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CYTOTOXIC EFFECT OF CHLOROFORM AND HEXANE EXTRACTS OF *ARNEBIA DENSIFLORA* ON NEUROBLASTOMACELL LINE

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ABSTRACT

Objective: We investigated to prepare chloroform and hexane extracts from root parts of *Arnebia densiflora* (*Boraginaceae*) plant, to determine cytotoxicity activity on neuroblastoma SH-SY5Y cell lines. **Materials and Methods:** *Arnebia densiflora* used in this study were collected from Kangal/Sivas, Turkey at a height of 1540 meters at the stage of full flowering and was identified by Prof. Dr. Akpulat HA, Cumhuriyet University Sivas, Turkey. Dried and powdered roots (5g) were extracted 50mL of chloroform and hexane separately, by maceration method for 24 h in a shaker at room temperature. The prepared extracts were applied to SH-SY5Yneuroblastoma cell line and the cytotoxic effect of these extracts were determined by using MTT method. The spectrophotometric readings at 570 nmwere recorded andanalysed with Graphpad Prism7. **Results:** In this study, the effects of chloroform and hexane extracts from roots of *Arnebia densiflora* on the SH-SY5Y cells were compared with the control group and IC_{50} values were determined for 24, 48 and 72 hours. **Conclusion:** In this study, it was shown that the effect of chloroform and hexane extracts on SH-SY5Y cells was inhibitory to growth in cancer cells when compared with control group.

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INTRODUCTION

Cancer is one of the leading diseases in both developed and developing countries. Chemotherapy is a widely used treatment for cancer and one of the biggest disadvantages is the toxicity on normal cells because chemical drugs cannot distinguish between normal and cancerous cells (Balamurugan *et al.,* 2014). Traditional drugs have long been investigated to obtain a more effective drug against cancer. Compounds isolated from plants are worth examining widely because of their content (Chun-Fai *et al.,* 2013). Investigation of plant extracts is of great interest to scientists in the discovery of new drugs that are effective in the treatment of various diseases (Cragg *et al.,* 1997). The world flora has one of the most comprehensive flora of the world with more than 1900 plant species (Davis, 1988).

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The herbal extract in Turkey antibacterial, anticancer, anti-inflammatory literature has been involved in activities related to the prevention and wound healing, but the majority has not yet been researched at all (Sokmen *et al.,* 1999; Dulger *et al.,* 2006). *Arnebiadensiflora* is a perennial species that is endemic to Turkey (Kneifel *et al.,* 1992). *Arnebia* is a member of the Boraginaceae family (Ghasemi-Pirbalouti, 2009). The root part of these species contains more shikonin / alkannin derivatives than the other members of the Boraginaceae family (Leifert*et al.,* 1992). It is well known that bioactive natural products (plant secondary metabolites) have therapeutic value for the prevention and treatment of various types and stages of cancer. Alkanines and shichonins are potent pharmaceuticals that exhibit important biological activities such as wound healing, antimicrobial, anti-inflammatory, antioxidant, anticancer and antithrombotic properties (Papageorgiou *et al.,* 1999; Pietrosiuk *et al.,* 2004; Papageorgiou *et al.,* 2006). In this study, we aimed to to prepare chloroform and hexane extracts from the root of the plant of Arnebia Densiflora

(Boraginaceae) and to determine their cytotoxicity in the SH-SY5Y cell line.

MATERIALS AND METHODS

Collection and identification of plant material: To *Arnebi Densiflora* (Boraginaceae) plant at a height of 1540 m Kangal (Sivas, Turkey) were collected at the stage of full flowering of the district. Akpulat HA, Cumhuriyet University in Sivas, was detected by Turkey. The plant that we used was photographed and was also confirmed as *Arnebi Densiflora* (Figure 1) by the patient herself.

Figure 1. Photos of *Arnebi Densiflora* post collection

Preparation of plant extracts: Dried and powdered root (5g) on 50 mL chloroform and hexane were added to the dried and powdered and allowed to separate at room temperature with shaker. After 24 hours, the extracts were filtered using What man filter paper (No.1) and then condensed at 40 °C under reduced pressure using a vacuum evaporator. These procedures were repeated twice. The resulting solid extracts were stored in a freezer at -20 °C until use.

Figure 2. Chloroform and hexane extracts of *Arnebi Densiflora*

Cell Culture: SH-SY5Y cells were maintained in DMEM medium, containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L). Cells were grown in at 37 °C, 5% CO_2 and 95 % air in a humidified incubator. For each cell line, 70-80% confluent cell culture flask was trypsinized and cells were seeded in 96 well plates.

Cytotoxic effect of Arnebi Densiflora chloroform and hexaneextracts on SH-SY5Ycells: The in vitro cytotoxicity of *Arnebi Densiflora*chloroform and hexane extracts against SH-SY5Ycell lines was performed with the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to the Skehan's method (Skehan *et al.,* 1990). 1 µL of test substance at concentrations ranging between 10-1000µg/mL was added into each well containing the cells. After mixing with a mechanical plate mixer for 15min, the absorbance of plates was recorded at 570 nm on a microplate reader (Bio-Tek, USA). All drug doses were parallel tested in triplicate and were performed at least 3 times; control samples were run with 1% sterilized water.

