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Original Article

Total Phenol, Antioxidant and Allelopathy Assay, and Meiosis Study of *Lampranthus spectabilis* and *Aptenia cordifolia*

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Abstract

Lamperanthus spectabilis and *Aptenia cordifolia* (Aiozaceae) are two ornamental plants. A little information is available about their active compounds and meiosis' process. In this study, the phenolic content, antioxidant activity, allelopathy effects and meiosis' process of pollen mother cells of *L. spectabilis* and *A. cordifolia* were studied. The alcohol extract was used for phenol, antioxidant and allelopathy assay along with young buds for meiosis study. The stamens and petals of both plants possessed the highest phenol and antioxidant effects. All extracts of *L. spectabilis* and *A. cordifolia* had small inhibitory effects on mungbeen seed germination and seedlings growth. The allelopathy effect of both plants on barley seed germination and seedlings growth was significant especially for *A. cordifolia*. The count of chromosomes in meiosis revealed that the chromosome number of *A. cordifolia* was $2n=12$ and $2n=18$ for *L. spetabilis*. The meiosis in both plants was normal and the chromosomes were very small. Our results revealed that the extracts of all organs of both plants; specially stamens and petals, have a lot antioxidant activity. They had not allelopathic effects on seed germination' and seedlings growth' mungbeen and barley. Chromosome number of two plants was different but meiosis process and chromosomes size was similar.

Keywords: active compounds; barley; chromosomes; germination; mungbean

Introduction

Aptenia cordifolia (L. fill) and *Lampranthus spectabilis* (N.E.Br) belonging to Aiozaceae family, are two glabrous perennials and succulent plants (Dellagareca *et al*., 2007). *L. spectabilis* (syn: *Mesembryanthemum spactabile*) is commonly known as a fig marigold (Braun and Winkelmann, 2016; Park *et al*., 2016). Heptadecyl caffeic acid and 2-(2-hydroxyethyl)-3-methyl fumaric acid from the aerial parts of *L. spectabilis*, did not prove to have a significant antioxidant activity or cytotoxicity on a lung cancer cell line (Samy *et al*., 2018). *L. francisci* is an ornamental succulent plant. The hydro-alcohol extracts of *L. francisci* caused hemolysis of sheep cells and had a fungicidal activity (Moyo and Mukanganyama, 2015). Betalines from phenolic compounds did not have toxic effects on human (DelgadoVargas *et al*., 2000) Betalins produce yellow, pink, red and orange colors in different organs Aizoaceae family plants. The betacyanins from betalines as hydroxycininamyl derivatives have been found in *Lampranthus* sp. flowers (Piatteli and Impellizzer, 1969).

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms (Xiao *et al*., 2019). Allelochemical compounds can be classified into organic acid, alcohols, long chain fatty acids, terpenoids, phenols, alkaloids and lactones (Li *et al*., 2010). Meiosis is a special type of cell division that reduces the chromosome. In plant sexual reproduction, normal meiosis of pollen grains is the most important factor of pollen fertility. Meiosis I and II stages (prophase, metaphase, anaphase and telophase) and chromosome behavior studies can be determined for normalized pollen. For this, studies have been still focusing on chromosome behavior during meiosis I and II stages through light microscopy and record the results with pictures (Gopala-Krishnan *et al*., 1964).

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Materials and Methods

Plant material

Shoots of *L. spectabilis* and *A. cordifolia* were trimmed from around the area of agriculture faculty of Azad university of Saveh.

Alcoholic extraction and total phenol

A total of 20 g of each organ (stems, leaves, petals, ovaries and stamens) of fresh plants were mixed with 100 ml ethanol 60%, stored in a bath 60 °C for 2 hours and filtered. The alcoholic extracts were concentrated using vacuum rotary evaporator, at 60 °C. They were used to measuring the total phenol, antioxidant activity, and allelopathic effects. Folin- Ciocalteu' reagent was used for total phenol determination (Singleton and Rossi, 1965). In 0.5 ml of the each sample, 2.5 ml of diluted Folin-Ciocalteu' reagent (1/10v/v) was added. After 5 min, 2 ml of aqueous Na_2CO_3 solution (1M) was added. The solution was stirred gently and incubated in a water bath at 45 °C for 15 minutes. Calibration curve was obtained from different concentrations of galic acid (0-250 µg/ml) by spectrophotometery at 765 nm.

