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CYTOTOXIC EFFECTS OF *PORTULACA OLERACEA* AND *CICHORIUM INTYBUS* SEEDS ON LAND SNAIL *MONACHA CARTUSIANA*

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ABSTRACT: The present study was designed to investigate the chemical composition, determination the phenolic contents in plant extracts and studying the cytotoxic effects of plant seeds against land snail. The plants under study were *Portulaca oleracea* and *Cichorium* intybus. Our results could be summarized that Portulaca oleracea (purslane) contain ash, fiber, crude lipids, protein and carbohydrate 2.15, 33, 26, 11.6 and 60.25 % and Cichorium intybus (chicory) contain 1.65, 35, 19, 10 and 69.35 % respectively. Purslane contain 13 phenolic compounds, meanwhile chicory contain 16 phenolic compounds, the main compounds in purslane seed extract were coumaric acid (129.62 µg/ml) and coffeic acid (108.98 µg/ml) and main compounds in chicory were naringenin (3631.04 µg/ml) and chlorogenic acid (2395.18 µg/ml). Results showed cytotoxic effects of extracts of purslane and chicory seeds on the land snails iv stage, whereas increases the extract concentration from (25 to 100%) led to decrease the egg ms/snail, egg no./ms, egg no. / snail, Inc. period (day) and hatch rate.

Key words: Purslane, Chicory, Land snails, Cytotoxic, Phenolic.

INTRODUCTION

Purslane (Portulaca oleracea L.), belonging to the *Portulacacea* family. Seven subspecies belong to this species, but the subspecies *sativa* is the most common. Tocher, (2019). Phytochemical screening of *Purtulaca oleracae* contain alkaloids, tannins, glycosides carbohydrates, flavonoids, terpenes, phenolics, saponins, proteins and steroids. Sabeeha and Nahida (2013). Methanolic extracts of leaves & seeds of *Portulaca oleracea* were assayed for insecticidal and wormicidal activities in different concentrations (1mg to 100 mg/mL) on *Tribolium castaneum*, *Sitophilus oryzae* and *Lumbricus terrestris* along with positive, negative control and standard drugs. The crude extract showed no mortality in all doses in insecticidal activity but significant dose dependent mortality was observed in wormicidal activity test. Shazia *et al.*, (2010).

Chicory (Cichorium intybus L.) belongs to the family Asteraceae and it is a small aromatic biennial or perennial herb. The whole plant contains a number of medicinally important compounds such as inulin, esculin, volatile compounds (monoterpenes and sesquiterpenes), coumarins, flavonoids and vitamins. It has also an industrial importance for the production of various food preparations like pure instant chicory powder, chicory powder, agglomerated chicory powder, chicory flour, roasted chicory cubes, chocolate flavor and liquid chicory extract. Subhash *et al.*, (2016). The fresh chicory typically contains 68% inulin, 14% sucrose, 5% cellulose, 6% protein, 4% ash, and 3% other compounds, while dried chicory contains approximately 98% inulin and 2% other compounds. Meehye and Shin, (1996). The main groups of compounds of the chicory root are carbohydrates, including saccharose, glucose and fructose, fructooligosaccharides and inulin, whose contents reach 21% on average. Inulin is a soluble polyfructan and belongs to a group of dietary fibre. Inulin chains consist of up to 100 D-fructofuranose units linked *via* β-(2→1) glycosidic bonds Monde *et al.*, (1990). Chicory contains some phytochemicals such as inulin (starch-like polysaccharide), coumarins, flavonoids, sesquiterpene lactones (lactucin and

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lactucopicrin), tannins, alkaloids, vitamins, minerals, and volatile oils. The secondary metabolites (flavonoids, tannins, and coumarins) found in chicory have been reported to demonstrate some biological activities such as antioxidant, anticancer, anti-inflammatory, antiparasitic, antihepatotoxic,. Hoste *et al.*, (2006).

MATERIALS AND METHODS

Materials

Seeds of *Portulaca oleracea* and *Cichorium intybus*. were obtained from research center department of medical and aromatic plants Giza, Egypt. The seeds dried at 50 ºC and grounded into a powder state using commercial blender and finally used for analysis.

Methods

Chemical composition

Determination of crude protein

Total nitrogen was determined (dry basis) according to the modified Kjeldahl pirjo, and pekka, (1996), the crude protein contents were calculated using the conversion factor 6.25. Protein % = TN (Total Nitrogen) x 6.25.

Determination of crude lipid.

A known weight of the samples (10 g) was extracted with n- Hexane for 6 hours in soxhlet apparatus. The solvent was evaporated and the residue was dried to constant weight at 95 ºC according to A.O.A.C., (2000).

Determination of total carbohydrate

Total carbohydrate or non-nitrogen extract determined by.

