

## Research Article

### Antioxidant and Antitumor Effect of Different Fractions of Ethyl Acetate Part from *Elaeagnus angustifolia* L.

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**Abstract:** To determine antitumor and antioxidant activities of the different fractions of ethyl acetate of *Elaeagnus angustifolia* L. extract, the MTT assay, the DPPH scavenging assay and the reducing power method were used. The results showed that as the concentration and the polarity were increasing, the antioxidant activity of the different fractions of ethyl acetate of *Elaeagnus angustifolia* L. extract was increased. The DPPH scavenging of the E and F fractions was 94.9%. The antitumor assay showed that the different fractions of ethyl acetate of *Elaeagnus angustifolia* L. exhibited the inhibition of the in vitro cultured Hela cell proliferation properties. During the first 48 h, there was a dependant relation of inhibition effect and the concentration and the polarity. The higher the concentration and the polarity, the stronger the inhibition effect was. The inhibition rate was 74.34% when the concentration of F fraction was 2.5 mg/mL.

**Keywords:** Antioxidant, antitumor activities, different fractions, DPPH, *Elaeagnus angustifolia* L., ethyl acetate part, reducing power

## INTRODUCTION

With the improvement of the socioeconomic level of people, their health requirements are also increasing. Health food is well known as the 21<sup>st</sup> century food. Untapped resources, particularly those from the wildlife, should be studied to meet the demands for natural green and health foods. The *Elaeagnus angustifolia* tree, also known for several names, such as Guixiangliu, Qilixiang and so on, belongs to the Elaeagnaceae plant family. This plant largely grows in the dry areas of western China (Mingsheng *et al.*, 2011). As a natural wild plant, the fruit of *Elaeagnus angustifolia* L. is a purely natural food which is rich in carbohydrates, tannins, flavonoids and other essential substances and has many physiological functions, such as stopping diarrhea, promoting diuresis and decreasing blood lipid and blood glucose levels. *Elaeagnus angustifolia* L. has high food and medicinal value; however, most of its fruits fall to the ground upon maturity and fail to be collected for consumption, causing severe wastage of wildlife resources. This paper studies the antioxygenic and antitumor properties in vitro of acetic ether of *Elaeagnus angustifolia* L. in different fractions and provides a certain theoretical basis for the development and application of *Elaeagnus angustifolia* L.

## MATERIALS AND METHODS

**Materials.** *Elaeagnus angustifolia* L. was collected from the Tengge Desert in Minqin, Gansu. The materials were dried naturally in the air. After separated from the nuclear, the fleshes (the edible part) were crush to 0.38 mm before use.

**Reagent and instruments.** Methylthiazolyldiphenyl-tetrazolium bromide (MTT) and DPPH was purchased from Sigma. RPMI-1640 cell culture medium (GIBCO Company), calf serum without mycoplasma (GIBCO Company) and trypsin (Hyclone Company) were used in the assay. Elisa detector (EL×808), inverted microscope (I×71S8F-2) and carbon dioxide incubator (CB210) were purchased from BioTek, OLYMPUS and Binder, respectively.

**Tumour.** Human cervical carcinoma cell strain was obtained from the cell room in Lanzhou University.

### Methods:

**The preparation of different fractions of ethyl acetate extract:** *Elaeagnus angustifolia* L. powder → percolation extraction (85% ethanol) → filtrate was concentrated under reduced pressure → extract (petroleum ether, acetic ether) → petroleum ether, acetic ether, obtained after being concentrated → acetic ether extracted by silica gel chromatography

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(petroleum ether: acetone (25:1, 15:1, 10:1, 8:1, 5:1, 3:1, 2:1, 1:1) gradient elution) → TLC identification, combining the similarities → get A to F (polarity increased gradually) 6 fractions.

**The antioxidant assay:** The DPPH scavenging assay and reducing power assay were conducted to evaluate the antioxidant activity.

**DPPH scavenging assay:** The DPPH radical scavenging assay was measured by the modified method of Aruoma (1994) and Sánchez-Moreno *et al.* (1998). The DPPH was dissolved with 80% (v/v) ethanol. First, 2.0 mL of ethanol solution containing samples at different concentrations was mixed with 2.0 mL of DPPH solution. This was the sample group. The assay also should contain the blank control and sample control. The blank control contained all reagents without sample, while the sample control contained all reagents except DPPH. The mixture was then incubated for 30 min at room temperature. The mixture was scanned at full wavelength. And then, the wavelength was determined at 520 nm. Following that, the absorbance at 520 nm was measured and the inhibition (%) of DPPH radical formation was calculated with the following equation:

$$SR = \left(1 - \frac{A_i - A_j}{A_0}\right) \times 100 \%$$

In which,

$A_0$  = The absorbance at 520 nm of the control group contained all reagents without the sample