Antioxidant

The antioxidant activities of the organs extracts of *L. spectabilis* and *A. cordifolia* were determined using 2, 2-diphenyl-1-1- picrylhyydrazyl (DPPH) (Ibtissem *et al*., 2009). The extracts were dissolved in alcohol. In 1 ml of the DPPH (0.1 mM) in ethanol, 2 ml of different concentrations (0.05- 0.1 mg/ml) of the extract was added. The solution was incubated in the dark at 25 °C for 30 minutes. The absorbance was obtained using a UV-VIS spectrophotometer (Shimatzu/JAP), at 517 nm. The percentage of DPPH scavenging activity was calculated using the following formula:

$$\% \, Antioxidant = \left[\frac{ABScontrol - ABS\ sample}{ABScontrol}\right] \times 100$$

Allelopathy

A total of 10 seeds of barley or mungbean were cultured in a Petri dish with 3 ml extract (0-0.1 mg/ml) and incubated at 25 °C, for 5 days in dark. Seed germination was determined as followed: the germinated seeds/ the total seeds. For seedlings growth, radicle and shoot length were measured.

Meiosis

Young flower buds (3- 4 mm long) were used for meiosis study of pollen mother cells. Pollen mother cells of the young anthers of the stamens were stained with 2% aceto carmine (Sharma *et al*., 1993).

Statically analysis

Analysis of variance and comparison of means were done using Tukey test and Minitab software (version 15), respectively.

Results

Total phenol

Total phenol of the organs of *L. spectabilis* and *A. cordifolia* ranged from 13.44 to 43.12 mg/g dry weight (DW) (Table 1). The maximum total phenol was present in the petal extract of *A. cordifolia*. Total phenol of petals and stamens of both plants were higher than in their leaves, stems and ovaries. The total phenol difference was significant among all organs of *L. spectabilis* and *A. cordifolia* but no significant difference was observed between the two plants except for petals (Table 1).

Antioxidant

The alcohol extracts of all organs of *L. spectabilis* and *A. cordifolia* had a significant antioxidant activity in both plants (Table 2). The stamens in both plants showed a higher antioxidant activity relative to their leaves, stems, ovaries and petals. The petals revealed a greater antioxidant activity than other organs did, but it was less than that of the stamens.

Allelopathy

Seed germination of mungbean did not decline with any of the organ extracts of *A. cordifolia* and *L. spectabilis* except for *A. cordifolia* ovary extract (Table 3). Growth of seedlings decreased in seeds in contact with alcohol extract. In the seedlings in contact with all extracts of *L. spectabilis*, the hypocotyl growth increased within 0.14- 1.1 cm, except for the stem extract; the hypocotyl growth of mungbean seedlings in contact with leave and stem extracts of *A. cordifolia* significantly declined. The radicles' length decreased for all extracts, except for stamen extracts of both plants (Table 3). Germination of barely seeds significantly diminished with all the extracts of *A. cordifolia* and *L. spectabilis* except for leaf extract of *L. spectabilis* (Table 4). The germination of the seeds in contact with the stem and petal extracts of both plants dropped by 35-50%. The radicles' length and youngling's length in each extract decreased by 1.3-3 cm and 2.01-3.05 cm, respectively (Table 4). Seedlings' growth for seeds in contact with alcohol extracts also declined.

Meiosis

Stage I (Meiosis I): In both plants, in prophase I, coupling of homologous chromosomes and synapse between them was observed. At metaphase I, bivalent chromosomes settled on the cell plate. In anaphase I, homologous chromosomes were separated and shifted towards opposite poles by the spindles. Two groups of chromosomes, each with 9 and 6 dyad chromosomes of *L. spectabilis* and *A. cordalis* respectively, were separated from each other. Two groups of chromosomes were obverted in cell at telophase I.