Difference = 100 – (% ash + % Protein + % Fat)

Determination of ash

Ash content determined by ignition of dried sample at 550 ºC until a constant weight according to A.O.A.C., (2000).

Determination of crud fiber

Crude fibre of the sample was estimated by using moisture and fat free samples.Fibre was estimated by boiling in acid (sulphuric acid 0.255 N) and subsequent alkali (0.31 N NaOH) using Gerhard fibre bag system. Then it was filtered and washed with distilled water and dried at 80 ^{o} C- 100 ^{o} C. Sample in crucible with residue (We). Ashing was done in muffle furnace. The ash content was cooled in desiccators and weighed (Wa). The difference represents the crude fibre content of the sample and was expressed as gram per 100g or per cent (ANNEXURE-II).

$$\text{Crude fibre (g/100g sample)} = \frac{100 - (\text{Moisture} + \text{fat})] \text{ X } (\text{We} - \text{Wa})}{\text{Weight of sample taken (moisture and fat free)}}$$

Preparation of extracts

Methanolic extract

500 grams of plant sample powders were steeped in 5000 ml of methanol 80% and the mixture was then kept in shaker incubator for 24 hours at room temperature. Then filtered through filter paper and centrifuged at 3000 rpm. the filtrate was placed in Rotary Vacum Evaporator to evaporate alcohol from it . We used to obtain a dried powder as by Mukhtar and Ghori, (2012).

Quantitative determination of phenolic compounds by HPLC

A modified method of (Zuo *et al.*, 2002) was used. A Shimadzu LC 20 AT HPLC filtered with a SIL 20 A auto sampler and a SPD-20 UV visible detector with a class LC 10 chromatography workstation was used for the analysis of the prepared sample. A luna TM 5 µM C18, 25cm ×4.6 mm i.d (phenomenex, Torrance, CA, USA) column with a Ryeodyne precolumn filter 7335 model was used .All solvents were filtered through a 0.45 µM Millipore membrane filter disk and degassed before injection into a HPLC system. Agradient elution was carried out using the following Solvent systems: Mobile phase A (acetonitrile / acetic acid /double distilled water -9/2/89 v/v/v). Mobile phase B (acetonitrile /acetic acid /double

distilled water -80/2/18 v/v/v). the mobile phase composition for a binary gradient to 60% mobile phase A, 32% mobile phase B and held at this composition for 10 min. The min before the next injection. the flow rate of mobile phase was 1mL / min and the temperature at the column was performed at 35 ±0.5^{o} C. the quantification of catechins was performed at 278 nm and was achieved using a caffeine external standard with a calibration curve $R_{2=}$ 0.9984 in conjunction with the consensus individual catechin reponce factor (RRF) values with respect to caffeine calculated on dry matter basis. Total catechins as percentage by mass a sample dry matter basis was given on the summition of individual catechins.

Cytotoxic effects of purslane and chicory seeds extracts

Collection and acclimatization of snails

Adults of land snail monacha cartusiana are collected from agricultural research national institute of Zagazig and the collected snails were transferred to laboratory in muslin bags and put in glass cages (40, 30, 30 cm) with moist soil about 10cm height, covered with muslin cloth and tied with rapper band to prevent escaping of them. They fed on fresh cappage leaves for two weeks. These snails were kept at 20°c ±2°c and relative humidity 80-90%.

Poisonous bait formulation

Poisonous baits were prepared by adding (chicory, and purslane extract g) with wheat bran to 5g of sugarcane syrup in order to obtain concentrations of (chicory and purslane %) while control group was obtained by adding 95g wheat bran to 5g of sugarcane syrup only without treating.

Experimental design

Three groups of *m. cartusiana* snails were prepared where the first group treated with(chicory) , the second one treated by (purslane) while the third one was not treated left as control group. Each concentration of the two groups in addition to the control one contained 5 replicates; each replicate contains 500g of moist soil, 4sails added to each replicate and left to feed on 10g of bait for 8weeks. Egg masses were removed periodically from soil and put in another dish and counted then left to be hatched then juveniles counted and incubation period and hatching rate are demonstrated.

RESULTS AND DISCUSSION

Proximate Analysis of *Portulaca oleracea* and *Cichorium intybus* seeds

Data given in Table (1) show the chemical composition of *Portulaca oleracea* and *Cichorium intybus* seeds. The obtained results indicated that: purslane seeds contain crude ash (2.15 %), crude protein (11.6 %), crude lipid (26 %) and total carbohydrate (60.25%). Chicory seeds consisted of crude ash (1.65 %), crude protein (10 %), crude lipid (19 %) and total carbohydrate (69.35%). Theses result were in accordance with Abd El-Latif, (2008). who reported that, Purslane seeds contain moisture 7.6%, crude protein 10.37%, crude fat 25.40%, total ash 9.66% and total carbohydrates 46.97%.

