In-vitro adventitious root production of *Cichorium endivia* L. and antioxidants, total phenolic, and total flavonoids assessments

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Received 19 February 2019
Accepted 23 March 2019

Egyptian Pharmaceutical Journal 2019, 18:216–227

Background and objectives
Chicory plant serves as a vegetable with higher nutritional value, having major antioxidant properties. The aim of this work was *in-vitro* production of adventitious roots from *Cichorium endivia* subsp., *pumelum* L. and exploring the capacity of adventitious roots for antioxidant activities as well as determine their contents of total phenolic and flavonoids compounds compared with different parts of *C. endivia*.
Materials and methods
For *in-vitro* adventitious root induction, root segments were cultured on half-strength Murashige and Skoog solid medium supplemented with different concentrations of indole-3-butyric acid and 0.1 mg/l α-Naphthalene acetic acid. The cultures were incubated under total darkness and or 16/8 h (light/dark) photoperiod. Murashige and Skoog liquid medium with different carbon sources was used for adventitious root production. Two different solvents (aqueous ethanol and chloroform) were used for bioactive components extraction process. 2, 2′-diphenyl 1-Picryl-hydrazyl radical scavenging capacity (RSC) as well as total phenolic and flavonoides contents were estimated in produced adventitious roots compared with different plant parts (seeds, leaves, and roots).
Results and conclusion
Medium supplemented with 0.1 mg/l α-Naphthalene acetic acid and 1.0 mg/l indole-3-butyric acid recorded maximum adventitious root induction percentage (100%) in the dark condition. High-yield production of adventitious roots (6.60±0.5 g) was found in the liquid medium that contains sucrose as the carbon source. The aqueous ethanol extracts recorded higher RSC% values than chloroform extracts in all plant parts. Aqueous ethanol extract of seeds recorded maximum RSC% (92.8%) at 2.5 mg/ml of extract. Total phenolic contents showed maximum value with aqueous ethanol extract of seeds (18.17±0.40 mg/g of extract), whereas minimum value recorded with chloroform extract of seeds (0.28±0.05 mg/g of extract). The flavonoids contents showed maximum value also with aqueous ethanol extract of seeds (94.43±1.00 mg/g of extract), followed by aqueous ethanol extract of leaves (93.68±0.1 mg/g of extract), and minimum value with chloroform extract of leaves (2.60±0.18 mg/g of extract).

Keywords:
Adventitious roots, Antioxidant activity, *Cichorium endivia*, Phenolic and flavonoids

Egypt Pharmaceut J 18:216–227

1687-4315

Introduction

Chicory (*Cichorium endivia* subsp., *pumelum* L.) is a vegetable plant that belongs to the family *Asteraceae* and characterized by its widespread presence in the west and south of Europe. It has achieved a communal food status owing to its nutritional value and bitter taste, eaten cooked or raw in salads [1]. *Cichorium* plant has medicinal importance owing to having a number of active compounds including alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins, and tannins [2–5]. Moreover, it has been used for treatment of fever, diarrhea, jaundice, and gallstones [6,7]. Some studies on rats showed that *Cichorium intybus* has antihepatotoxic and anti-diabetic activities [8,9]. Moreover, others have showed its own antibacterial properties [3,10], anti-inflammatory [11,12], hyperglycemic [13], and anti-ulcerogenic activities [14].

Polyphenols of chicory plant including flavonoids act as an effective agent to promote public health, because it possesses many important influences such as antiviral, anti-carcinogenic, antibacterial, anti-inflammatory, antifungal, antimutagenic, immunostimulating, and antioxidant effects; moreover, it can conserve the

alimentary tract and reduce cholesterol level in the blood [15–17].

Antioxidants are essential to protect biological systems from harmful free radicals. The human organs are qualified to deal with peroxidative activities of Reactive oxygen species (ROS) owing to the existence of endogenous enzymes, including superoxide dismutase, catalase, and glutathione peroxidase [18]. However, when the ROS level increases, the cells need external supply of antioxidant molecules, which can be obtained from plants, including phenols, flavonoids, carotenoids, and vitamins (C and E). Therefore, favorable nutrition is an important factor needed to provide effective antioxidant compounds [19,20].

In this respect, induction of adventitious roots by *In vitro* methods showed high rates of production of active secondary metabolites, and therefore cultivation of adventitious roots has been proposed as a substitution for natural compound production [21].

The current study aimed to improve the *In vitro* production of adventitious roots from *C. endivia* subsp., *pumelum* L. and exploring their capacity for antioxidant activity compared with their different parts of *C. endivia* as well as to determine their contents of total phenolic and flavonoid compounds.

Materials and methods

Plant material and explants preparation

Seeds of *C. endivia* subsp., *pumelum* L. were obtained from Agricultural Research Center, Ministry of Agriculture, Egypt. Seeds were immersed in 70% ethanol for 2–3 min and then rinsed three times in sterile distilled water. The seeds were then sterilized for 30 min in 20% commercial Clorox (5% NaOCl) containing 0.5% Tween 20. After rinsing three times with sterile distilled water, seeds were cultured on Murashige and Skoog (MS) medium [22] containing 3% (w/v) sucrose and solidified with 0.7% (w/v) agar. Culture medium was adjusted to pH 5.8. The seeds were incubated in a culture room at 24±2°C and were kept under 16-h photoperiod of fluorescent 45-µmol cool white light tubes and 8 h dark. Root segments were excised (3 cm) from 3-week-old seedlings as explants for the present study.