Stage II (Meiosis II): After telophase I or during prophase II, chromosomes were observed as partially separated due to compaction.

In metaphase II, the chromosomes compaction continues to reach their most degree of contraction. The single chromosome settled on the cell plate, the two chromatids were adhered together at centromere. At anaphase II, sister chromatids were dissociated and moved by the spindles. As a result, telophase II cells contained four groups of 9 (*L. spectabilis*) and 6 (for *A. cordifolia*) newly formed chromosomes. The four groups of chromosomes were expanded and enveloped to form of the haploid nuclei. The cytoplasm was partitioned by fragmoplast formation in cell plate during cytokinesis, resulting in the formation of tetrad (Fig. 2). Subsequently, the four haploid microspores were separated from each other and continued their development in to mature pollen grains (Fig. 3).

Chromosome number: The number of chromosomes in *L. spectabilis* and *A. cordifolia* was $n = 9$ ($2n = 18$) and $n = 6$ ($2n = 12$). The chromosomes were small in both plants (Figs. 1, 3).

Table 1. Total phenol of the organs of *L. spectabilis* and *A. cordifolia*

Plant	Leave	Stem	Petal	Ovary	Stamen
L. spectabilis	17.32d	23.33d	36.64b	13.44e	35.32c
A. cordifolia	16.87d	21.15d	43.12a	14.00e	32.70c

Note: different letters denote significant differences (Turkey test, P<0.05)

Table 2. Antioxidant effects of the organs of *L. spectabilis* and *A. cordifolia*

Plant	Extract mg/ml	Leave	Stem	Petal	Ovary	Stamen
L. pectabilis	0.05	28. 5k	51i	360f	36j	1386b
	0.1	32j	86h	396f	52i	1469a
A. ordifolia	0.05	22.5l	31j	654e	78h	1129c
	0.1	33.5j	44.5ij	1197d	192g	1345b

Note: different letters denote significant differences (Turkey test, P<0.05)

Table 3. Allelopathy effect of 0.1 mg/ml dry alcohol extract of *L. spectabilis* and *A. cordifolia* on germination of mungbean seeds and seedling growth, after 5 days

Plant	Mungbean	Control	Leave	Stem	Petal	Ovary	Stamen
L. spectabilis	Germination%	100a	95ab	95ab	100a	92ba	100a
	Shoot (cm)	0b	0b	0b	0b	0b	0.04a
	Hypocotyle(cm)	0.75e	1.85a	0.5f	1.25c	0.89d	1.4b
	Radicle(cm)	2.35a	0.65c	1.75b	1.85b	1.83b	2.30a
A. cordifolia	Germination%	100	97a	94ab	100a	90b	100a
	Shoot (cm)	0	0b	0b	0b	0b	0.1a
	Hypocotyle(cm)	0.75a	0.1d	0.15d	1.35b	0.92c	1.54b
	Radicle(cm)	2.35a	0.25d	0.27d	1.74c	1.83b	2.27a

Note: different letters denote significant differences (Turkey test, P<0.05)

Table 4. Allelopathy effect of 0.1 mg/ml dry alcohol extract of *L. spectabilis* and *A. cordifolia* on germination of barely grain and seedling growth

Plant	Barely	Control	Leave	Stem	Petal	Ovary	Stamen
L. spectabilis	Germination%	90a	95a	55c	45d	83b	80b
	Shoot (cm)	3a	0.28	1.7b	0d	0.25c	0.3c
	Radicle(cm)	3.15a	2b	2b	0.3d	1.5c	1.1c
A. cordifolia	Germination%	90a	55c	53c	39c	79b	83b
	Shoot (cm)	3a	0.1de	0.15d	0e	0.96b	0.28c
	Radicle(cm)	3.15a	0.15d	0.14d	0.1d	0.87c	1.14b

