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Cytotoxic profile activities of ethanolic and methanolic extracts of chicory plant (*Cichorium intybus* L.)

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**ABSTRACT**

This study aims to highlight the antioxidant and cytotoxic effects of *Cichorium intybus* L. The cytotoxic activity for cancer cell lines was evaluated by MTT assay. Total phenol and flavonoid content were examined using chemical analysis methodology. The highest content of phenols and flavonoids have occurred in the methanolic extract (1162.29 ± 103.4 µg·g⁻¹ D.W), respectively, followed by ethanolic extract (153.83 ± 10.25 µg GAE·g⁻¹ D.W), respectively. The cytotoxic effect of methanolic extract on MCF-7 cell line was achieved at 300 µg·mL⁻¹. The results showed that methanolic extract possessed potent cytotoxic activity while ethanolic extract possessed antioxidant activity. In conclusion, natural plant extract might be prevent and working as a defense by enhancing the immune system against cancer.

**KEYWORDS**

Antioxidant; cytotoxic activity; MCF-7 cell line; AML cells; TLC technique

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**1. Introduction**

Breast cancer has surged worldwide, and is the second reason for female mortality (Hortobagyi et al., 2005), especially in Americans and Asians countries (Jemal et al., 2008). Various therapy methods have been applied to decline the percentage of the mortality caused by breast cancer (Kim & Choi, 2013).

Acute myeloid leukemia (AML) is a highly heterogeneous disease; AML cells is the most common acute leukemia in adults. The incidence of AML was ranged from 3 to 5 patients per 100,000 of the population in the United States (Yamamoto & Goodman, 2008).

Oxidative damage is attributed to free radical compounds, which eventually leads to worsening diseases especially cancer (Miller, Rigelhof, Marquart, Prakash, & Kanter, 2000). Antioxidant compounds have the ability to prevent or delay the oxidative stress through free radical trap (Halliwell, Gutteridge, & Cross, 1992).

Medicinal plants have been used in folk medicine for thousands of years as natural products which play a key role in pharmaceutical biology. According to the World Health Organization (WHO), the active ingredients of anticancer drugs have been extracted from natural products (Siegel, Miller, & Jemal, 2015). In addition, the current drugs share fully or some parts of structures with natural compounds.

The current treatments of cancer showed severe side effects, so the use of natural products such as Vinorelbine and Paclitaxel with chemotherapy gave promising results (Jose & Rao, 2006).

Chicory, *Cichorium intybus*, belongs to the Asteraceae family and is used in folk medicines all over Asia, Europe and Egypt (Blumenthal, 1998). It contains a high amount of important secondary metabolites (phenols, flavonoids, and polyamines) (Lante, Nardi, Zocca, Giacomini, & Corich, 2011). Anticancer activity of *C. intybus* has registered against various cancer cell lines such as Amelanoic melanoma C32 (Conforti et al., 2008), breast cancer MCF-7 (Abu-Dahab & Affifi, 2007), and renal adenocarcinoma, prostate cancer (Saleem et al., 2014).

This plant has many anticancer compounds which showed antiproliferative activity against some cancer cell lines (Zhang, Yan, Wang, & Liu, 2016; Zhou et al., 2010). Therefore, this work aims to study the cytotoxic activity of ethanolic, methanolic extracts of *C. intybus* against both MCF-7 cell line and AML cells. The extracts were also evaluated for their antioxidant capacities. The separation and fractionation of active ingredient from ethanolic extract was processed using TLC technique.

**2. Materials and methods**

**2.1. Sample collection and authentication**

Fresh leaves of *C. intybus* L were collected from Faculty of Agriculture farm in March 2017, and the plant was identified and authenticated at department of Vegetable Crops, Faculty of Agriculture – Cairo University, Giza Egypt.

**2.2. Preparation of plant extracts and fractions**

Specific weight of *C. intybus* leaves was ground. Then chloroform, ethyl acetate, acetone, ethanol 80% and...
methanol were added sequentially and soaked for 24 h at a refrigerator at 10°C. Ethanol and methanol extracts gave the highest phenolic content and antioxidant activity, so we decline the other extracts. The ethanol extract was further applied to thin-layer chromatography (TLC) technique to separate active ingredients using silica gel 60 F254 aluminum sheets 20 × 20 (Merck, Darmstadt, Germany), by using system solvents petroleum ether: ethyl acetate (1:1v/v). The fractions were detected by UV lamp at 240 nm and scratched. The scratched fractions were dissolved in ethanol 80%, filtered and evaporated to dryness and stored at −20°C until used.