In-vitro adventitious root induction

Root explants were cultured on half-strength MS solid medium supplemented with different concentrations of indole-3-butyric acid (IBA) (0.25, 0.5 and 1.0 mg/l) and 0.1 mg/l α-Naphthalene acetic acid (NAA). All culture media were adjusted to pH 5.8, 0.7% (w/v) agar and 3% (w/v) sucrose were added. The cultures were incubated at 24±2°C under 16/8 h (light/dark) photoperiod with white fluorescent lights or under total darkness for 4 weeks. The responded explants that succeeded to induce adventitious roots were scored as follows:.

$$\text{Adventitious roots induction}(\%) = \frac{\text{Total number of induced adventitious roots}}{\text{Total number of roots}} \times 100$$

Effect of different carbon sources on adventitious root production

This experiment aimed to evaluate the effect of different types of carbon sources in the culture media on adventitious root production. For this purpose, 1.0 g of adventitious roots was cultured on half-strength MS solid medium contained 0.1 mg/l NAA and 1.0 mg/l IBA and supplemented with 30 g of different carbon sources (glucose, fructose, sucrose, and maltose) and solidified with 0.7 g agar. Similar previous procedure and condition were done on liquid media without the gelling agent. The cultures were kept in total dark condition, and adventitious roots fresh weights (AFW) were recorded after 4 weeks of culturing.

Extraction method

Leaves and roots of one month seedling were separated from *C. endivia,* and adventitious roots were collected from the best culture medium. All samples were dried at 40°C. Moreover, seeds were taken as it is. All dried samples were grounded in the mortar. Grounded powder of the samples was weighed and macerated with 200 ml of 80% ethanol and chloroform separately and kept overnight in shaker at 110 rpm. The extracts were collected after filtration using Whatman No. 1 filter paper; this procedure was repeated once again. The extracts were collected and evaporated below 40°C. Each residue was dissolved in the same extract solvent and stored at 4°C until further use.

Percentage of the extract yield was calculated using the formula:.

$$\text{Extraction yield}(\%) = \frac{\text{Weight of the extract}}{\text{Weight of ground plant material}} \times 100$$

DPPH radical scavenging capacity

Radical scavenging activity of plant extracts against stable 2, 2′-diphenyl 1-Picryl-hydrazyl (DPPH) was determined by a slightly modified method [23]. Different concentrations of each extract (0.5, 1.0,

1.5, 2.0 and 2.5 mg/ml) were used to evaluate antioxidant capacity. Overall, 500 µl of each extract was added to 2.5 ml of methanolic solution of DPPH (0.3 mM). After 30 min at room temperature, the absorbance values were measured at 517 nm on the spectrophotometer. Radical scavenging activity (%) was calculated by the following formula:.

$$\mathrm{RSC}(\%) = \left[\frac{A_{\mathrm{DPPH}}A_s)}{A_{\mathrm{DPPH}}}\right] \times 100$$

where A_s is the absorbance of plant extract and A_{DPPH} is the absorbance of DPPH solution.

Total phenolic and total flavonoids contents

The concentration of total phenolic compounds was determined by spectrophotometric method using the Folin–Ciocalteu reagent [24]. A calibration curve of gallic acid (20, 40, 40, 60, 80, and 100 µg/ml) was prepared, and the absorbance for tests and standard solutions was determined against the reagent blank at 550 nm with an ultraviolet/visible spectrophotometer. Total phenolic content was expressed as milligram of gallic acid equivalent/g of dry weight (DW) plant material.

The total flavonoids content was measured using a modified colorimetric [25]. The standard curve was prepared using different concentration of quercetin. The flavonoids content was expressed as milligrams quercetin equivalents/g of plant material DW.

Statistical analysis

The experiments were carried out in triplicate, and the results were expressed as means±SE. Statistical analysis of variance was performed by analysis of variance, and least significant differences at P value less than or equal to 0.05 between the means and were determined by Duncan's Multiple Range Test.

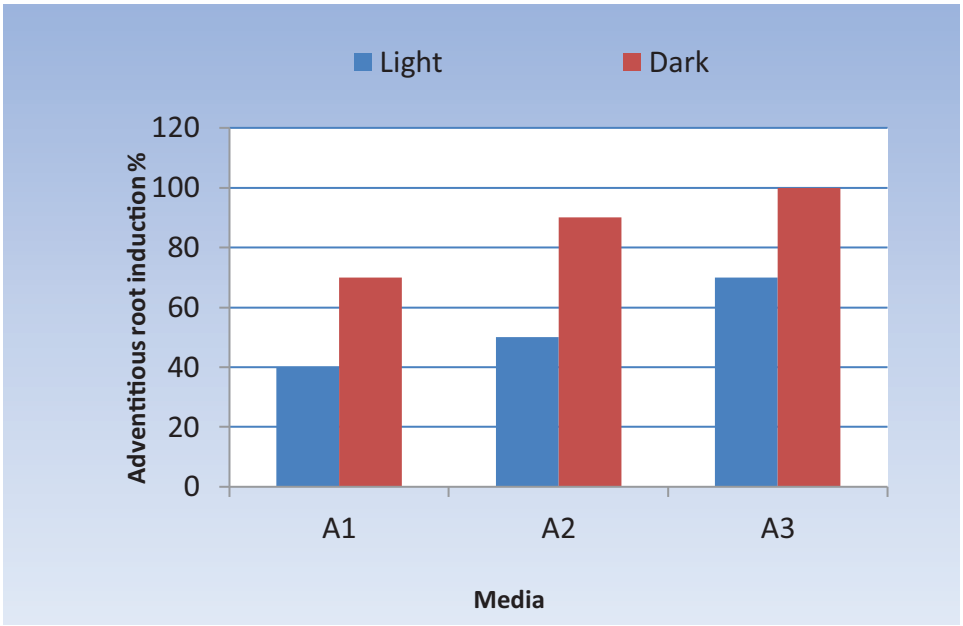
Results and discussion

In-vitro adventitious root induction

For adventitious root induction, root explants were cultured on MS medium supplemented with 0.1 mg/l NAA and different concentration of IBA (0.25, 0.5 and 1.0 mg/l) and incubated under light (16/8 h) photoperiod and total dark conditions. Percentages of adventitious roots were recorded after 4 weeks of cultivation (Fig. 1).