$A_j$  = The absorbance at 520 nm of the sample group contained all reagents without DPPH

$A_i$  = The absorbance at 520 nm of the sample group

**Reducing power:** The reducing power assay was referred to Oyaizu (1986) and Kosanić *et al.* (2011). Each extract (2.5 mL) with different concentration was mixed with 2.5 mL of 0.2 mol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1.0%). The mixture was incubated for 20 min at 50°C. Then, 2.5 mL of trichloroacetic acid (10%) was added. The mixture was centrifuged at 6000 r/min for 10 min. The distilled water (10 mL) and 0.5 mL of ferric chloride (0.1%) were added to the supernate (10 mL). After the mixture was incubated for 10 min, the absorbance was measured at 700 nm. The equation of linear regression was obtained within the certain concentration. The  $EC_{50}$  was calculated according to the equation of linear regression.

**MTT assay:** The effects of cell viability were measured by the MTT assay. The human cervical cancer Hela cells of logarithmic phase were digested by the trypsin (0.25%). The Hela cells were cultured at  $5 \times 10^3$  cells/mL with DMEM nutrient solution (contained 10% calf serum) in 96-well plates. After 4 h in culture, cells were treated with 100  $\mu$ L of sample

(1.5 mg/mL) for 48 h. The blank control was set and every group was set in 5 wells. After observed, 20  $\mu$ L of MTT solution (5 mg/mL) was added to each well. After incubation for 4 h, the medium was gently removed and 100  $\mu$ L of 5 mg/mL DMSO was added. After vibration for 10 min, the absorbance of each well was measured at 490 nm using a enzyme-linked immune detector.

## RESULTS AND DISCUSSION

**The antioxidant activity of different fractions of ethyl acetate extract:**

**The DPPH scavenging assay of different fractions of ethyl acetate extract:** Table 1 shows that the  $IC_{50}$  decreases gradually from fractions A to F for acetic ether of *Elaeagnus angustifolia* L. The  $IC_{50}$  value is smaller, but its ability to remove DPPH free radicals is stronger, with stronger antioxidation activity. Therefore, the antioxidation activity gradually increases from fractions A to F in acetic ether of *Elaeagnus angustifolia* L., wherein the relatively weakest part is in fraction A and the strongest is in fraction F.

Figure 1 shows that along the gradual increase in concentration from 0.4 mg/mL to 2 mg/mL, the ability of acetic ether of *Elaeagnus angustifolia* L. to clean out DPPH also gradually increases from fractions A to D and this ability is closely related to the concentration. For fraction F, the clearance is more than 90% under this concentration. The clearance of fraction E is more than 90% in 0.8 mg/mL to 2 mg/mL. The ability of fractions E and F to clean out DPPH is as high as up to 94.9%. This finding is probably related to the polarity and structure of active substances in acetic ether of *Elaeagnus angustifolia* L. If the polarity from fractions A to F is higher, the ability to clean out DPPH and the antioxidation activity would also be higher.

**The reducing power of different fractions of ethyl acetate extract.** Table 2 shows that  $EC_{50}$  gradually decreases from fractions A to F. If  $EC_{50}$  is smaller, the reducing capacity would be stronger with higher antioxidation activity. Therefore, the reducing capacity in fraction A of acetic ether of *Elaeagnus angustifolia* L. is relatively the weakest, whereas the strongest is in fraction F.

Figure 2 shows that with gradual increase in concentration from 0.8 mg/mL to 4 mg/mL, the absorbance values of all fractions are also increased. It indicates that all fractions in acetic ether of *Elaeagnus angustifolia* L. show a certain reducing capacity and this capacity is closely related to the concentration. From fractions A to F, the polarity of samples increases gradually and the reducing capacity improves gradually, as the antioxidation activity also gradually increases.

**The antitumor activity of different fractions of ethyl acetate extract.** Table 3 shows that the  $IC_{50}$  value of

Table 1: The compare of IC<sub>50</sub> of different fractions of ethyl acetate part

Fractions	Equation of linear regression	IC <sub>50</sub> /mg·mL <sup>-1</sup>
A	Y = 11.024X+14.782 R <sup>2</sup> = 0.9817	3.1947
B	Y = 19.516X+13.966 R <sup>2</sup> = 0.955	1.8464
C	Y = 19.106X+30.927 R <sup>2</sup> = 0.9703	0.9983
D	Y = 24.100X+48.809 R <sup>2</sup> = 0.9257	0.0494
E	Y = 13.333X+74.296 R <sup>2</sup> = 0.5217	-1.8223
F	Y = 2.2241X+91.308 R <sup>2</sup> = 0.6005	-18.5729