RESULTS AND DISCUSSION

Cytotoxic effect of Arnebi Densiflora chloroform extract on SH-SY5Y cells: Figure 1 shows changes in cell inhibition for 24, 48 and 72 hours versus increasing concentrations of SH-SY5Y cell lines. Compared to the control group, chloroform extract treatedSH-SY5Yneuroblastoma cells showed significantly decreased tumor survival rate after 24h, 48h and 72h of incubation. Cell survival rates in all groups after 24h, 48h and 72 h of incubation were significantly decreased than those in the control group. With elongated treatment time, the survival rate of tumor cells was significantly reduced. Chloroform extract on SH-SY5Y cells was the most active for 72 h of incubation. In addition, the most active chloroform extract and IC_{50} values for 24, 48 and 72 hours were 45,40 µg/ml, 35,37 µg/ml and 10,79 µg/ml respectively (Table 1).

Table 1. Comparison of IC_{50} values between chloroform extract of *Arnebiadensiflora*on SH-SY5Y after 24 h, 48 h and 72 h of incubation

Time	Chloroform extract IC_{50} (µg/mL)
24h	45,40
48h	35,37
72h	10,79

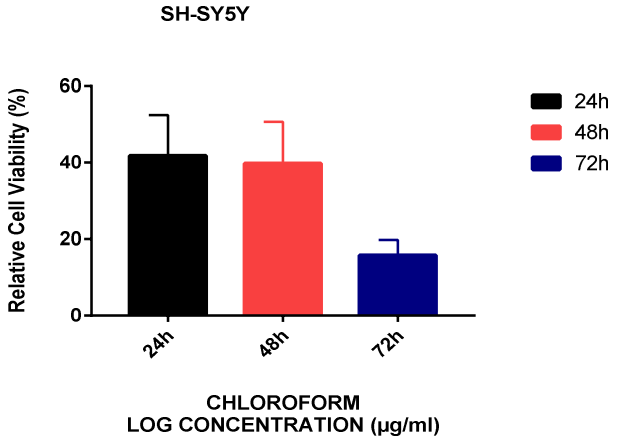

Fig.1. Cytotoxity activities of chloroform extract of *Arnebiadensiflora*on SH-SY5Y cell line

Cytotoxic effect of Arnebi Densiflora hexane extract on SH-SY5Y cells: Hexaneextract of *Arnebia densiflora* treated human SH-SY5Y neuroblastoma cells showed significantly decreased tumor survival rate after 24h, 48h and 72h of incubation. Effect of hexane extract SH-SY5Ycells were exposed to a range of concentrations of this extract rate was examined by MTT (Figure 2). Compared to the control group, hexane extract of Arnebia densiflora treated human SH-SY5Ycells showed significantly decreased tumor survival rate after 24h, 48h and 72h of incubation. Withelongatedtreatment time, thesurvival rate of tumor cells was significantly reduced. Hexane extract of *Arnebiadensiflora* on SH-SY5Y cells were the most active for 72 h of incubation. In addition, IC_{50} values of hexane extract for 24, 48 and 72 hours were 116,74 µg/ml, 88,54 µg/ml and 77,61 µg/ml respectively (Table 2).

Table 2. Comparison of I_{C50} values between hexane extract of *Arnebia densiflora*on SH-SY5Y after 24 h, 48 h and 72 h of incubation

Time	Hexane extract IC_{50} (µg/mL)
24h	116,74
48h	88,54
72h	77,61

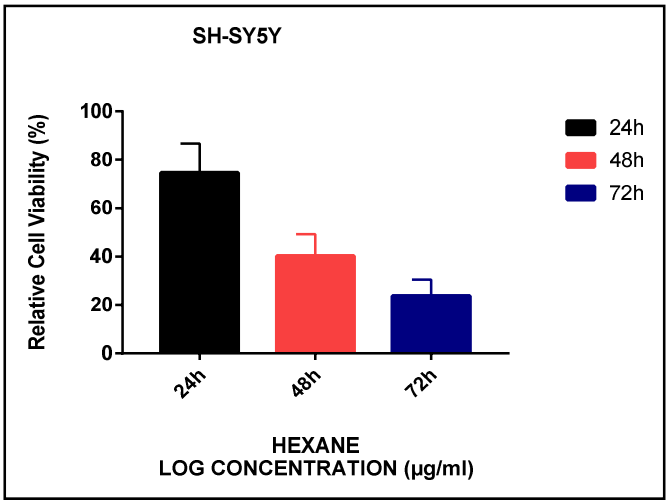

Fig. 2. Cytotoxity activities of of hexane extract of *Arnebiadensiflora* on SH-SY5Y cell line

Conventional plant-derived compounds are of great clinical importance in the development of drugs effective against cancer.Natural bioactive compounds have attracted the attention of researchers to overcome the problems associated with chemotherapy (Chun-Fai *et al.,* 2013). Cell culture studies are conducted to determine the efficacy of raw plant extracts as a potential alternative drug and also to control their effectiveness in clinical practice (Balamurugan *et al.,* 2014). In our study, the anticancer activity of the chloroform extract was found to be higher after incubation for 72 hours on SH-SY5Y cells compared to the hexane extract. According to the data obtained from this study, separation of chloroform extract into fractions will be determined by determining the most active fraction. The active substance will then be purified from the active fraction.

Conclusion

In this study, we determined cytotoxic activity of chloroform and hexane extracts of *Arnebiadensiflora*on SH-SY5Y. Chloroform and hexane extracts of Arnebi Densifloraon SH-SY5Y cells was the most active for 72 h of incubation. When the chloroform and hexane extracts of *Arnebi Densiflora* were compared on SH-SY5Y cells, chloroform extract was more active after 72 hours of incubation. In order to the chloroform and hexane extracts of *Arnebiadensiflora* to be a future cancer drug candidate, there is a need for purification of the active substance, in vitro and in vivo studies with this active substance.

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Conflcit of interest: Theauthorsdeclarenoconflict of interest.

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