Two factors (light conditions and IBA concentrations) were taken into account to study their effect on adventitious root induction *in vitro*. Data presented in Fig. 1 reveal that, root segments succeed to induce adventitious roots in all different media under both dark and light (16/8 h photoperiod) conditions. The percentage of adventitious root induction was generally increased when root segments were cultivated in dark condition; they were 70, 90, and 100% on A1 (0.1 mg/l NAA +0.25 mg/l IBA), A2 (0.1 mg/l NAA+0.5 mg/l IBA), and A3 (0.1 mg/l NAA+1.0 mg/l IBA) media,

Figure 1



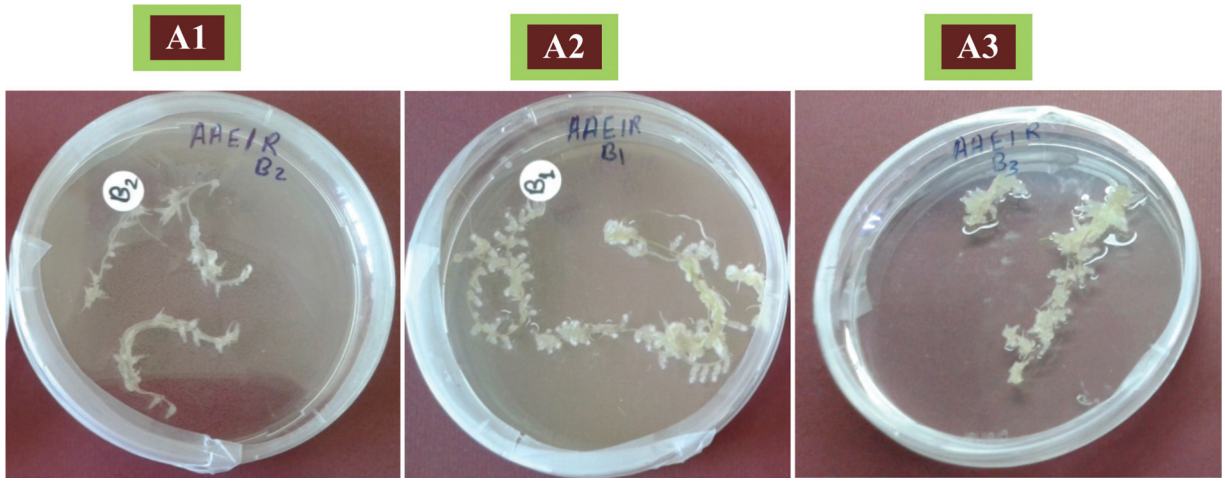
Percentage of adventitious root induction from root explant on A1 (0.1 mg/l NAA+0.25 mg/l IBA), A2 (0.1 mg/l NAA+0.5 mg/l IBA), and A3 (0.1 mg/l NAA+1.0 mg/l IBA) media cultivated under complete dark and light (16 h) conditions. IBA, indole-3-butyric acid; NAA, α-Naphthalene acetic acid.

respectively, versus 40, 50, and 70%, respectively, on the same three media at light condition (16/8 h photoperiod).

Concerning the effect of IBA concentration, it was noticeable that, adventitious root induction percentage increased with IBA concentration increasing, which reached the maximum value on A3 medium in both dark and light conditions, recorded at 100 and 70%, respectively. However, minimum adventitious root induction (40%) was recorded with A1 medium cultivated under light condition (16/8 h photoperiod). Therefore, it is noticeable that the two factors (light conditions and IBA concentration) have a clear effect on adventitious root induction, where it was found that, the maximum percentage of adventitious root induction was achieved when IBA was at the highest concentration (1.0 mg/l), moreover, it attained the maximum response in the dark compared with light at incubation period (16/8 h photoperiod). Such treatment (0.1 mg/l NAA +1.0 mg/l IBA) was subjected in the following experiment for adventitious root production. Induced adventitious roots on dark and light conditions with different media supplementation are displayed in Figs 2 and 3.

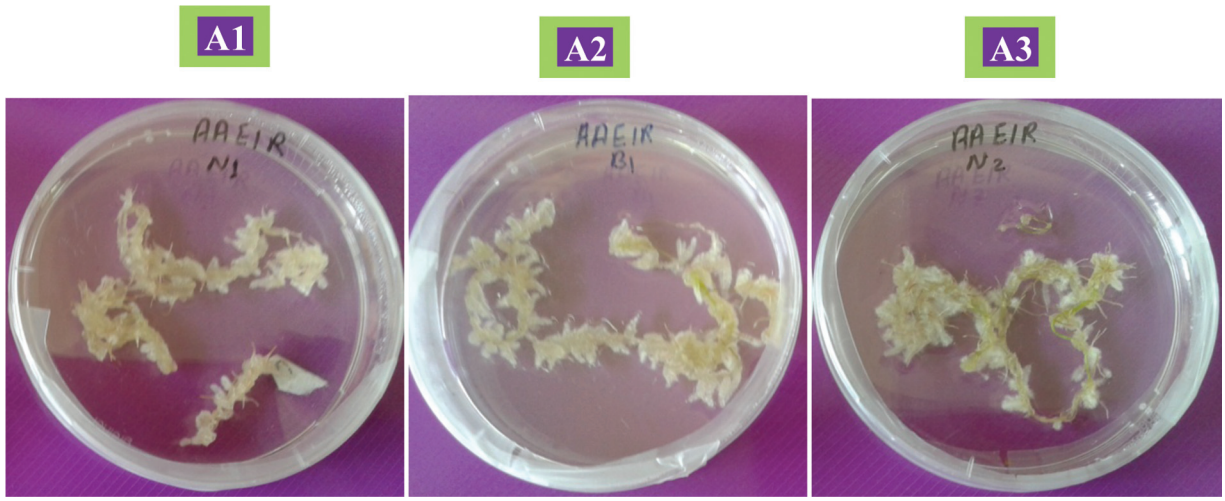
Auxin has a key role in root formation by its involvement in successive and interdependent phases [26]. The researchers reported that, the differentiation of the roots depends upon the type and concentration of auxin, because most suitable auxins are a demand for

