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Research Article

Phytochemical Profile, Antioxidant and Cytotoxic Activities of *Launaea acanthodes*; an Endemic Species of Iran

Running title: Biological Activities of *Launaea acanthodes* extract

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ABSTRACT

Many diseases are associated with oxidative stress caused by accumulation of free radicals in the creatures body. The purpose of this study was to evaluate the *in vitro* cytotoxic and antioxidant activity and phytochemical screening of compounds in methanolic extract of the *Launaea acanthodes* aerial parts. The antiproliferative activity of a methanolic extract from the aerial parts of *Lanuaea acanthodes* was assessed with the MCF 7, KYSE 30 and HEK 293 cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test for cell viability and cytotoxicity indices. In addition, the ability of free radical scavenging of extract was evaluated by use of DPPH test, followed by phytochemical screening tests for determining the group of compounds in the extract. The results showed that methanolic extract of aerial parts of the *Launaea acanthodes* has antioxidant potetial with the half percent inhibition of free radicals (SC50) that is equal to 84.34 µg/ml and 74.36 µg/ml for 60 and 90 minutes, respectively. Also there was not any strong cytotoxity in concentrations used in this study. The results showed that methanol extract of the aerial parts of the *Launaea acanthodes* is a good source of natural antioxidant compounds with weak cytotoxic effect.

Keywords: Launaea acanthodes, cytotoxic activity, antioxidant activity, phytochemical screening, MCF7 cell line.

INTRODUCTION

Cancer is one of the main causes of decease worldwide. It is assessmented that approximately 180 million people will die due to malignant sickness between 2005 to 2015 [1]. Medicinal and traditional plants contain several phytochemicals with enormous therapeutic importance and considered to be natural, harmless in comparison with artificial drugs [2, 3].Derived drugs of plants like Vinblastine, Vincristine and Taxol made the greatest extend within vicinity of antitumors which are used in chemotherapy of various cancers[4]. The study make known that free radicals are complicated in the beginning and promotion of cancer[1]. They are usually formed through metabolic activities in body, however excessive amount of these free radicals can cause oxidative stress and lead to several diseases such as cancers [5]. Known radicals including hypochloro, oxygen peroxide and others and antioxidants including active free

radicals such as hydroxyls, super oxides, peroxyls, alkoxyls which can interact with proteins, lipids and nucleic acids oxidative break induced by active oxygen [6]. Natural antioxidants raise plasma antioxidant capability and decrease the risk of cancers. Alternatively, a number of secondary metabolites in plants, which are regularly discovered in each part of plants, such as phenolic and flavonoid compounds, have high capacity in restraining free radicals [7].

Lanuaea is a small genus of Asteraceae family, including 54 species. Therefore, there is a report on 10 species in Iran; 3 of them are monoplaized plants of Iran. *Lanuaea acanthodes* is one of these species which has glabrous, branching shoots and dense bushes. Flowering of this plant is in May and June [8]. This plant is one of the resistant species and grows in moderate superficial areas such as central desert of Iran [9]. In Iranian traditional medicine, the resin of this plant is used for back and foot hurt relief, digestive tract illnesses such as gastric and duodenal ulcer and also wound healing as an oral or topical remedy [10, 11].

A literature survey did not reveal any reference to previous work describing the antioxidant and cytotoxic activity of methanolic extract of aerial parts *launaea acanthodes L.*, because its not well-known and very limited distribution around the world. For this reason, and because of lack of any report about the antioxidant capacity, cytotoxicity and phytochemical compounds of the mentioned spices, present report will discuss the forenamed effects on methanolic extract of aerial parts of this traditional native plant.

MATERIALS AND METHODS

Plant Collection

Aerial parts of *launaea acanthodes L.* were collected in June 2012 from the regions around Sabzevar in Iran. The voucher specimen of *launaea acanthodes L.* was confirmed and deposited in Herbarium at the *Herbarium* of the Ferdowsi University of *Mashhad* (FUMH). The collected plant parts were air-dried in darkness at room temperature (25 °C).

Preparation of Extract

The substance was 10g of filtered, dried aerial parts which were powdered and extracted with absolute methanol (2×60ml) by shaking for 48h at room temperature. Then the extracts were filtered with a filter paper and evaporated in vacuo by using rotary evaporator below 40°C. The obtained plant extract was used for the next stages of testing [12].

Qualitative analysis of phytochemical constituents

Phytochemical analysis was carried out qualitatively to recognize the existence of different secondary metabolites [12].

