IN VITRO ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF *ECLIPTA PROSTRATA* L. AERIAL PART EXTRACTS

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The phytochemical exploration in medicinal plants is increasingly recognized for its diverse pharmacological benefits. This research aims to evaluate the antioxidant potential and anticancer properties of Eclipta prostrata L. (EPL) extracts on human breast adenocarcinoma (MCF7), hepatocellular carcinoma (HepG2), and human gastric carcinoma (MKN-7) cell lines. The dried plant material was extracted in 70% ethanol, n-hexane, ethyl acetate, and n-butanol solvents. The antioxidative properties of EPL extracts were assessed through ABTS and FRAP assays, while the antiproliferative effects were measured using the sulforhodamine B assay. Findings indicated that the ethyl acetate extract exhibited higher antioxidant and cytotoxic activities against the HepG2 cell line compared to other extracts. Future research should aim to isolate and characterize the specific bioactive compounds responsible for the observed anticancer and antioxidant effects, and clarify their mechanisms of action.

Keywords: *Eclipta prostrata*, cytotoxicity, SRB, antioxidant, HepG2.

I. INTRODUCTION

Human beings frequently encounter free radicals produced during normal metabolic activities within the body. While free radicals are not inherently detrimental, an excessive accumulation can disrupt normal cellular functions by causing oxidative damage to DNA, proteins, and lipids, leading to chromosomal instability and mutations.[1] Additionally, an overabundance of free radicals can impair the effectiveness of the body's biological defense mechanisms designed to neutralize these radicals, resulting in oxidative stress. This oxidative stress may contribute to the increased risk of developing various metabolic diseases, including cancer, cardiovascular conditions, diabetes, obesity, neurodegenerative disorders, and age-related ailments.[1,2]

The antioxidant mechanism plays a crucial role in delaying or preventing the formation of free radicals, thereby mitigating the risk of life-threatening diseases. Additionally, it can inhibit cancer progression by ensuring proper cell cycle regulation, reducing cell proliferation, and promoting apoptosis.[3,4] A variety of plants and their active compounds have been identified as possessing antioxidant properties that can manage numerous health conditions. Consequently, natural antioxidant compounds have been explored for their potential in various treatment approaches, including cancer therapy.[5] In this regard, we have chosen the traditional medicinal plant *Eclipta prostrata* Linn. (EPL) for our research. EPL is a member of the Asteraceae family and is found in tropical and subtropical regions across Asia, Africa, and South America, commonly referred to as "Co

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nho noi" or "Co muc" in Vietnamese and "False daisy" or "Ink plant" in English.

EPL is extensively utilized in traditional medicine to treat different ailments, including liver and kidney diseases, respiratory issues, skin conditions, fever, diabetes, hypertension, wounds, and to improve memory and cognitive abilities. Given its diverse applications, numerous studies have been undertaken to isolate and identify its biologically active components and assess its therapeutic potential. Research has shown that crude extracts and specific compounds derived from EPL demonstrate hopeful pharmacological activities, including anti-inflammatory, antioxidant, anticancer, antimicrobial, hair growth stimulation, hepatoprotective, anti-diabetic, and neuroprotective effects.[6]

Although some studies have been conducted, there remains insufficient understanding of the antioxidant and cytotoxic properties of various fractions of EPL extract. Consequently, this study aims to assess and compare the *in vitro* antioxidant and cytotoxic effects of the total ethanolic extract alongside its multiple fractions (n-hexane, ethyl acetate, and butanol) obtained from the aerial parts of EPL.

II. MATERIALS AND METHODS

1. Plant collection and extraction

E. prostrata was gathered in Hanoi, Vietnam, in November 2024. The specimen was verified and archived at the University of Medicine and Pharmacy, Vietnam National University, Hanoi. Subsequently, the collected plant underwent cleaning, washing, and air-drying processes. The dried plant material was then ground into a fine powder and stored at room temperature. A total of 1kg of this powdered medicinal substance was soaked in 70% ethanol at room temperature for seven days, after which it was filtered through a cotton plug and Whatman Grade 1 Qualitative Filter Papers. The extracts obtained were combined and subjected to distillation under reduced pressure to produce the total ethanol extract (EPTE). A portion of this extract (5g) was fractionated using a modified Kupchan partitioning method into n-hexane, ethyl acetate, and n-butanol fractions. The solvents were completely evaporated under reduced pressure, yielding the respective fractions of n-hexane (EPH, 0.55g), ethyl acetate (EPEt, 1.50g), and n-butanol (EPB, 1.50g).