Figure 2



Adventitious root cultures of *Cichorium endivia* cultured 4 weeks on MS medium supplemented with A1 (0.1 mg/l NAA+0.25 mg/l IBA), A2 (0.1 mg/l NAA+0.5 mg/l IBA), and A3 (0.1 mg/l NAA+1.0 mg/l IBA) media under light (16 h) condition.

Figure 3



Adventitious root cultures of *Cichorium endivia* cultured 4 weeks on MS medium supplemented with A1 (0.1 mg/l NAA+0.25 mg/l IBA), A2 (0.1 mg/l NAA+0.5 mg/l IBA), and A3 (0.1 mg/l NAA+1.0 mg/l IBA) media under total dark condition.

differentiating cells to become competent to respond to the organogenic signal [27,28].

In this regard, adventitious roots were established cultures from *C. intybus* L. on MS medium with different IBA and NAA combinations. They reported that 0.5 mg/l NAA and 1.0 mg/l IBA combination was the best suited for growth promotion [3].

Adventitious roots were induced from *Centella asiatica* on MS medium containing different auxins [indole-3-acetic acid (IAA), IBA, and NAA]. They showed that, among these different auxins, IBA achieved the highest adventitious root induction [29].

On the contrary, adventitious roots were induced from *Aloe vera*. There are no or very little adventitious roots on the media containing IBA and IAA, whereas there was induction on the medium containing NAA only [30]. Moreover, NAA is the only one that was able to induce adventitious root from *Andrographis paniculata* among different studied auxins (IAA and IBA) [31].

Recently, a study was conducted on adventitious root formation from *C. intybus* on MS medium containing different IAA and NAA concentrations and reached the highest induction percentage on MS medium with 1.0 mg/l NAA [32]. Moreover, adventitious roots were induced from *C. endivia* on MS medium with 0.5 mg/l NAA and different concentrations of IAA and IBA [33]. They found that, the combination of 0.5 mg/l NAA and 1.0 mg/l IBA was more responsive for root induction. Moreover, they showed the induction of adventitious roots was enhanced in total dark condition compared with the use of light condition of 16/8 h (light/dark) photoperiod. This result is perfectly consistent with our result.

In this area as well, half-strength MS medium supplemented with IBA was the most effective to promote adventitious roots of *Couroupita guianensis* than other auxins (IAA and NAA) [34].

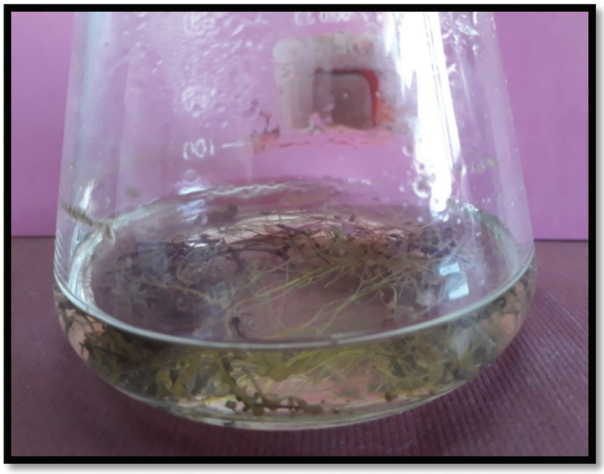
Effect of different carbon sources on adventitious roots production

Root initiation and growth require high energy, where carbohydrates are the primary source in the metabolic substrates [35]. In this experiment, different carbon sources (sucrose, fructose, glucose, and maltose) were used to evaluate which one increases the adventitious roots on the solid and liquid medium supplemented with 0.1 mg/l NAA+1.0 mg/l IBA and incubated under dark conditions. AFWs were recorded after 4 weeks of cultivation and are shown in Table 1.

Table 1 Effect of different carbon sources on adventitious roots' fresh weights of *Cichorium endivia* cultured 4 weeks on solid and liquid Murashige and Skoog medium supplemented with 0.1 mg/l α-Naphthalene acetic acid+1.0 mg/l indole-3-butyric acid.

Type of carbon sources	Fresh weights (g±SE)	
	Solid media	Liquid media
Sucrose	3.57±0.30	6.60±0.5
Glucose	4.25±0.20	6.32±0.3
Fructose	0.69±0.06	2.44±0.2
Maltose	0.99±0.09	3.11±0.3

Figure 4



Adventitious root cultures of *Cichorium endivia* cultured on MS liquid medium containing sucrose as the carbon source.

As is clear from the results, AFWs were obviously increased in liquid media compared with solid media with all studied sugars; this is explained by the availability, ease of uptake of water and nutrients, and closer contact between explants and the medium if it is liquid [36].

Many studies were conducted to use adventitious roots to produce biomass and secondary metabolite in liquid culture, such as in *Glycyrrhiza uralensis*, *Eurycoma longifolia*, *Hypericum perforatum*, *Prunella vulgaris* L., and *Eleutherococcus koreanum* [37–41].