Table 2: The compare of EC<sub>50</sub> of different fractions of ethyl acetate part

Constituent	When cultured linear regression equation (h)	EC <sub>50</sub> /mg·mL <sup>-1</sup>
A	Y = 0.0985X-0.0833 R <sup>2</sup> = 0.9569	5.9218
B	Y = 0.1815X-0.0659 R <sup>2</sup> = 0.9905	3.1179
C	Y = 0.1397X+0.0868 R <sup>2</sup> = 0.9905	2.9578
D	Y = 0.2612X-0.1479 R <sup>2</sup> = 0.9956	2.4805
E	Y = 0.3032X-0.2158 R <sup>2</sup> = 0.9825	2.3608
F	Y = 0.4262X-0.3255 R <sup>2</sup> = 0.9887	1.9369

Table 3: The compare of IC<sub>50</sub> to Hela cell (mg/mL)

Incubation	A	B	C	D	E	F
24	4.06	3.42	2.91	2.66	1.99	1.82
48	3.06	2.45	2.22	2.01	1.62	1.40

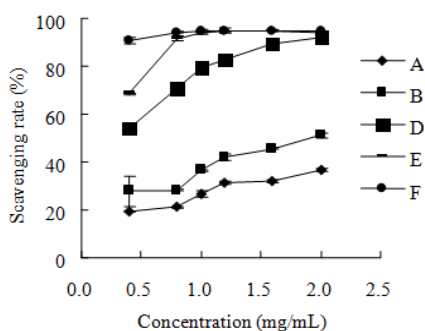


Fig. 1: The DPPH free radical scavenging of different fractions of ethyl acetate part

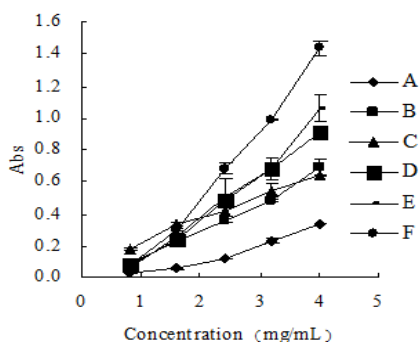


Fig. 2: The reducing power of different fractions of ethyl acetate part

the same fraction decreases with increased culture time within 24 h to 48 h. If IC<sub>50</sub> value is smaller, the antitumor activity of a sample is stronger; under the same culture time, IC<sub>50</sub> values from fractions A to F are gradually decreased. Table 3 also shows that the inhibition on the proliferation of human cervical carcinoma (Hela) cells increases gradually from fractions A to F. This finding probably indicated that the substance with the higher polarity in acetic ether of

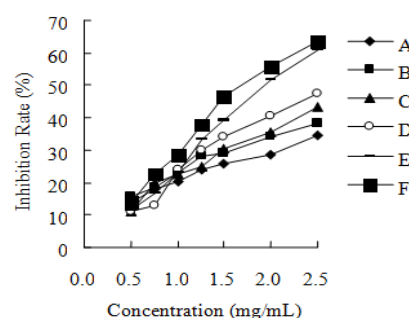


Fig. 3: The inhibition rate to Hela cell after 24h

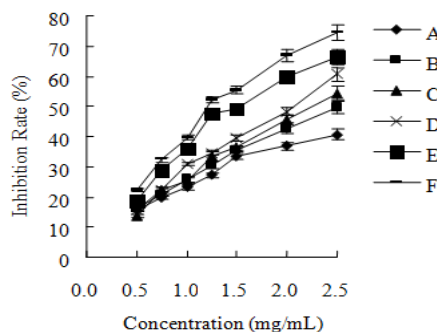


Fig. 4: The inhibition rate to Hela cell after 48h

*Elaeagnus angustifolia* L. exerts a stronger inhibition effect on the proliferation of Hela cells.

Figure 3 and 4 showed that different fractions in acetic ether of *Elaeagnus angustifolia* L. have certain degrees of inhibitory effects. The inhibitory effects depend on time, concentration and polarity. The inhibitory effects of all fractions on the proliferation of Hela cells increase with increased concentration and polarity levels. Among which, the inhibition rate of fraction F is the highest at 74.34% in the concentration of 2.5 mg/mL.

### CONCLUSION

With increased concentration and polarity levels of each sample, the abilities of different fractions in acetic

ether of *Elaeagnus angustifolia* L. to clear out DPPH also increase, along with the gradual increase in the reduction and antioxidation activities of the fractions. The abilities of fractions E and F to clear out DPPH is as high as up to 94.9%; different fractions have significant inhibitory effects on the proliferation of Hela cells in vitro. The inhibitory effects depend on the concentration and polarity of each sample. If concentration and polarity levels are higher, the inhibition effect would be better.

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