2.3. Determination of total phenolic content
\( Y = 0.0036x - 0.007 \) at \( R^2 = 0.9897 \)

The total phenol (TP) of both extracts was estimated (Singleton & Rossi, 1965). 200 µL of extract or standard gallic acid (20–240 mg L⁻¹) was added to 3 mL distilled water and then 250 µL Folin-Ciocalteu reagent/2 min was added. Then 750 µL sodium carbonate 20% was added and the mixture was made up to 5 mL with distilled water. The mixture was incubated for 2 h and the absorbance was measured at 750 nm.

TF content was expressed as gallic equivalent (µg GAE.g⁻¹ D.W).

2.4. Determination of total flavonoid
\( Y = 0.02111x + 0.0184 \) at \( R^2 = 0.9759 \)

The total flavonoid (TF) of both extracts was estimated (Ordonez, Gomez, Vattuone, & Others, 2006). 0.5 mL of extract or standard solution of quercetin was added to 0.5 mL of AlCl₃ (2%). After 1 h, absorbance was measured at 420 nm. TF content was expressed as quercetin equivalent (µg g⁻¹ D.W).

2.5. DPPH radical scavenging activity

Quantitative measurement of % inhibition was recorded (Burits & Bucar, 2000). 1 mL of DPPH solution (0.002%) was added with 1 mL of plant extracts at different concentrations, and butylated hydroxyl toluene (BHT) was used as positive control. The absorbance was measured after 30 min at 517 nm. The percentage of DPPH radical scavenging was estimated using the following formula:

\[
\% \text{ inhibition} = \frac{[(A_0 - A_1)/A_0] \times 100}{100}
\]

where \( A_0 \) is the control absorbance and \( A_1 \) is the sample absorbance. \( IC_{50} \) value was calculated using the dose inhibition curves \( Y = 0.0332x + 9.041 \) at \( R^2 = 0.5296 \) for ethanolic extract, \( Y = 0.0541x + 9.806 \) at \( R^2 = 0.9046 \) for methanolic extract.

2.6. Reducing power activity
\( y = 0.0292x, \text{at } R^2 = 0.9936 \)

The reducing power of extracts was determined according to Dorman, Kosar, Kahlos, Holm, and Hiltunen (2003). 500 µL of standard or plant extracts at different concentrations was mixed with 500 µL phosphate buffer and then 500 µL potassium ferricyanide was added. The mixture was incubated at 50°C for 20 min. Then 500 µL trichloroacetic acid (TCA) was added to the mixture. 1400 µL of supernatant was taken and then 200 µL FeCl₃ was added and absorbance was measured at 700 nm. Reducing power was expressed as gallic acid equivalent.

2.7. Cytotoxic activity

The cell line MCF-7 was obtained from VACSERA, AML cells from Kasr El-Ainy Giza, Egypt. Two cancer cells were maintained in RPMI media with 10% fetal bovine serum and streptomycin. The cell viability was determined by MTT assay (Wilson, 2000). Cells (MCF-7 only) were seeded overnight in 96-well culture plates at a density of 2 × 10⁴ cell/well with 200 µL media. Cells (MCF-7 and AML) were treated with extracts at different concentrations. After 24 h of incubation, 20 µL of 5 mg/mL MTT was added to each well and incubated at 37°C for 4 h. DMSO (100 µL) was added to wells to solubilize formazan crystals. The absorbance was measured at 630 nm (Wilson, 2000).

2.8. Statistical analysis

Experimental results are shown as mean ± standard deviation (SD) of three replications. Data were analyzed with analysis of variance (ANOVA), Duncan test, using ASSISTAT Version 7.7 beta. The \( IC_{50} \) values were calculated from linear regression analysis.

3. Results

3.1. Total phenol (TP) and flavonoid (TF)

The total phenol and flavonoid were recorded in ethanolic and methanolic extracts of C. intybus. Data highlighted that the highest phenolic content was showed in the methanolic extract (1162.29 ± 103.46 µg·g⁻¹ D.W), while the lower was in the ethanol extract (153.83 ± 10.25 µg·g⁻¹ D.W) (Table 1). The methanolic extract exhibited that the highest flavonoid content (167.47 ± 5.83 µg·g⁻¹ D.W) followed by ethanolic extract (36.56 ± 5.95 µg·g⁻¹ D.W) (Table 1).

3.2. DPPH radical scavenging activity

In the present work, the DPPH radical scavenging assay was used for evaluation of the antioxidant activity of C.
### Table 1. Total phenol and flavonoid content of the extracts of *Cichorium intybus*.

| Plant extract          | Phenols (mg/g d.w) | Flavonoids (mg/g d.w) |
|------------------------|-------------------|----------------------|
| *C. intybus* (Et.OH)   | 153.83 ± 10.25    | 36.56 ± 5.95         |
| *C. intybus* (Me.OH)   | 1162.29 ± 103.46  | 167.47 ± 5.83        |

Data represent mean ± SD (n = 3). GAE, gallic acid equivalents. QE, quercetin equivalents.