The use of sucrose as a carbon source achieved the maximum AFW in the liquid medium recorded at 6.60 ±0.5 g (Table 1 and Fig. 4), followed by use of glucose as a carbon source (6.32±0.3 g). However, in the case of solid medium, the glucose recorded highest AFW (4.25±0.20 g) followed by sucrose (3.57±0.30 g). On the contrary, fructose and maltose recorded AFW lower than sucrose and glucose in both solid and liquid medium.

This result is quite consistent with Hussein *et al.* [42]. They reported that, among different studied carbon sources (sucrose, glucose, fructose, galactose, and sorbitol) to induce adventitious roots from the leaf explants of *E. longifolia*, both sucrose and glucose are the most influential.

Sucrose is the most used carbon source in tissue cultures, owing to the active absorption through the cell membrane [43]. Sucrose is hydrolyzed to glucose and fructose by the plant cells during assimilation, and the rate of uptake varies in different plant cells [44]. Sugars are considered as a main carbon sources and osmotic regulators [45].

The type of carbon source is an impact factor on adventitious root induction in many plant species [46]. The roots' frequency and quality can be enhanced by modify different types of carbohydrates in the culture medium [47]. The useful effects of sucrose on rooting had been reported in apple and *Gladiolus hybridus* [48]. Glucose and fructose had showed to be better carbon source in the few reports [49]. They reported that the uptake of glucose instead of sucrose could induce higher rooting frequency.

Adventitious root production was achieved from *C. intybus* on half-strength MS liquid medium supplemented with 0.2 mg/l NAA and 0.5 mg/l IBA under total dark condition [3]. Fresh weight of adventitious root reached to 5.82 g after 6 weeks of cultivation.

Adventitious roots were also produced from *C. intybus* L. on MS medium containing different auxins [32]. The highest fresh weight and DW was obtained in MS liquid medium containing 0.3 mg/l NAA and 1.5 mg/l IBA.

Moreover, the highest biomass production of adventitious roots from *C. endivia* (4.5 g) was achieved by using root explant on the medium containing 0.5 mg/l NAA and 0.8 mg/l IBA after 6 weeks of culture [33]. However, in the present study, the production of adventitious roots reached to 6.5 g after 4 weeks of cultivation by using sucrose as a carbon source and AFW reached to 6.3 g by using the glucose.

The highest accumulation of adventitious roots in the liquid media could be owing to the exposure of periphery tissues to sufficient nutrients and oxygen in the liquid medium; in contrast, the center of the cultures had restricted supply of nutrients and oxygen [50].

Extraction-yield percent estimation

Extraction step is an important for discovery of bioactive constituents from the plants. Active compounds in plants are usually found in low concentration, and an extraction technique especially type of solvent is able to obtain high yield of extract [51]. Various studies have reported the variations in extract preparation cause difference in the biological activities, therefore, it is needful to the selection of extraction method and solvent depending on sample matrix properties, chemical properties of the analytes, matrix, efficiency, and desired properties [52,53].

In this study, extraction-yield percent obtained by using two extraction solvents (aqueous ethanol and chloroform) was estimated in adventitious root extracts compared with different parts of *C. endivia* (seeds, leaves, and roots) extracts. For aqueous ethanol used as a solvent, the extraction-yield percent of the four extracts was obviously elevated and compared with those of chloroform. Data in Table 2 show that, in aqueous ethanol extract, the maximum value of extraction-yield (%) was found in adventitious root extract (52.5%) and root extract (51.4%), followed by leaves and seeds extracts (30.0 and 25.0%, respectively). The same order was obtained by using chloroform solvent, where adventitious root extract recorded maximum extraction-yield percent followed by root extract then leaves and seed extracts (25.0, 18.5, 12.7, and 12.5%, respectively).

This result is consistent with Milala *et al.* [54], and also explain our results, where they showed that, different parts of *C. intybus* L. (root, leaves, and seeds) which subjected to the extraction by using water–ethanol resulted in the root fraction having higher fructooligosaccharides, which predominated than leave and seed fractions.

Table 2 Extraction yields percent of four different parts of *Cichorium endivia* extracted by aqueous ethanol and chloroform

Solvent	Part of plant	Percentage of extraction-yield
Aqueous ethanol	Seeds	25.0
	Leaves	30.0
	Roots	51.4
	Adventitious roots	52.5
Chloroform	Seeds	12.5
	Leaves	12.7
	Roots	18.5
	Adventitious roots	25.0

The main compounds of chicory root are carbohydrates, inclusive, saccharose, glucose, and fructose [55]. Fructooligosaccharides and inulin represented 21% on average.

Regarding our finding, aqueous ethanol extract recorded extraction-yield percent higher than chloroform extract comparable to their counterpart to the same plant part; this has been explained by Willeman *et al.* [56]. They reported that the high solubility of some compounds in alcoholic solvent may be owing to the high surface polarity especially induced by hydroxyl group.

2,2-diphenyl 1- picryl- hydrazyl with DPPH

In this experiment, radical scavenging capacity (RSC %) of the different *C. endivia* extracts was estimated, and the data are presented in Figs 5–8. In all extracts of *C. endivia*, the aqueous ethanol generally recorded RSC% higher than those of chloroform extracts. This remark is entirely consistent with Montefusco *et al.* [57]. They found that, hydrophilic antioxidant activity of different chicory varieties was generally higher than lipophilic extract.

Figure 5 shows RSC% of aqueous ethanol and chloroform extracts of seeds comparable with L-ascorbic acid, where it was found that, RSC% values of seeds extracted with aqueous ethanol approach to the values of L-ascorbic acid at different concentrations. The maximum RSC% reached to 92.8% at 2.5 mg/ml versus 95.3% for L-ascorbic acid at the same concentration. It is clearly observed that, aqueous ethanol extract of seeds recorded high RSC% even at the lowest concentration, where it recorded 90.2% at 0.5 mg/ml. As for chloroform extract of seeds, it reached to maximum value (60.6%) at 2.5 mg/ml.