2. Quantification of antioxidant activity

ABTS radical scavenging assay

The ABTS assay was conducted following the methodology established in a prior study.[9] The ABTS radical cation was generated by combining equal volumes of a 7mM ABTS stock solution and a 2.45mM potassium persulfate solution. To prepare the 7mM ABTS solution, 0.360g of ABTS salt was dissolved in 100mL of distilled water. Similarly, a 2.45mM potassium persulfate solution was created by dissolving 0.066g of the salt in 100mL of distilled water. Subsequently, the ABTS cation radical solution was formed by gently mixing 10mL of the 7mM ABTS solution with 10mL of the 2.45mM potassium persulfate solution. This mixture was then allowed to sit in the dark at room temperature for 12 hours to ensure the reaction was complete and the absorbance stabilized.

The radical cation generated was subsequently diluted in a 1:1 ratio with acetate buffer (pH = 4.5) to achieve an absorbance value of 0.74 ± 0.03 at 734nm, measured using a UV–Vis Spectrophotometer. The EPL extract was combined with the $ABTS^{+\bullet}$ solution and allowed to incubate in the dark for 7 minutes at room temperature. Absorbance readings were taken at 734 nm with a UV–Vis Spectrophotometer. The reactivity of different concentrations of each solvent extract was compared to ascorbic acid (ranging from 0.625 to 10.0 µg/mL). A control sample was prepared following the

same procedure without any extract.

The percentage of $ABTS^+$ radical scavenging was calculated for various concentrations of the extract (from 6.25 to 100 µg/mL) and standard using the specified equation:

$$ABTS\% \text{ scavenging} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

The IC_{50} ABTS values, which represent the sample concentration needed to inhibit 50% of ABTS radicals, were determined by extrapolating data from regression analysis. The assessment of antioxidant activity was conducted using this IC_{50} value.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was conducted following a modified protocol based on the method proposed by Benzie and Strain.[10] Initially, a TPTZ solution was prepared by mixing the following chemicals in equal proportions: 300mM acetate buffer, 10mM TPTZ in 40mM HCl, and 20mM $FeCl_3.6H_2O$. Vitamin C (3.125 to 50 µg/mL) was used as a positive control. The extracts were dissolved in DMSO and subsequently diluted with water to achieve a final concentration of 50 µg/mL. Once the extract reached this concentration, 1.5mL of the TPTZ solution was added and thoroughly mixed with 0.5 mL of the extract. The assay was performed in triplicate. The FRAP values were calculated using a standard curve based on vitamin C and expressed as mg vitamin C equivalent (AAE) per gram.

3. In vitro assay for cytotoxic activity

Cell line and culture

Human breast adenocarcinoma (MCF7), hepatocellular carcinoma (HepG2), and human gastric carcinoma (MKN-7) cell lines were obtained from Prof. Dr. Pezzuto JM at Long Island University in the United States and Prof. Jeanette M at the University of Milan in Italy. These cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich Co. LLC, St. Louis, MO, USA), which was supplemented with 10% Fetal Bovine Serum (FBS) (HiMedia Laboratories, Mumbai, India) and 1× Penicillin/Streptomycin. The cultures were maintained at 37°C in a CO_2 incubator (NU-5501E/G, NuAire Inc., Plymouth, MN, USA) under a humidified atmosphere containing 5% CO_2 and 95% air. Routine sub-culturing was performed in 25 cm² tissue culture flasks to maintain the cells.