Figure 1. DPPH radical scavenging activity (%) of *Cichorium intybus* at different concentrations (µg.mL⁻¹) of methanolic, ethanolic 80% leaf extracts and BHT as positive control. Data represent mean ± SD (n = 3). Significant at P < 0.05.

*intybus* extracts (Figure 1). The DPPH radical scavenging of both extracts was showed across extents of concentrations (200–1000 µg.mL⁻¹). The values of percent DPPH scavenging activities of both methanol and ethanol extracts of *C. intybus* are summarized in Figure 1 as compared with positive control (BHT). The highest percent DPPH radical scavenging activity was observed in methanol extract 70.83 ± 2.95% followed by ethanol extract 54.65 ± 2.94% at 1000 µg.mL⁻¹. BHT had the highest scavenging activity 88.28 ± 0.21% at 800 µg.mL⁻¹ (Figure 1).

There was eventually a higher antioxidant power recorded by the methanol extract as compared to the ethanol extract and characterized by the calculated IC₅₀ values 742.96 µg.mL⁻¹ and 1098.90 µg.mL⁻¹, respectively.

### 3.3. Reducing power activity

The reducing power of the ethanolic and methanolic extracts of *C. intybus* was estimated relative to that of the gallic. The reducing power was 58.24 ± 4.30 in the methanolic extract and 29.77 ± 0.90 in ethanol extract at 1000 µg.mL⁻¹ as GAE. It was clear that the methanolic extract possesses a higher reducing power than ethanolic extracts with IC₅₀ (814.54 and 1719.59 µg.mL⁻¹), respectively (Table 2). There is a dose relation between the reducing power and the concentration of the both extracts.

| Concentration of extracts µg.mL⁻¹ (as GAE) | Ext. 200 | Ext. 400 | Ext. 600 | Ext. 800 | Ext. 1000 |
|-------------------------------------------|---------|---------|----------|----------|----------|
| (Et.OH)                                   | 5.98 ± 0.63 | 12.34 ± 3.80 | 16.83 ± 2.21 | 22.80 ± 2.06 | 29.77 ± 0.90 |
| (Me.OH)                                   | 18.07 ± 0.25 | 29.14 ± 2.29 | 37.46 ± 1.74 | 51.71 ± 2.32 | 58.24 ± 4.30 |

Data represent mean ± SD (n = 3).

### 3.4. Cytotoxic activity

In this study, the cytotoxic activities of methanol and ethanol extracts of *C. intybus* were tested against MCF-7 cell line and AML cells (Figures 2 and 3) at incubation period of 24 h. A wide range of extract concentrations were used ranging from 250 to 750 µg.mL⁻¹ of extracts. The data revealed that both extracts had cytotoxic activities against both cancer cell types. The results of the cytotoxic effect of ethanol extract showed the moderate effect at 750 µg.mL⁻¹ (83.98 ± 1.80%) against AML cells in (Figure 2), while the methanol extract recorded 80.71 ± 3.10% at 500 µg.mL⁻¹. There was no significant difference of cytotoxic activity between two extracts at different concentrations.

The methanol extract showed the cytotoxic effect at 500 µg.mL⁻¹ which reached to 88.38 ± 0.45% against MCF-7 cell line (Figure 3), while ethanol extract displayed 84.74 ± 1.51% at 750 µg.mL⁻¹.

Three ethanolic fractions were tested against MCF-7 cell line (Table 3). Pure fraction F1 had the highest cytotoxic activity against breast cancer cells (94.18 ± 3.93%) at 80 µg.mL⁻¹ and F3 gave (62.37 ± 0.66%) at 40 µg.mL⁻¹. The results indicated a generally moderate cytotoxic activity in the *C. intybus* extracts and highly in fractions F1 and F3.

Figure 2. Cytotoxic activity (%) of *Cichorium intybus* at different concentrations (µg.mL⁻¹) of methanolic and ethanolic 80% leaf extracts and data represent mean ± SD (n = 3) against AML cells.

Figure 3. Reducing power activity of the extracts of *Cichorium intybus*. Concentration of extracts µg.mL⁻¹ (as GAE).
Figure 3. Cytotoxic activity (%) of *Cichorium intybus* at different concentrations (µg.mL⁻¹) of methanolic and ethanolic 80% leaf extracts and data represent mean ± SD (n = 3) against MCF-7 cell line.