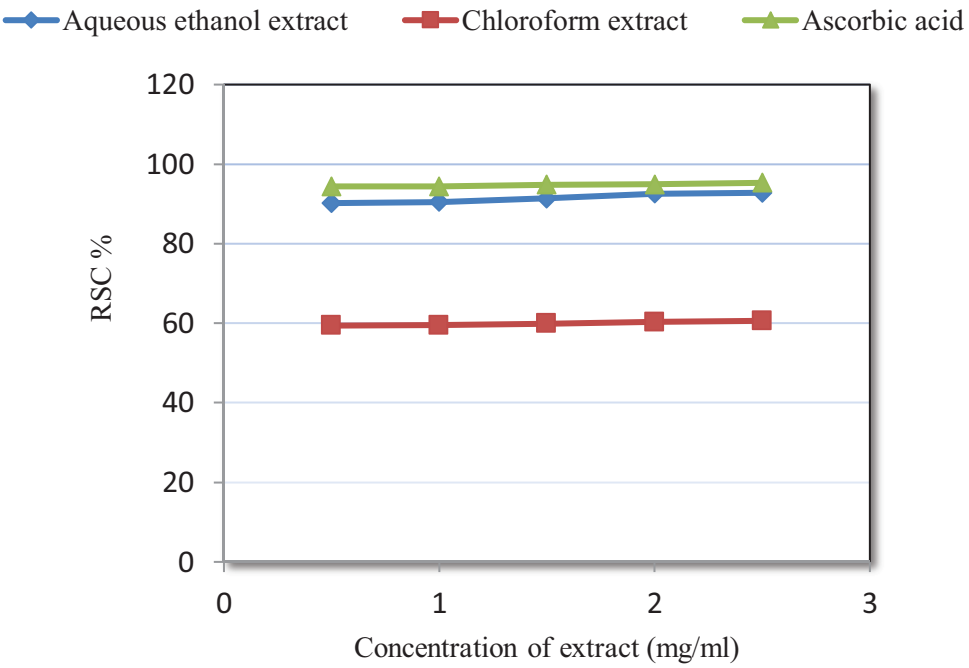
RSC% of leaves which presented in Fig. 6 shows that, in case of aqueous ethanol extract, the high values of RSC% (89.3 and 87.7%) were recorded at 2.0 and 2.5 mg/ml, respectively, whereas the lowest extract concentration (0.5 mg/ml) showed minimum RSC% (55%). However, chloroform extract of leaves reached to maximum RSC% (48.3%) at 1.0 mg/ml, and then declined at the higher concentrations.

Regarding RSC% of roots (Fig. 7), the values of aqueous ethanol extract approached to the values of chloroform extract especially at concentrations of 0.5 and 1.0 mg/ml, where it was found that, RSC% of aqueous ethanol recorded maximum value (59.4%) at 2.0 mg/ml and the chloroform extract (50.6%) at 2.5 mg/ml.

Aqueous ethanol extract of adventitious roots reached to maximum RSC% (63.1%) at 2.5 mg/ml, whereas chloroform extract showed lower value comparable with aqueous ethanol extract, recorded 38.8% at 2.5 mg/ml (Fig. 8).

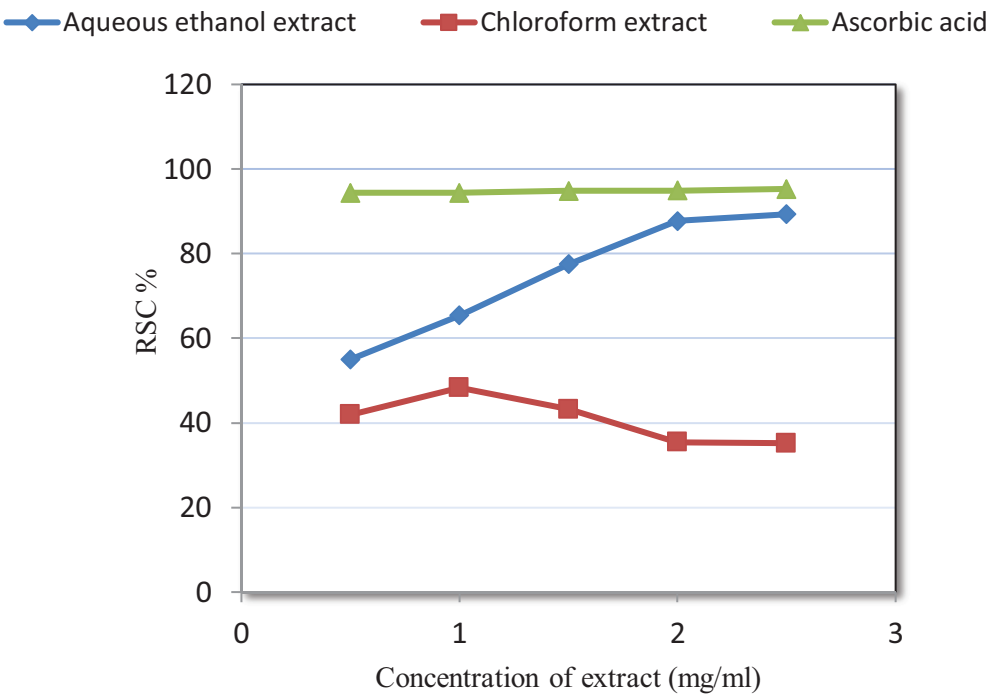
These results can be summarized as such, aqueous ethanol extract of seeds recorded maximum RSC%, followed by aqueous ethanol extract of leaves, and then