Sulforhodamine B (SRB) assay

The SRB assay evaluated the extract's capacity to inhibit cell proliferation by quantifying the protein content within the cells. Cancer cells were plated at a density of 10,000 cells per well in a 96-well plate (Thermo Scientific) and allowed to adhere overnight at 37°C. Subsequently, the cells were treated with a series of dilutions of the plant extracts (100, 20, 4, 0.8 µg/mL). After incubation, the cells were fixed using a 20% trichloroacetic acid solution for one hour. Following a wash with water, the cellular protein was stained with SRB solution and incubated at room temperature for 30 minutes before being placed at 37°C. The plate was then washed three times with 1% acetic acid, and any unbound dye was removed by flicking. The plates were air-dried, and 100µL of Tris buffer (10mM; pH 10.5) was added to each well. The plates were gently shaken for 10 minutes on a mechanical shaker. Control wells, which contained cells without any test samples, served as the Day 0 control. The optical density (OD) of the wells was measured using a microplate reader at 540nm (BioTek Instruments Inc., Winooski, VT, USA).[7,8] Growth inhibition was determined using the equation provided below:

$$\%\text{Growth inhibition} = 100\% - \frac{OD\ (\text{test sample}) - OD\ (\text{Day 0})}{OD\ (\text{control}) - OD\ (\text{Day 0})}$$

The IC_{50} value, which indicates the concentration required to inhibit 50% of cell proliferation, was determined using TableCurve 2Dv4 software.

4. Statistical analysis

Numerical data collected from the three independent experiments were presented as mean ± standard deviation (SD). Statistical significance was assessed for each treated group in comparison to the control group using Student's t-test, with a p-value threshold of less than 0.05 considered significant.

III. RESULTS

1. Antioxidant activities

The total antioxidant capacity of the EPTE, EPEt, EPH, and EPB was assessed using the $ABTS^+$ radical and ferric-reducing power assay.

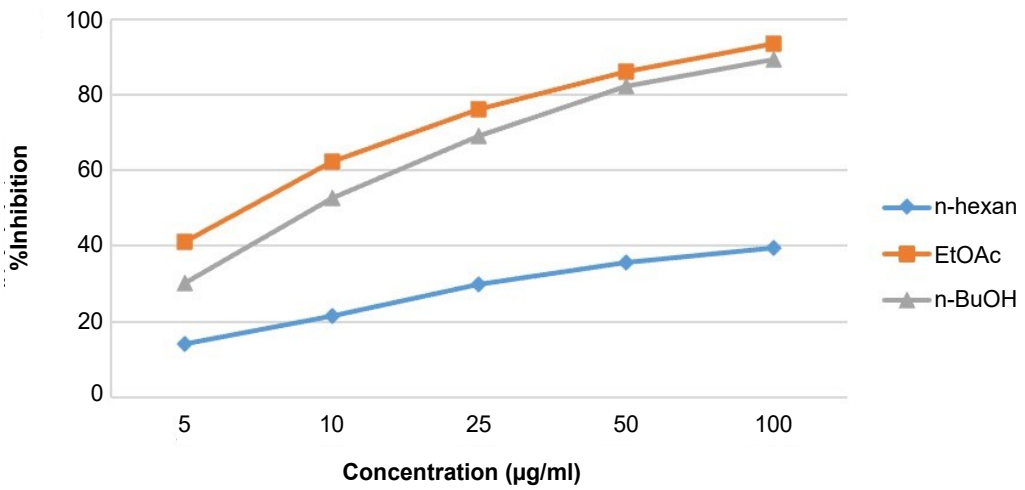

Chart 1. ABTS radical scavenging activity (% inhibition) of various extracts

In the ABTS assay, the $ABTS^+$ radical was generated by combining ABTS with potassium persulfate and allowing it to react overnight. The antioxidant compounds convert the blue $ABTS^+$ radical into ABTS, resulting in a reduction in blue color intensity. As illustrated in Chart 1, the relative IC50 values for all extracts were ranked as follows: EtOAc > n-BuOH > n-hexane. Notably, the n-hexane extract exhibited no antioxidant activity, with an IC50 value surpassing 100 µg/mL. The EtOAc extract demonstrated the highest antioxidant activity, with an IC50 of 7.97 ± 0.41 µg/mL, followed by the n-BuOH extract, which had an IC50 of 11.87 ± 1.12 µg/mL.

Based on the vitamin C standard curve ($y = 0.0236x + 0.0978$; $R^2 = 0.9976$), the FRAP value is expressed as the vitamin C mg (AAE)/g dry mass. Similar to data obtained in the ABTS assay, the EtOAc extract significantly reduced iron ions, with the highest FRAP value of 512.23mg AAE/g (Chart 2).