Antioxidant activity

The antioxidant capacity of the plant extract was evaluated by using the 2, 2'diphenylpicrylhydrazyl (DPPH) assay [13]. In brief, a 1.5 ml aliquot of each extract in methanol at 10, 20, 40, 80, 160 and 320 µg/ml was added to 1 ml of 0.1 mM DPPH in methanol. The mixture was shaken for 1 min and allowed to stand in dark for 60 and 90 min at room temperature. The absorbance was read at 517 nm. Ascorbic acid was used as positive control. The percent inhibition of free radical formation (I %) was calculated as;

I% = (A blank- A sample / A blank) × 100;

Where; A blank is the absorbance of the control reaction (containing all reagents except the extract) and A sample is the absorbance of the mixture containing the extract. The SC_{50} (defined as the concentration required to scavenge 50% of the free radicals) was calculated from graphing inhibition percentage against extract concentration. Determinations were carried out in triplicate.

Determination of total phenolic content (TPC)

The total phenolic content of the extract was measured according to Folin-Ciocalteu's reagent method. Briefly, a 0.5ml of FCR (10% in distilled water) was added to a vial containing 0.5ml of

each extract (800µg/ml in methanol) and 1.5ml of distilled water. The combination was severe shaken. After 5 min, 2ml of 10% sodium carbonate solution was added and the mixture was shaken another time. The combination incubated in dark for 2h at room temperature. The absorbance was measured at 760 nm with UV-VIS spectrophotometer. The analyses were carried out in triplicate. A gallic acid standard curve was made (5-100µg/ml). Total phenolic content (TPC) was determined as µg of gallic acid equivalents (GAE)/g [14, 15].

MTT bioassay

The cytotoxic effect of *Launaea acanthodes* extract against MCF-7, KYSE30 and HEK293 cell lines was evaluated using MTT bioassay. This assay was performed in consistent with a fiddling change of the process reported by Mosmann [16]. Two human cancer cell lines MCF-7 and KYSE30 and non-malignant control cell line, HEK293, were obtained from National cell bank of Iran (Pasteur institute), and cultured in RPMI1640 medium (Gibco, UK) complemented with 10% fetal bovine serum (FBS), 100 mg/mL streptomycin and 100 units/ml penicillin G. The cells were cultured at 37°C in a 5% CO_2 incubator. The cells were detached with 0.05% trypsin/EDTA when they reached ~90% confluency. Then, the cells were seeded in 96-well plate (200 µl/well) with concentration of 4×10^4 cells/cm^2. At 70-80% confluency, the cultivated cells were exposed to various concentrations of the methanolic extract (5, 10, 20, 30, 40, 50, 75, 100 mg/ml) preparedin 1% dimethylsulfoxide (DMSO) and were incubated for different periods of time (48 and 72h). Control groups received the same amounts of DMSO with three wells remained untreated as control. After the treatment, normal culture medium was substituted with 200 µl freshmedia and 50 µl MTT reagent (2mg/ml in PBS), except the cell-free blank control wells. Cells were maintained in 37°C with 95% air, 5% CO_2 and complete humidity for 4h. Subsequently, the MTT solution was replaced with 200 µl of DMSO and 25 µl sorenson buffer (0.1M NaCl, 0.1M glycine regulated to pH: 10.5 with 1M NaOH), incubated for 15 min at 37°C. Finally, the optical density of the wells was measured at 570 nm by means of a spectrophotometric plate reader (Sunrise Tecan, Austria). The growth of tumoral cells and viability of the cells was determined using the formula: Viability%= (optical density of sample/optical density of control) ×100. Furthermore, the cytotoxicity of the extract was determined by plotting of the percent cytotoxicity index, CI %=[1-(optical density of sample/optical density of control)]×100, versus concentrations of the methanolic extract of *L.acanthodes.*

Statistical Analysis

Information shown is the means and standard errors of three or more independent experiments. Statistical comparisons between groups were made by Student's t-test using *SPSS version* 18 program, and a P-value<0.05 considered to be statistically significant.

RESULTS

Phytochemical Screening and Antioxidant activity

Preliminary phytochemical analysis of aerial parts of methanolic extracts revealed the presence of tannins, flavonoids, saponins, terpenoids. Where, Alkaloids, coumarins, cardiac glycoside and Anthroquinones are completely absent in extract. The potential of antioxidant activity of methanolic extract of *Launaea acanthodes* evaluated by using DPPH free radical scavenging activity assay. By comparing the percent inhibition of DPPH methanol extracts tested against ascorbic acid (control), antioxidant activity *dose*-dependent was observed. Fifty persents of radical *scavenging* concentration(SC_{50}) obtained from aerail parts of methanolic extract in 60 and 90 min equal to 84.342±1.180 and 74.360±0.868 µg/ml, respectively (Table 1 and 2). It was found that

increasing the concentration was significantly associated with increas free radical scavenging ability.