Table (1) Proximate analysis of purslane and chicory seeds:

Components	Purslane	Chicory
Ash	2.15	1.65
Crude lipid	26	19
Crude protein	11.6	10
Total carbohydrate	60.25	69.35

Identification of phenolic compounds of purslane and chicory seeds extracts

Phenolic compounds in methanol extracts of seeds for purslane and chicory were analyzed by High Performance Liquid Chromatography (HPLC) , and concentration of all tested phenolic compounds are given in Table (2).

From Table (2) it was found that purslane seeds extract contains 13 phenolic compounds , analysis of purslane seeds extract showed that the major compounds found in the extract, were coumaric acid (129.62 µg/ml), coffeic acid (108.98 µg/ml), vanillin (106.112 µg/ml), naringenin (84.08 µg/ml), rutin (72.20 µg/ml), ellagic acid (36.74 µg/ml), gallic acid (36.72 µg/ml), cinnamic acid (28.68 µg/ml), hesperetin)25.40 µg/ml), methyl gallate (21.60 µg/ml), while compounds presented in amounts less than (10 µg/ml), occurred as trace materials such as, ferulic acid (8.60 µg/ml), apigenin (3.38 µg/ml), and daidzein (0.80 µg/ml). These results agree with Hanan *et al.,* (2014). In which they mentioned that phenolic compound of purshane seeds fractionated to catechein, chlorogenic, salcylic and pyrogalol while flavonoids were fractionated to rosmarinic, rutin, quercitrin.

Table (2) showed that, chicory seeds extract contains 16 phenolic compounds, the major compounds were: naringenin (3631.04 µg/ml), chlorogenic acid (2395.18 µg/ml), ferulic acid (125.08 µg/ml), in themean while gallic acid (106.90 µg/ml), coffeic acid (75.20 µg/ml) , daidzein(30.06 µg/ml), and vanillin (27.92 µg/ml), while compounds were presented in amounts less than (10 µg/ml), occurred as trace materials such as and ellagic acid esperetin (6.74 µg/ml), methyl gallate (6.72 µg/ml), querectin (6.14 µg/ml), cinnamic acid (3.98 µg/500 µg), and coumaric acid (1.90 µg/ml. The results of the chemical composition of chicory seeds extract were in accordance with those reported by Zhang, *et al.*, (2014). In which they mentioned that the composition of bitter chicory seeds was total phenolic content (higher in seeds) was gallic acid, catechein, catechol, epicatachin and coumarin; flavonoid content was quercetin.

Table (2): Phenolic compounds of *Portulaca oleracea* and *Cichorium intybus* seeds.

Phenolic compounds	*Portulaca oleracea* (µg/ml)	*Cichorium intybus* (µg/ml)
Gallic acid	36.72	106.90
Catechin	ND	ND
Syringic acid	ND	18.56
Coumaric acid	129.62	1.90
Naringenin	84.08	3631.04
Vanillin	106.112	27.92
Methyl gallate	21.60	6.72
Ferulic acid	8.60	125.08
Querectin	ND	6.14
Apigenin	3.38	ND
Cinnamic acid	28.68	3.98
Coffeic acid	108.98	75.20
Rutin	72.20	12.32
Ellagic acid	36.74	24.66
Hesperetin	25.40	6.74
Daidzein	0.80	30.06
Kaempferol	ND	7.10
Chlorogenic acid	ND	2395.18

Cytotoxic effects of purslane and chicory seeds extracts

Results tabulated in Table (3) showed the effect of the extract of purslane seeds on the land snails iv stage. Increases the extract concentration from (25 to 100%) led to decrease the egg ms/snail, egg no./ms, egg no. / snail, Inc. period (day) and hatch rate. From Table (3), the egg ms/snail for control group recorded 1.5 and groups treated with purslane seeds extract at concentrations 25 ,50 and 100% recorded 0.4 , 0.35 and 0.35 respectively , also egg number/ms for control group was 49.48 and groups treated with purslane seeds extract at concentrations 25 ,50 and 100% recorded 48.2 , 41.7 and 30.4 respectively, and egg number / snail for control group was 68.35 and groups treated with purslane seeds extract at concentrations 25 ,50 and 100% recorded 20.35 , 18.25 and 14.45 respectively, also data showed decrease in incubation period from 12.32 day for control group to 11.2 , 10.5 and 9.3 day for groups treated with purslane seeds extract at concentrations 25 ,50 and 100% respectively, also hatch rate decrease from 96.24% for control group to 65.6 , 56.21 and 55.26 % for groups treated with purslane seeds extract at concentrations 25 ,50 and 100% respectively.