2. Cytotoxicity activity by SRB assay

The antiproliferative effects of total ethanol extract of *Eclipta prostrata* (EPTE) were assessed using the SRB assay across three cancer cell lines: HepG2, MCF-7, and MKN-7. As indicated in Table 1, EPTE demonstrated no cytotoxicity towards the MCF-7 and MKN-7 cell lines, while it showed toxicity to HepG2, with an IC50 value of 59.21 ± 4.17 µg/mL. The positive control displayed the most significant toxicity across all cancer cell lines, with an IC_{50} value of less than 0.4 µg/mL.

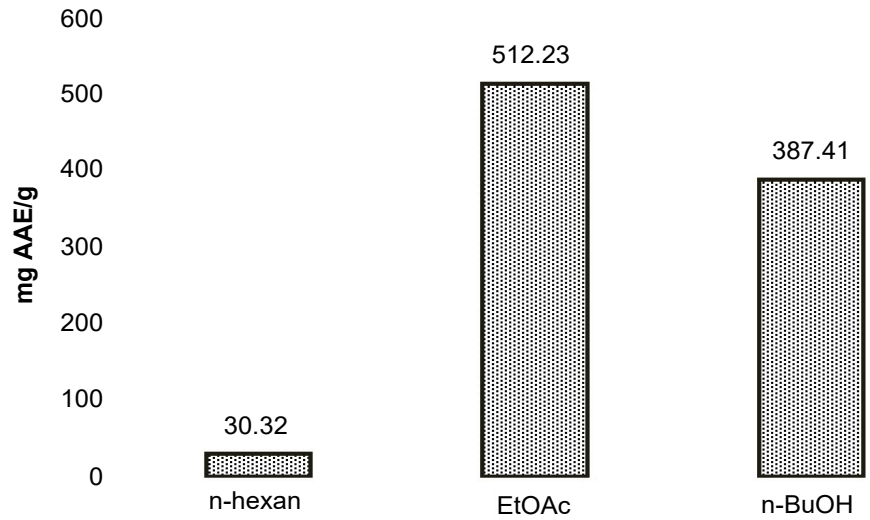

Chart 2. FRAP values of the various extracts

Table 1. *In vitro* cytotoxicity activity (IC50 µg/mL ± standard deviation) of total ethanol extract of *E. prostrata* against three types of human cancer cells with exposure time 72h.

	IC_{50} (mg/mL)		
	HepG2	**MCF-7**	**MKN-7**
EPTE	59.21 ± 4.17	> 100	> 100
Ellipticine	0.31± 0.01	0.39 ± 0.02	0.31 ± 0.01

Table 2. *In vitro* cytotoxicity activity (IC_{50} µg/mL ± standard deviation) of EP fractions against HepG2 cell line with exposure time 72 h

	n-Hexane (EPH)	**Ethyl acetate (EPEt)**	**Butanol (EPB)**
IC_{50} (mg/mL)	21.12 ± 0.87	29.45 ± 2.11	> 100

The EPTE demonstrated a reduction in cell proliferation within the HepG2 cell line. Consequently, three fractions derived from EPTE, including EPH, EPEt, and EPB, were subsequently evaluated for their cytotoxic effects on this cell line. As presented in Figure 1 and Table 2, the EPB was declared inactive when the IC50 value of the HepG2 cell line was more than 100 µg/mL. The IC_{50} values for EPH and EPEt were determined to be 21.12 ± 0.87 and 29.45 ± 2.11 µg/mL, respectively, demonstrating that the n-hexane fraction of the EP extract exhibits greater cytotoxic potency.