Total Phenolic Content (TPC)

The total phenolic content of methanolic extract of *Launaea acanthodes*aerial parts was determined by folin-ciocalteau assay. For this purpose, the calibration curve of gallic acid was drawn (Figure 1). The amount of phenolic in methanolic extract was 62.838 ± 3.528 mg GAE/g extract.

Cytotoxicity effect of plant extract

The results of cytotoxic activity of a methanolic extract from the aerial parts of *Lanuaea acanthodes* after 48 and 72h on MCF7, KYSE30 and HEK293 cell lines is drawn (Figure 2). Methanolic extract induced a distinct dose and time dependent diminution of cell viability. The IC_{50} values of methanolic extract on studied cell lines were not determined as after 48 and 72h of treatments with applied doses in this study.

Extract exhibited cytotoxic activity against apoptosis-proficient all cell lines, with IC_{50}values ranging >100 μg/ml. Values were mean ± standard error of the *mean* (SEM) of at least three independent experiments, each in triplicates.

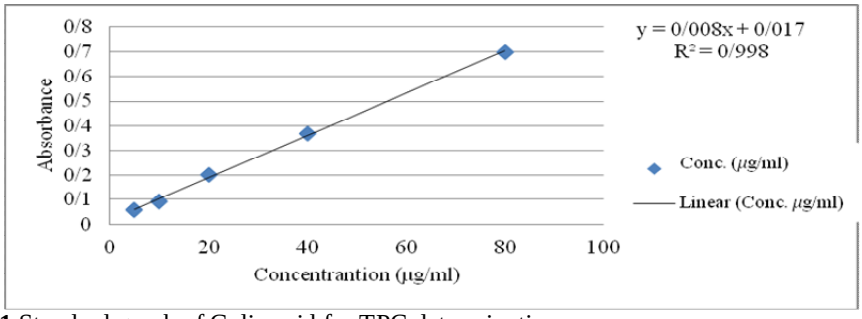
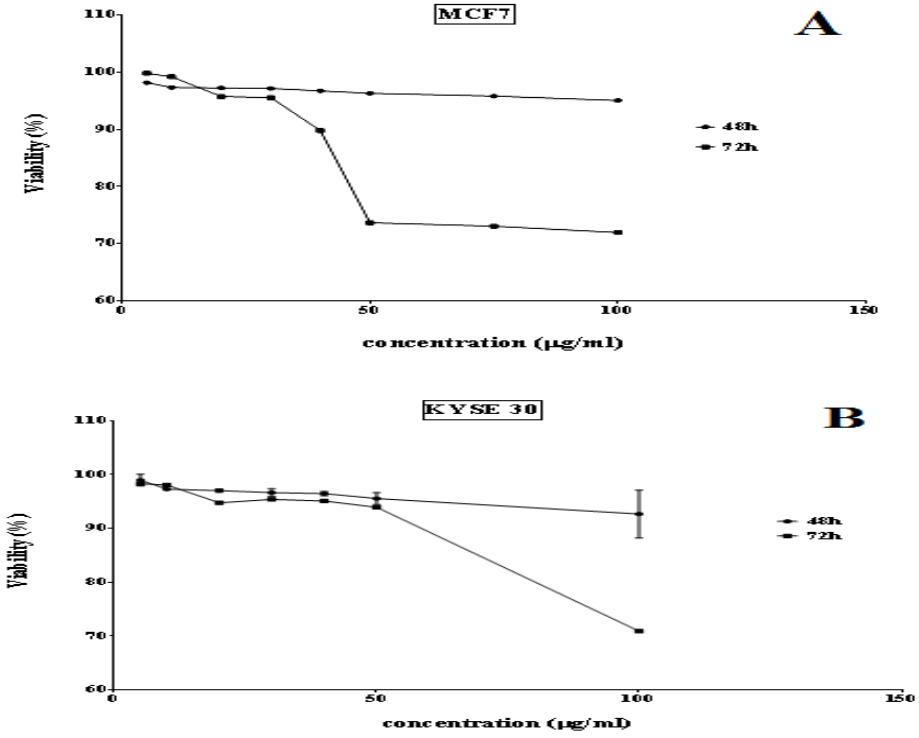


Figure 1. Standard graph of Galic acid for TPC determination.