Figure 5



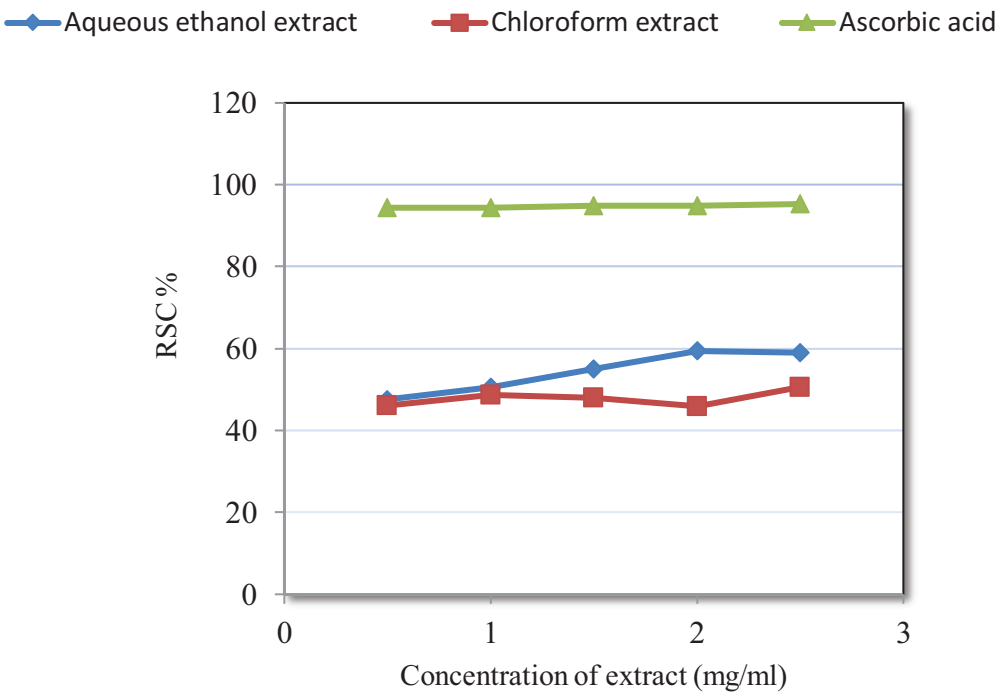
Radical scavenging capacity (RSC%) of *Cichorium endivia* seeds extracted with aqueous ethanol and chloroform.

Figure 6



Radical scavenging capacity (RSC%) of *Cichorium endivia* leaves extracted with aqueous ethanol and chloroform.

Figure 7

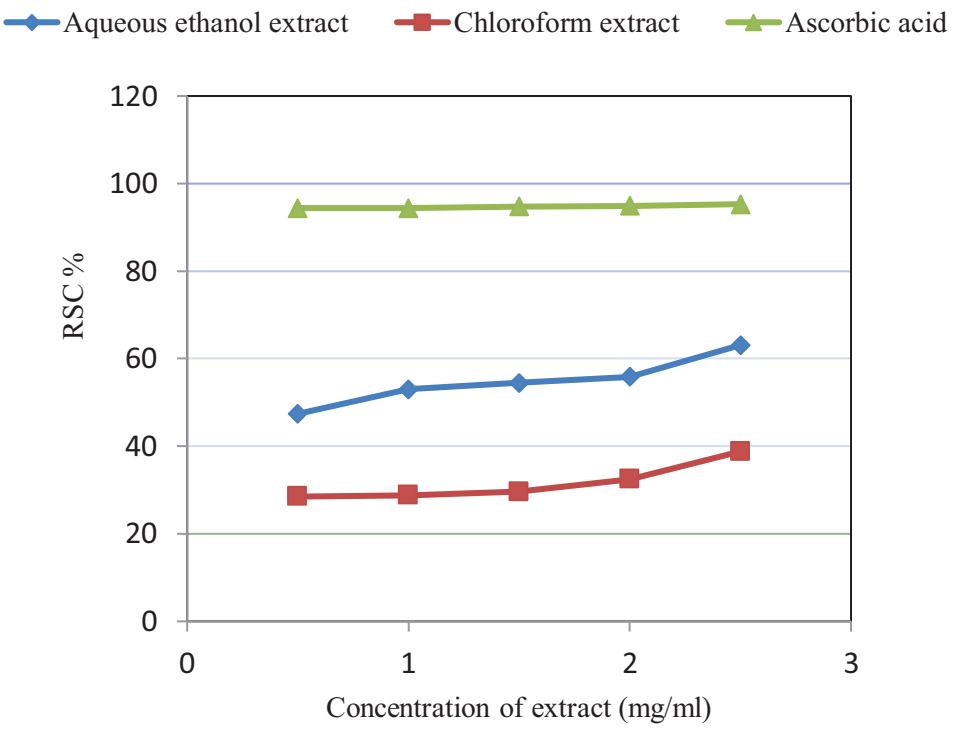


Radical scavenging capacity (RSC%) of *Cichorium endivia* roots extracted with aqueous ethanol and chloroform.

RSC% values of aqueous ethanol extracts of adventitious roots and roots, which recorded the lowest values.

Recently, attention has increased extremely in finding naturally occurring antioxidants for use in foods or medicinal materials. Adventitious roots are considered

Figure 8



Radical scavenging capacity (%) of *Cichorium endivia* adventitious roots extracted with aqueous ethanol and chloroform.

factories for biosynthetic and production of health promoting secondary metabolites, including phenolics, flavonoids, and alkaloids. *In-vitro* root culture is an efficient protocol with faster growth rate and active secondary metabolites production [58].

The previous studies indicated that, chicory plant could be a perfect source of natural antioxidants and it is considered as remedial factor in inhibiting or slowing down oxidative stress caused by diseases. This may be attributed to the occurrence of antioxidant compounds in this plant such as phenols, flavonoids, alkaloids, tannins, coumarins, and terpenoids [59].