Note: different letters denote significant differences (Turkey test, P<0.05)

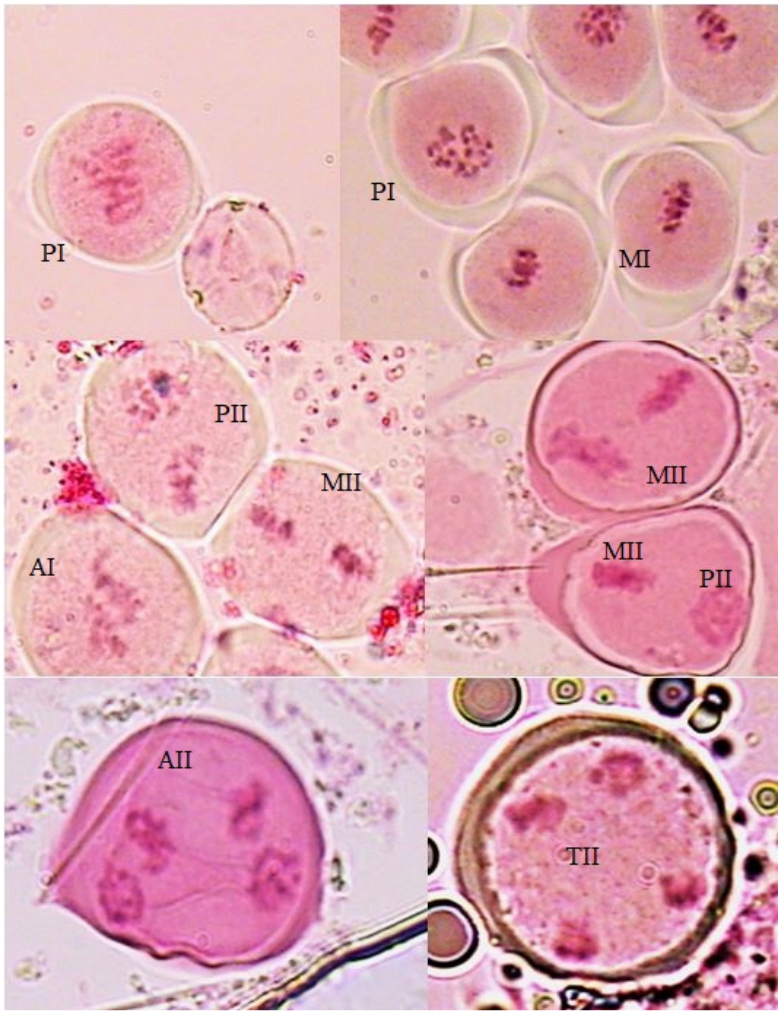

Fig 1. Meiotic stages of *L. spectabilis*: (PI) Prophase I: 9 compaction bivalent chromosomes. (MI) Metaphase I: 9 compaction bivalent chromosomes, stood at spindle equator. (AI) Anaphase I: dissociation of the 9 chromosomes shifting towards each spindle pole and Telophase I with two polar groups of chromosomes. (PII) prophase II: two groups of chromosomes. (MII) Metaphase II: two groups of compaction chromosomes at the spindle equators. (AII) Anaphase II: dissociation of chromatids towards each spindle pole. (TII) Telophase II: four groups of sister chromatids.