Results tabulated in Table (4) showed the effect of the extract of chicory seeds on the land snails iv stage. Increases the extract concentration from (25 to 100%) led to decrease the egg ms/snail, egg no./ms, egg no. / snail, Inc. period (day) and hatch rate. From Table (4), the egg ms/snail for control group recorded 1.5 and groups treated with chicory seeds extract at concentrations 25 ,50 and 100% recorded 1.25, 1.1 and 1 respectively, also egg number/ms for control group was 49.48 and groups treated with chicory seeds extract at concentrations 25, 50 and 100% recorded 48.7, 45.6 and 45.4 respectively, and egg number / snail for control group was 68.35 and groups treated with chicory seeds extract at concentrations 25, 50 and 100% recorded 61.57, 60.4 and 59.45 respectively, also data showed decrease in incubation period from 12.32 day for control group to 11.3, 11.2 and 10.9 day for groups treated with chicory seeds extract at concentrations 25 ,50 and 100% respectively, also hatch rate decrease from 96.24% for control group to 90.1, 89.9 and 86.23 % for groups treated with chicory seeds extract at concentrations 25, 50 and 100% respectively.

Table (3): Effect of purslane seeds extract on (Egg Ms/Snail), (Egg No./Ms) (Egg No./Snail), (Inc. Period (day), and (Hatch. Rate%).

Treat	Egg Ms/Snail	Egg No./Ms	Egg No./Snail	Inc. Period (day)	Hatch. Rate%
Control	1.5±0.05	49.48±1.47	68.35±3.65	12.32±0.05	96.24±0.34
25%	0.4±0.45	48.2±2.89	20.35±2.56	11.2±0.11	65.6±1.44
50%	0.35±0.23	41.7±3.56	18.25±2.11	10.5±0.13	56.21±1.56
100%	0.35±0.13	30.4±2.34	14.45±2.45	9.3±0.16	55.26±1.31

Table (4): Effect of chicory seeds extract on (Egg Ms/Snail), (Egg No./Ms) (Egg No./Snail), (Inc. Period (day), and (Hatch. Rate%):

Treat	Egg Ms/Snail	Egg No./Ms	Egg No./Snail No./Snail	Inc. Period (day)	Hatch. Rate%
Control	1.5±0.05	49.48±1.47	68.35±3.65	12.32±0.05	96.24±0.34
25%	1.25±0.01	48.7±4.67	61.57±0.9	11.3±0.29	90.1±2.45
50%	1.1±0.22	45.6±2.25	60.4±1.34	11.2±0.56	89.9±2.21
100%	1±0.02	45.4±4.78	59.45±1.23	10.9±0.35	86.23±3.65

Insecticidal activity of extracts, even from the same source, can be inherently variable for many reasons. The chemical composition and broad spectrum of biological activity for extracts can vary with plant age, the plant tissues or organs used in the distillation process, the type of distillation and the species and age of a targeted pest organism. Ahmed *et al.,* (2008).

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التأثير السام لبذور الرجلة والشيكوريا على الحلزون الارضى

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الملخص العربى

تهدف هذة الدراسة إلى معرفة التركيب الكيميائى وتقدير المركبات الفينولية وكذلك التأثير السام لمستخلصات بذور كلا من الرجلة والشيكوريا على الحلزون الارضى. ويمكن تلخيص النتائج المتحصل عليها فى الاتى:

تحتوى بذور الرجلة على رماد , الياف خام , ليبيدات خام , بروتين خام , كربوهيدرات بنسب ٢,١٥ - ٣٣ - ٢٦ - ١١,٦ و ٦٠,٢٥ % على التوالى وأحتوت بذور الشيكوريا على ١,٦٥ - ٣٥ - ١٩ - ١٠ و ٦٩,٣٥ % على التوالى ، وبتقدير المركبات الفينولية احتوى المستخلص الميثانولى لبذور الرجلة على ١٣ مركب فينولى فى حين احتوى المستخلص الميثانولى لبذور الشيكوريا على ١٦ مركب فينولى وكان المركب الرئيسى فى بذور الرجلة هو حمض الكوماريك وحمض الكافيك بنسب (١٢٩,٦٢ و ١٠٨,٩٨ ميكروجرام/مل) واحتوى مستخلص الشيكوريا على مركب النارنجينين وحمض الكلوروجينك بنسب (٣٦٣١,٠٤ و ٢٣٩٥,١٨ ميكروجرام/مل) ، وأظهرت النتائج التأثير السام للمستخلصات ضد الحلزون الارضى حيث زيادة التركيز المستخدم من ٢٥ % إلى ١٠٠ % من المستخلص أدى إلى إنخفاض فى كتلة البيض وعدد البيض وكذلك فترة الحضانة ومعدل الفقس.