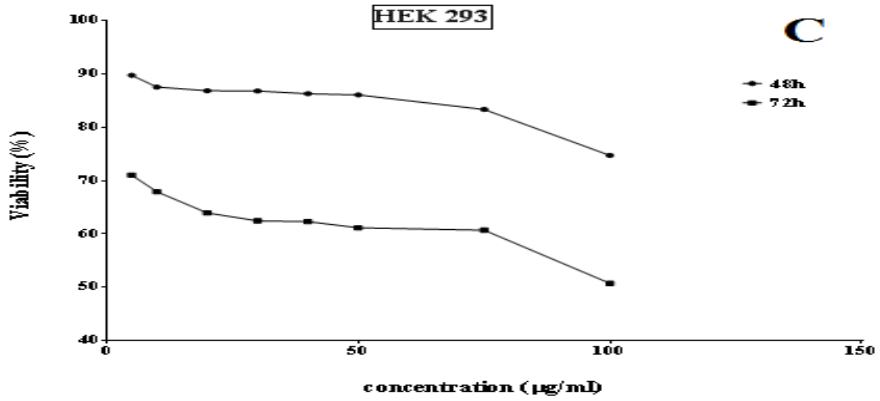


Figure 2. The dose-dependent effects of methanolic extract from the aerial parts of *Lanuaea acanthodes* after 48 and 72h on the growth of MCF7, KYSE30 cells and non-malignant HEK293 cells (A, B, C).

Table1. Free radical scavenging (% inhibition) of methanolic extract of *Launaea acanthodes* aerial parts in incubation for 60 minutes.

Concentration (µg/ml)	**Antioxidant activity (% inhibition)**	
	Ascorbic acid	**Methanolic extract of *Launaea acanthodes***
10	95.227±1.132	9.769±3.019
20	95.388±0.140	15.109±0.413
40	96.926±0.080	27.123±0.207
80	97.735±0.080	48.726±0.899
160	97.735±0.080	84.951±1.103
320	97.896±0.080	92.415±0.479

Table 2. Free radical scavenging (% inhibition) of methanolic extract of *Launaea acanthodes* aerial parts in icubation time for 90 minutes.

Concentration (µg/ml)	**Antioxidant activity (% inhibition)**	
	Ascorbic acid	**Methanolic extract of *Launaea acanthodes***
10	91.141±1.199	9.527±2.753
20	94337±0.428	16.141±0.305
40	95.955±0.157	29.854±0.171
80	96.056±0.116	54.369±0.823
160	96.177±0.116	91.262±0.475
320	95.146±0.420	92.476±1.053

DISCUSSION

Strong data supports the significant role of apoptosis in the pathology of various diseases including cancer. Hence, pharmacological modulation of apoptosis is probably the most important approach for searching well-organized anticancer therapeutics [17]. A lot of compounds found in plants with anticancer properties such as terpenoids, alkaloids and phenylpropanoid [18, 19]. There is a strong need for natural effective antioxidants as analternatives to synthetic ones in order to prevent free radicals implicated diseases [20].

Phytochemical studied on some species of lactuceae shown that the members of this family are enriched with secondary metabolites such as

sesquiterpene lactone [21, 22]. Phytochemical analysis of *L .acanthodes* in this study showed that extract of aerial parts of this plant is containing of tannin, flavenoid and saponin. These secondary metabolites also have been found in the similar plant spieces [23, 24].

So far, the number of flavenoid isolated from lactuceae species [25] and specified that these compounds, through the creation of anionic radicals serve as significant possible combinations in the promotion of natural remedies [26]. In previous studies, effects of tannins on treatmenting inflammation, recovery of injured tissue and also striking role of this compounds in preventing cancer [27, 28].According to this study, methanolic extract of aerial parts of *L. acanthodes* can be a good investigation about a possible anticancer activity in future. On the other hand, due to clear inhibitory effects of saponins compounds on inflammation [29] and also biological properties of this compounds, isolated from plants [30], it could be said that identify the high level of saponins in this plant in our study provides that so more confidently focused about anti-inflammatory effects of plant considered [31].

MTT assay is one of the best techniques among various methods of cytotoxicity assays [32, 33]. Mitochondria is crucial organell that playsvital role in cell metabolism. The MTT assay represents significant process in evaluating mitochondrial damage. In this study, we evaluated the MTT reduction activity of MCF 7, KYSE 30 and HEK 293 cells to verify the cytotoxic effects of methanolic extract from the aerial parts of *Lanuaea acanthodes*. The loss of viability was not visibly evident after treatment times, exposure. Based on the results, the extract was low toxic for studied cell lines. This suggests that the toxic compounds in this extract is likely to have a low concentration.

The investigation provided evidence of low cytotoxicity in MCF-7, KYSE30 and HEK293 which may be due to existing different values of phytochemical compounds in the extract since *L. acanthodes* as mention previously. The sensitivities of cancer cells to cell death by flavanoids are accordance with this finding from previous reports in literature [34].

CONCLUSION

This finding suggests that the reduction observed in the viable cells following treatment with *L. acanthodes* extract is due to cell death. Further experiments are needed, both *in vitro* and *in vivo* to obtain more detailed mechanisms of action.

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