Table 3. Cytotoxic activity (%) of separated fractions of ethanolic extract against MCF-7 cell line.

| Fractions | Concentration of fractions µg.mL⁻¹ |
|-----------|----------------------------------|
|           | 40                               | 60                         | 80                         |
| F1        | 49.76 ± 1.17                     | 59.10 ± 1.88               | 94.18 ± 3.93               |
| F2        | 38.93 ± 5.635                    | 50.00 ± 0.00               | 60.67 ± 5.17               |
| F3        | 62.37 ± 0.66                     | 56.57 ± 1.08               | 48.36 ± 0.23               |

Data represent mean ± SD (n = 3). Significant at P < 0.05.

4. Discussion

It is observed from the results of this study that the highest phenolic and flavonoid content of chicory extracts exhibited high antioxidant activities. Previous publications found that methanolic extract of *C. intybus* possessed the highest content of phytochemicals (Jasim, 2018).

It was reported that the presence of phenolic compounds such as coumaric, protocatechuic, vanillic, p-hydroxybenzoic, ferulic, syringic and caffeic (Malik, Pirzadah, Tahir, & Rehman, 2017; Mansouri, Embarek, Kokkalou, & Kefalas, 2005) was thought to contribute in their antiradical potency which appeared in method of DPPH assay (Hsu, Coupar, & Ng, 2006).

According to Jabłońska-Trypuć et al. (2018), cichoric acid showed the highest percent of DPPH radical inhibition, and the scavenging mechanisms for antioxidant of flavonoids were detected (Pai et al., 2015). Therefore, the reducing power activity of chicory extracts was recognized due to the presence of phenolic and flavonoid compounds percentage (Mansouri et al., 2005).

Additionally, both extracts of chicory recorded cytotoxic activity against MCF-7 cell line and AML cells which indicated the importance of natural crude extract for cancer reduction.

These results were cited with other types of cancer cell line. The cytotoxic activity of *C. intybus* against Ehrlich ascites carcinoma cells (EACC) and SKBR3 cell line has been studied (Al-Snafi, 2016; Conforti et al., 2008; Guide, 2002; Mehrandish, Mellati, Rahimipour, & Nayeri, 2017), as well, the aqueous extract of chicory can induce caspase-3 activation (Nawab, Yunus, Mahdi, & Gupta, 2011).

Recently, there were numerous studies which showed that phenolic compounds such as lectin (Deeba, Sureshkumar, Satheeshkumar, & Priya, 2012), ascorbic acid (Thomas, Vezyraki, Kalfakakou, & Evangelou, 2005), and chlorogenic acid (Liu, Zhou, Qi, Lu, & Wang, 2013) could have a promising effect on arresting the cell cycle at different stages of cell proliferation. The study also showed that anthocyanins could decrease *in vitro* invasiveness of cancer cells (Huang, Chang, Wu, Hung, & Wang, 2011; Huang, Shih, Chang, Hung, & Wang, 2008). Furthermore, chicory-contained photosensitive compounds such as cichorin, lactucin, and lactucopirin could significantly decrease the number of estrogen receptors ER-positive cells in induced breast tumor masses in female Sprague–Dawley rats (Al-Akhras et al., 2012).

For more scientific evidences regarding the cytotoxic effect of chicory extracts against cancer cell lines from molecular point of view (Bøe et al., 1991). This molecular mechanism involves many caspase genes, for example, caspase-3 which has been reported to cleave a number of substrates response to DNA strand breaks leading to apoptosis (Mancini et al., 1998; Nicholson & Thornberry, 1997).

The present findings have recorded a tremendous relation between antioxidant, cytotoxic activities of methanolic extracts of *C. intybus*. Oxidative stress is a main factor for cancer progression and treatment. In addition, many signaling pathways that are responsible for carcinogenesis can also control reactive oxygen species (ROS) production and ROS downstream mechanisms in which the excess of ROS levels can trigger cancer initiation by enhancing DNA mutation and pro-oncogenic signaling. The majority of the natural active antioxidant compounds, such as flavonoids,
5. Conclusion

The objective of this work was to study the antioxidant and cytotoxic activities of two extracts of C. intybus. The data revealed that the ethanolic and methanolic extracts have cytotoxic and antioxidant activities. Hence, it was anticipated that C. intybus extracts can be a useful pharmaceutical material to protect or treat cancer cells. Two fractions of ethanolic extracts exhibited anticancer activity more than 50%. Therefore, the results of the current study also suggest that inclusion of anticancer-rich extract or fractions of C. intybus can be used as a dietary supplement. Further studies will be conducted using these plant extracts to identify the molecular mechanism of cytotoxic effect. The expression of some apoptotic and anti-apoptotic genes using RT-PCR is under investigation to know the possible mechanism of cytotoxic effect of C. intybus extracts against MCF-7 cell line and AML cells.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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