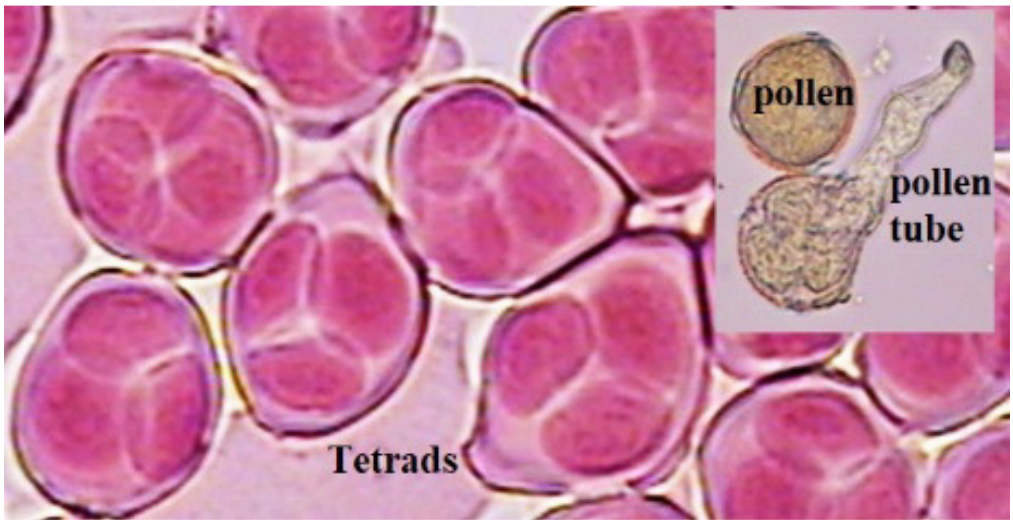

Fig. 2. Late telophase II of *L. spectabilis*: tetrad of four haploid nuclei ad adult pollen grain

