6.28	0.00
Klebsiella pneumoniae (ATCC 700603)	0.00	11.38±3.54	34.63±0.22	0.00	5.25±0.43	1.13±0.65	0.00	0.00	0.00
Streptococcus pneumoniae (ATCC 49619)	8.25±0.00	54.88±2.09	28.25±5.92	0.00	5.75±1.01	8.88±0.36	0.00	0.00	0.00
Staphylococcus aureus (ATCC 25923)	11.25±0.00	11.13±1.08	28.50±2.74	14.00±2.60	21.00±5.77	8.50±0.00	2.25±0.00	0.00	0.00
Streptococcus pyogenes (ATCC 49623)	0.00	31.25±7.94	6.00±0.00	14.63±5.56	25.63±3.54	0.00	0.00	0.00	0.00

Table 2. Antioxidant activity of seedlings extract (total antioxidant activity and DPPH scavenging activity methods). 1. *In vitro* grown seedlings on MS media (10 days old); 2. *In vitro* grown seedlings on MS media (30 days old); 3.*In vivo* grown seedlings (20 days old); 4. *In vivo* grown seedlings (30 days old).

Sample number	Total antioxidant activity method	DPPH scavenging activity method
1	237.50±6.13	6.61±0.10
2	117.88±6.73	6.07±0.10
3	1810.00±60.12	1.26±0.02
4	1133.13±55.35	1.16±0.02
Quercetin	-	0.80±0.26
LSD (0.05)	155.93	0.28
LSD (0.01)	233.89	0.42

economic importance. In *R. vesicairus, the* ethanol extract of 30 days old *in vitro* germinated seedlings on solidified MS hormone free medium showed high antibacterial activity against *P. aeruginosa* and *E. coli.* Comparing such activity with that of all plant parts at different stages of growth (Mostafa et al., 2011; Mostafa et al., 2012), it could be concluded that seedlings had higher antibacterial activity than those of all plant parts at all stages of growth in case of *P. aeruginosa* and *S. pneumoniae* (inhibition zones = 55.00 ± 0.00 and 26.25 ± 1.58 mm, activity index = 4.15 and 0.48, respectively), and equal to the methanol extract of leaves at late vegetative stage of growth (inhibition zone = 55.00 ± 0.00 mm, activity index = 1.67) in case of *E. coli*.

Antibacterial effects of these seedlings extract on *E. coli* and *P. aeruginosa* were higher than all positive controls in these experiments (quercetin and emodin, as authentic samples of natural products, cefotaxime, amoxycilin, flucloxacilin and cephradine, as authentic samples of synthetic drugs). Results confirmed the findings of Panduraju et al. (2009) who found that aqueous, methanol and petroleum ether extracts of leaves had variable effects against both Gram-positive bacteria (*S. aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Elegami et al. (2001) found that chloroform extract of *R. vesicarius* L. (whole plant parts) had positive effect against *B. subtilis*. Several previous investigators (Nishina et al., 1993; Yildirim et al., 2001; Al-zoreky and Nakahara, 2002; Harshaw et al., 2010) investigated different plant parts of different species of *Rumex* (*R. japonicas, R. crispus, R. nervosus* and *R. obtusifolius*, respectively). Their results revealed that they were potent anti-bacterial agents against a number of both Gram-

positive and Gram-negative bacteria.

Comparing results of total antioxidant activity of seedlings with those of all plant parts, at reproductive (flowering and fruiting) and at vegetative (early and late) stages of growth (Mostafa et al., 2011; El-Bakry et al., 2012; Alam, 2012) showed that seedlings contained higher amounts than all plant parts at reproductive stages (flowering and fruiting) of growth, but lower than at vegetative (early and late) stages of growth. Studied seedlings varied regarding their DPPH scavenging activity from equal to nearly half that of all plant parts, at reproductive (flowering and fruiting) and at vegetative (early and late) stages of growth. Leaf extract at early vegetative stage of growth were the highest plant parts regarding DPPH scavenging activity which was more active nearly four times than seedlings. Seedlings were potent antioxidant agents when compared with quercetin as positive control.

The present antioxidant activity results of *in vitro* germinated seedlings of *R. vesicarius* L. were in agreement with Nishina et al. (1991), Demirezer et al. (2001), Al-Ismail et al. (2006), Özen (2010) and Li and Liu (2009). They investigated different extracts of roots of *R. japonicus* and R*. patientia*, leaves of *R. pulcher* and *R. acetosella* and whole plant parts of *R. dentatus,* respectively. Their results revealed that these species were considered to be antioxidant agents.

Differences in growth parameters and in phytochemical composition in seedling of *R. vesicarius* grown *in vitro* were reported by El-Bakry et al. (2011). Phytochemical screening of 10, 20 and 30 days old seedlings showed variations in the presence and/or amount of some biologically active constituents such as flavonoids, saponins, alkaloids, tannins, chlorides and sulphates. These variations indicated that the formation of these active constituents is either positively or negatively related to time. Total phenolics and total flavonoids in seedlings grown on MS medium or agar showed variations between these seedlings at different ages.

Conclusion

The present study showed the feasibility of producing biologically active compounds, which are antibacterial and antioxidant, from the seedling stage of *R. vesicarius* L. Both the *in vitro* and *in vivo* systems used are simple, efficient and time saving. It also confirms the importance of studying the developmental and temporal regulation of secondary metabolites in higher plants. The study of the phytochemical and biological activities manifested by the different developmental stages allows the selection of the stage that produces the highest activity of the target bioactive compound. It also allows the study of the environmental and physiological control of the production during the plant life cycle. This would consequently contribute to the efficient production of the bioactive compound.

ACKNOWLEGMENTS

The authors wish to thank Ms Farkhonda Rafaat, Helwan University, for her help in preparing the manuscript.

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