IV. DISCUSSION

Throughout history, plants have been acknowledged for their therapeutic properties in addressing numerous health issues, including cancer, primarily attributed to their phytochemical content. The anticancer capabilities of plant-based substances have garnered considerable interest among researchers globally. Comprehensive literature reviews have underscored the anticancer efficacy of phytochemical-rich plants, demonstrating their cytotoxic effects on a range of cancer cell lines.[12] In addition to their

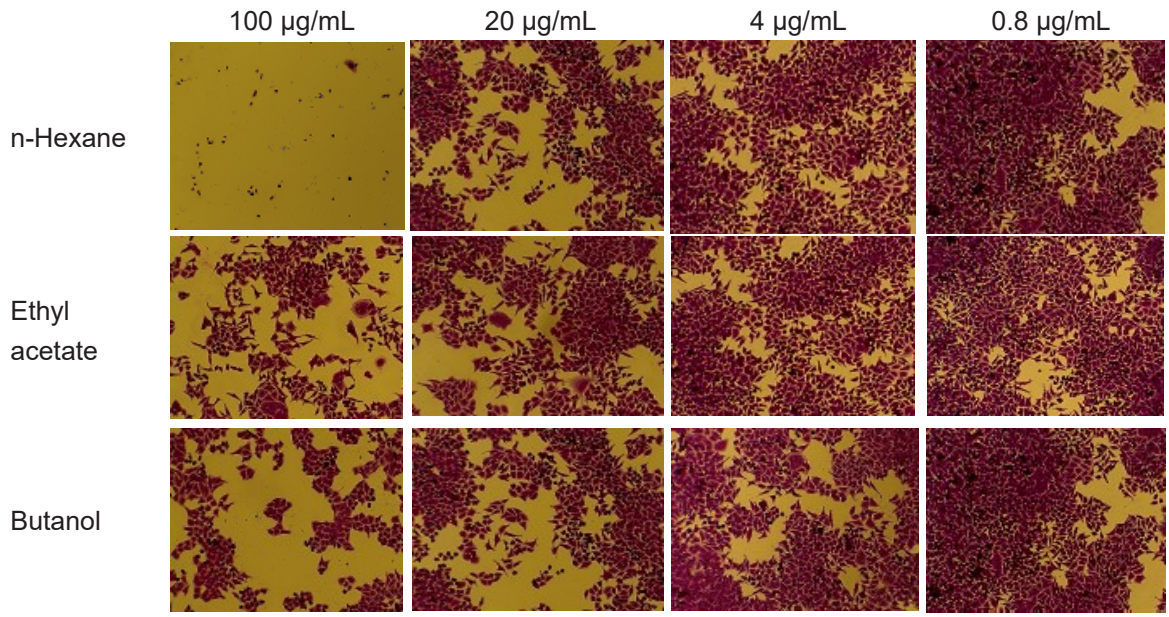

Figure 1. Changes in HepG2 cell density followed by 72-hour incubation with tested fractions at different concentrations

anticancer properties, these medicinal plants have been found to influence signaling pathways related to cell cycle regulation and apoptosis, exhibiting strong anti-inflammatory, antioxidant, and chemotherapeutic effects. Notably, these phytochemicals tend to exert greater cytotoxic effects on cancer cells compared to normal cells, a phenomenon that can be attributed to their pro-oxidant or antioxidant roles, which vary based on dosage and environmental context.[13]

This study assessed and contrasted the antioxidant and cytotoxic properties of the ethanolic extract and its three fractions derived from the entire EPL plant. The EtOA extract demonstrated simultaneously higher antioxidant and cytotoxic activities when compared to the other fractions.

To evaluate the antioxidant potential of EPL extracts, both ABTS and FRAP assays were conducted. These assays are commonly employed as in vitro techniques to measure the antioxidant activity of plant extracts and their components. Antioxidant activity reflects the capacity to neutralize reactive oxygen species (ROS), thus reducing oxidative stress. It is widely recognized that medicinal plants serve as significant sources of natural antioxidants.

In the $ABTS^+$ decolorization method, the presence of antioxidants facilitated the reduction of the blue chromophores $ABTS^+$ to a colorless form. The $ABTS^+$ decolorization method serves as a reliable approach for evaluating the antioxidant activity of plant extracts, applicable to both lipophilic and hydrophilic antioxidants. As illustrated in Chart 1, the scavenging activity percentage of ABTS free radical molecules rises as the concentration of the extracts increases. Notably, the ethyl acetate extract exhibited the lowest IC_{50} value at 7.97 ± 0.41 µg/mL (Chart 1). The ABTS free radical scavenging activity of the ethyl acetate extract from the leaves and stems of *E. prostrata* was previously documented, showing an IC_{50} value of 72.41 µg/mL, which was found to be higher in our current study.[14] This discrepancy may be attributed to the varying geographical locations from which the

herbs were collected, potentially influencing the concentration of active compounds. Additionally, protocatechuic acid extracted from *E. prostrata* was identified to possess significant antioxidant capabilities, exhibiting an IC50 value of 16.23 ± 2.10 µg/mL.[14]