Various organic fractions of *C. intybus* seeds were evaluated [60]. They reported that, seed extract showed good DPPH radical scavenging activity. This is in agreement with our results. Moreover, methanolic and ethyl acetate extracts exhibited the maximum antioxidant activity compared with other fractions (*n*-hexane, chloroform, and *n*-butanol).

Our results are also confirmed with the study of Milala *et al*. [54]. They elucidated that, aqueous ethanol preparation from seeds of *C. intybus* was distinguished with the highest antioxidant activity compared with other plant parts (leaves, peels, and roots), whereas lowest antioxidant activity was observed in the preparation of roots.

Antioxidant activity of different parts of *C. intybus* L. extracted with methanol was evaluated and compared [61]. They reported that, leaves have a distinctive ability in RSC compared with other plant parts (root, stem, and seeds), which showed lower percentage of RSC. This result was different from that we obtained, where the seeds recorded higher RSC than the other plant parts in the present study. This may be owing to the difference in the type of solvent or the variation of plant variety.

Another comparative study between leaves and root of *C. intybus* had been performed using different solvents (water, methanol, and chloroform) [62] and it showed that, maximum RSC% of leaves and roots was recorded with methanol extract (78.62 and 71.88%, respectively), followed by chloroform and water, which both recorded minimum values. This result is quite similar to ours; however, hydrophilic extract (aqueous ethanol) recorded RSC% higher than chloroform extract.

Total phenolic and flavonoids contents

Phenolic and flavonoids are a class of phytochemical compounds that have antioxidant properties and

possess the ability to inhibit apoptosis, where the oxidative stress is the first step which caused apoptosis [63]. In this part of the study, total phenolic and flavonoids contents were estimated in the different *C. endivia* extracts. Data presented in Table 3 explained that, maximum phenolic and flavonoids contents were recorded with seeds extracted with aqueous ethanol (18.17±0.40 and 94.43±1.00 mg/g extract) respectively. This is perfectly consistent with DPPH antioxidant evaluation, whereas also recorded maximum RSC% value. Regarding phenolic content, there were no significant differences between leaves and adventitious root extracted with aqueous ethanol, recorded at 6.11±0.40 and 6.23±0.20, respectively; however, aqueous ethanol extract of root recorded minimum value.

Regarding flavonoids contents, the aqueous ethanol extract of leaves recorded higher value than root and adventitious root (31.68±0.10, 20.87±0.30, and 21.62 ±0.30 mg/g extract), respectively. Chloroform extracts of the different plant parts recorded minor proportions of phenolic and flavonoids compounds compared with aqueous ethanol extracts, and there were no significant differences between the different plant parts.

In this area, the cultures of adventitious roots are an important source for the production of valuable plant secondary metabolites on a commercial scale [50]. Moreover, ~60% of medicinal plants used roots in the drug preparation [64]. Therefore, the in-vitro root culture development is a highly economic source for the production of valuable plant secondary metabolites, and it used as also alternative method for clonal propagation and germplasm conservation.

Small letter express LSD significant differences ($LSD_{0.050}$ value = 0.8723 for phenolic and 1.782 for flavonids. A considerable importance of *C. intybus* has been found owing to it containing high phenolic and flavonoid compounds. Leaves' extract is a good source for obtaining important pharmaceutical compounds which play role in improve public health [61].

Table 3 Total phenolic and flavonoids contents of the four different *Cichorium endivia* extracts

Solvent	Part of plant	Phenolics (mg/g extract)	Flavonoids (mg/g extract)
Aqueous ethanol	Seeds	18.17±0.40[a]	94.43±1.00[a]
	Leaves	6.11±0.40[b]	31.68±0.10[b]
	Roots	3.78±0.30[c]	20.87±0.30[c]
	Adventitious roots	6.23±0.20[b]	21.62±0.30[c]
Chloroform	Seeds	0.28±0.05[d]	3.21±0.20[de]
	Leaves	0.99±0.20[d]	2.60±0.18[e]
	Roots	0.61±0.10[d]	3.50±0.20[de]
	Adventitious roots	0.62±0.08[d]	4.90±0.20[d]

Moreover, *C. intybus* is rich in total phenolic compounds. Kaur and Singh [62] achieved a comparable study between leaves and roots extracted with three different solvents (methanol, chloroform, and water). Moreover, they detected that methanol extracts contain maximum values followed by chloroform extracts and then water extracts, which revealed minimum values. In three extracts, the leaves' extracts had own phenolic compounds greater than root extracts. These results are similar to some extent with the results we obtained.

Furthermore, a comparable study was done on different parts (seeds, leaves, roots, and peels) of *C. intybus,* extracted with aqueous ethanol [54]. The study concluded that the seed extract contained more than 10% of total polyphenols. The major components were dicaffeoylquinic acids (71% of total polyphenols). Seeds of chicory are considered as a good source for obtaining preparations rich in polyphenols, especially dicaffeoylquinic acids. These results are confirmed with our results.

The ethanolic and methanol fractions from seeds containing polyphenols showed higher antihepatotoxic properties than ethyl acetate and petroleum ether fractions [8].

Conclusion

In this study, the root explant responded to induce adventitious roots more better in the dark condition and highest IBA concentration. Adventitious root production was enhanced and attained good mass production in the liquid medium containing the sucrose as the carbon source. All studied *C. endivia* parts extracted with aqueous ethanol achieved considerable antioxidant activity more those extracted with chloroform; among these different extracts, aqueous ethanol extract of seeds possessed the highest antioxidant activity. This high activity can be confirmed and explained through containing the highest amount of total phenolic and flavonoids.

Acknowledgements

This research is quite compatible within the research plan of National Research Centre. The authors are greatly indebted to the NRC for the generous financing support that enabled us to achieve these results.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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