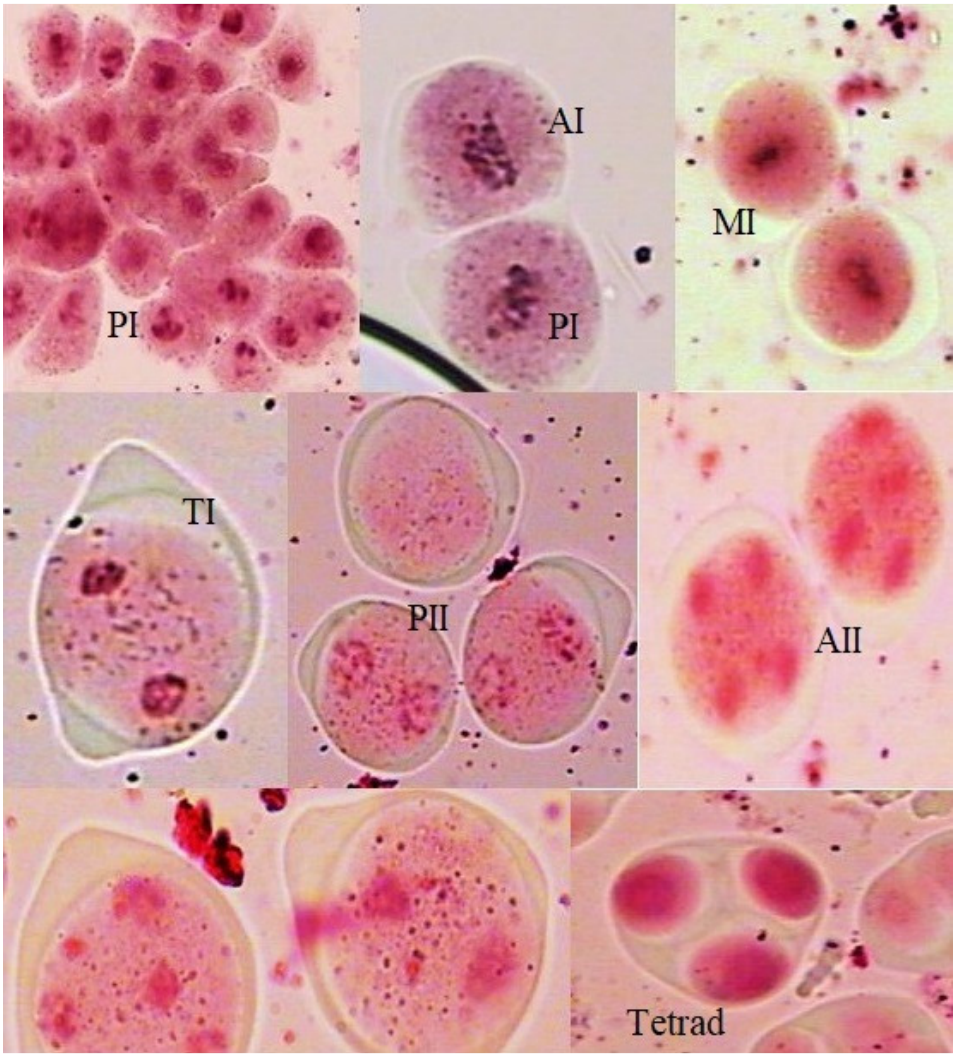

Fig 3. Meiotic stages of *A. cordifolia*: (PI) Prophase I, (MI) Metaphase I, (AI) Anaphase I, (TI) Telophase I, (PII) Prophase II, (MII) Metaphase II, (AII) Anaphase II and Tetrad

Discussion

Total phenols of the organs of *A. cordifolia* and *L. spectabilis* (*syn*: *Mesembryanthemum spectabila*) were 13.44 and 43.12 mg/g, respectively. The leaf of *Sesvium portulacastrum* contained phenol 8.9 mg/g and a high antioxidant activity (Mohan, 2013) but it was lower than our results (leaf phenol=17.32 mg/g). All organs of *M. edule* showed a high antioxidant activity, with a considerable efficiency for stems followed by leaves and roots. The stems with 86.5 mg/g phenol and leaves with 68.7 mg/g phenol had the highest phenol content (Falleh *et al*., 2011). Nevertheless, in this study, phenol content of the organs of *L. spectabilis* and *A. cordifolia* were very lower than that of organs of *M. edule*.

In particular, the antioxidant activity was higher in the stamens than in the other organs, and this difference seems to be due to the presence of betacyanins in organs of plants species of Aiozaceae family as shown by Piatteli and Impellizzeri (1969). Nevertheless, some of them did not show any significant antioxidant and toxicity activities (Samy *et al*., 2018). The greatest antioxidant activity was found in organic solution extracts of *M. crystallinum* and *Carpobrotus edulis* (Bouftira *et al*., 2009). In this study, the alcoholic extract of the stamens had the highest antioxidant activity. Allelopathy effects of *L. spectabilis* and *A. cordifolia* were associated with small inhibitory effects on seed germination and seedlings growth of mungbaen. Allelopathic effects of both plant extracts, in particular *A. cordifolia*, were significant on barley seed germination and growth of seedlings.The extracts of *Triantheum portulacastrum* (Aizoaceae) reduced seed germination and seedling growth of *Sesamum indicum* (Huang *et al*., 2017). Different extracts of *Cassia tora* organs inhibited seed germination and seedling growth of mustard (Sarkar *et al*.,) of mustard (Sarkar *et al*., 2012). The strength of the allelopathic effects of roots', leaves' and stems' extracts of *A. philoxeroides* on *Z. matrella* diminished subsequently (Huang *et al*., 2017) but no information was found about the allelopathic effects of *L. spectabilis* and *A. cordifolia*.

The number of chromosomes in meiosis revealed that the number of chromosomes of *A. cordifolia* is n=6 (2n=12) and n=9 (2n=18) for *L. spectabilis* (*syn*: *mesembryanthemum spetabila*). The meiosis number of chromosomes in 12 genera of *Mesembryanthemum* was found to n=9 (Sugiura, 1940). *M. crystallinum* and *M. froskaoli* had 2n= 18 in mitosis division (Soliman *et al*., 2017). The meiosis of *A. cordifolia* and *L. spectabilis* was normal and the chromosomes were very small. The results of Pagliarini (1990) indicated that *A. cordifolia* was abnormal in terms of the meiosis's process of pollen mother cells. On the other hand, our results suggested that meiosis stages are normal for both plants. In pollen mother cells division of *L. spectabilis* and *A. cordalis*, all stages of meiosis I and II were observed (Dawe, 1998).

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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