The antioxidant potential of *E. prostrata* extract was further assessed using the ferric reducing antioxidant power (FRAP) method. This method relies on the reduction of a ferroin analogue, specifically the Fe^{3+} complex of tripyridyltriazine $Fe(TPTZ)^{3+}$, to a vivid blue Fe^{2+} complex ($Fe(TPTZ)^{2+}$) in an acidic environment, facilitated by antioxidants. The results indicated a direct correlation between the antioxidant activity and the reducing capacity of the extracts. In this analysis, the ethyl acetate fraction continued to exhibit the greatest antioxidant activity, as evidenced by the maximum concentration of mg vitamin C equivalents (AAE) per gram (Chart 2). The current research indicates that the aerial portion of *E. prostrata* serves as a valuable source of antioxidant compounds, demonstrating effective scavenging activity in both ABTS and FRAP assays.

The cytotoxic activity results of the ethanolic crude extract of EPL against three human cancer cell lines using SRB assay are presented in Table 1 as IC50 values in µg/ml. Crude extracts with IC_{50} values exceeding 100 µg/ml were deemed ineffective in inhibiting cancer cell proliferation.[15] The extract demonstrated the strongest antiproliferative effect on HepG2, with an IC_{50} value of 59.21 ± 4.17 µg/mL, while showing no cytotoxic effects on MCF-7 and MKN-7 cell lines. Consequently, we concentrated on assessing the cytotoxic activity of the fractions derived from the ethanol crude extract of EPL on the hepatocellular carcinoma cell line. The cytotoxicity levels of the fractions were ranked as follows: EPH (IC_{50} = 21.12 ± 0.87 µg/mL) > EPEt (IC_{50} = 29.45 ± 2.11 µg/mL) > EPB (IC_{50} > 100 µg/mL) (Table 2). According to the National Cancer Institute (NCI) criteria, a plant crude extract is classified as toxic if the IC_{50} value is ≤ 30 µg/ml after 72 hours of treatment.[16] Notably, the n-hexane and ethyl acetate extracts exhibited promising anticancer activity, with IC_{50} values below 30 µg/ml, indicating their cytotoxic effects on the HepG2 cancer cell line.

The cytotoxic effects of various extracts from *E. prostrata* L. have been documented. Consistent with the findings of this study, the hydroalcoholic extract of *E. prostrata* demonstrated significant inhibitory effects on HepG2 cell proliferation, with an IC_{50} value of 22 ± 2.9 µg/mL.[17] Furthermore, this extract also showed considerable cytotoxicity against A498 and C6 glioma cell lines.[17] The methanol extract of *E. prostrata* proved effective against Ehrlich ascites carcinoma (EAC) cells in murine models.[18] Aryea et al. (2015) reported that the chloroform fraction of *Eclipta alba* inhibited breast cancer cells.[19] Additionally, constituents derived from the aerial parts of *E. prostrata* have been shown to be cytotoxic against human ovarian cancer cell lines, specifically SKOV3.[20] These findings lend support to the traditional application of this plant in cancer treatment. The compound wedelolactone, derived from *Eclipta prostrata*, has demonstrated significant anti-cancer properties and has become a focal point for numerous research teams globally. Additional investigations are required to assess the concentration of this crucial active component in *Eclipta prostrata* extracts that exhibited anti-proliferative effects in our research.

V. CONCLUSION

The *in vitro* antioxidant and cytotoxic

properties of *E. prostrata* extracts, particularly the ethyl acetate extract, demonstrated against the HepG2 cell line suggest a promising therapeutic avenue for developing novel alternative treatments for liver cancer. Identifying compounds that exhibit a range of beneficial biological activities would be highly advantageous. Future studies should focus on isolating and characterizing the specific bioactive compounds that contribute to the observed anticancer and antioxidant effects, as well as elucidating their mechanisms of action.

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