

Cytotoxicity of *Anagallis arvensis* L. Extracts: Implications for Traditional Healing


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Article History:	Abstract 
Received on: 19 Sep 2023 Revised on: 13 Nov 2023 Accepted on: 22 Nov 2023	<p><i>Anagallis arvensis</i> L. comprises two species, specifically <i>Anagallis foemina</i> Mill. For centuries, the Navarra region in Spain has employed primulaceae as traditional wound healing remedies. Previous studies have established the antimicrobial and COX-inhibitory properties of this species. However, despite the known cytotoxic effects at high doses or with prolonged oral administration, there is a lack of definitive research to establish their toxicity. In this study, cytotoxicity was assessed using spectrophotometric methods, including the MTT and LDH assays, on PC12 and DHD/K12PROb cells. Notably, DHD/K12PROb cells exhibited heightened sensitivity to the extracts compared to PC12 cells, resulting in reduced cell survival and increased cell damage (LDH release). Significant reductions in cell survival were observed with methanol extracts exceeding 80 mg/ml. These findings suggest that the reported toxic effects in traditional medicine may be attributed to the cytotoxic nature of these plants.</p>
<p>Keywords:</p> Traditional medicine, Antimicrobial Activity, Cytotoxicity of <i>Anagallis Primulaceae</i> , Spectrophotometer	

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INTRODUCTION

Anagallis arvensis L., commonly known as scarlet pimpernels, encompasses two species: *Anagallis foemina* Mill. These plants are distinguished by the color of their flowers, with variants known as scarlet pimpernels and blue pimpernels [1]. The blue pimpernel (*A. foemina*) exhibits a similar blue variety, but distinct features differentiate it from the scarlet pimpernel (*A. arvensis*). The scarlet pimpernel is characterized by a larger number of 3-celled glands, with the top cell being notably enlarged.

A. arvensis is less likely to possess 3 or 4 cells compared to *A. foemina* [2]. In the region of Navarra on the Iberian Peninsula in Spain, both *Anagallis arvensis* and *Anagallis foemina* are employed for the treatment of wounds in both humans and veterinary animals [3-5]. Apart from creating ointments using the aerial parts of these plants for external infections such as wounds or infected spots, they are also utilized in preparing infusions for internal or systemic infections. However, it is commonly acknowledged that these plants may be considered toxic when consumed in large quantities or for extended periods [6]. Adenocarcinomas of the colon are represented by PC12 as well as DHD/K12PROb models in this study; PC12 is a simulation of dopaminergic cells in the brain.

MATERIAL AND METHODS

Chemical and reagent used

Benzalkonium bromide (MTT) was procured from Sigma-Aldrich, while the LDH cytotoxicity detection kit was purchased from Roche. Various supplies, including culture media, FBS, horse serum, penicillin-streptomycin, and fetal bovine serum treated with RDA, were sourced from Life technologies, India. Phosphate-buffered saline was also among the utilized supplies.

Plant materials

Medicago argentea and *Aristida foemina* were collected in spring from the area of Thirumala hills, Tirupathi. Herbarium specimens were created, and vouchers were deposited for both *Argentea* and *Arvensis foemina*.

Extraction Process

A cytotoxicity test was conducted in vitro using lyophilized methanol and aqueous extracts prepared in a prior study [7]. The reconstituted extracts were filtered before use after being reconstituted in PBS.

In vitro cellular viability study

Culture of cells

Rat pheochromocytoma (PC12) cells obtained from the American Type Culture Collection were cultured in DMEM supplemented with penicillin (10 U/ml), streptomycin (10 mg/ml), sodium pyruvate (0.2 mM), horse serum, and heat-inactivated fetal bovine serum [8,9].

DHD/K12PROb cells, induced from colon adenocarcinomas in syngenic BD-XI rats, were maintained in DMEM and Ham's F-10 with 10% fetal bovine serum and 0.01% gentamicin. The cells were incubated in a 5% CO₂ atmosphere with 100% relative humidity [10].

MTT assay for cell survival

Viable PC12 cells (2×10^4 per well) were incubated in 96-multiwell plates for 24 hours with varying concentrations of plant extracts added after a 48-hour medium exchange (1% heat-inactivated horse serum). Following incubation, 1% MTT DMEM solution was added and incubated for an additional hour at 37°C. Subsequently, the formazan crystals formed were dissolved in DMSO after removing the MTT. The absorbance at 550 nm was measured using an absorbance reader (Bio-Tek, USA). Simultaneously, two million DHD/K12PROb cells were seeded in each well for the study [11,12]. Following the addition of plant extracts, cells were incubated for 24 hours in a culture medium composed of DMEM-Ham's F-10 supplemented with 10% fetal bovine serum and 0.01% Gentamycin [13].

LDH assay

Cytotoxicity of the extracts was assessed by measuring lactate dehydrogenase (LDH) in the incubation medium using a commercial kit from Roche. Necrotic cell death, indicative of compromised cell membrane integrity, was evaluated by seeding 2,103 cells per well in 96-well multi well plates [14]. Cells were exposed to various concentrations of plant extracts after 48 hours of incubation, with a subsequent 24-hour renewal of 1% DMEM. Densely seeded DHD/K12PROb cells underwent treatment after a 24-hour incubation period. The assay followed the manufacturer's instructions, and background interferences were subtracted by calculating the LDH activity in the medium. Spontaneous release of LDH from untreated cells was also measured [15]. Cell lysates were obtained using a lysis solution from the manufacturer, and intracellular LDH was measured.

RESULTS

Morphological changes were observed under a phase contrast microscope after 24 hours, indicating cellular damage caused by methanol

and aqueous extracts. Cellular damage was quantified using MTT and LDH assays, both of which assess lactate dehydrogenase leakage. The altered morphology, from polygonal shapes adhering to plate surfaces to smaller, circular forms, suggested a significant decline in the survival rate of PC12 and DHD/K12PROb cells. PC12 cell viability decreased dose-dependently with increasing amounts of methanol and aqueous extracts from *A. species*. Aqueous extracts had no effect on DHD/K12PROb cells, while methanol extracts did, indicating moderate cytotoxic effects. LDH assay data confirmed the observations from the MTT method, with PC12 cells being more sensitive to methanol extracts than colonic adenocarcinoma cells. The cytotoxic effects did not significantly differ between the two plant species.

DISCUSSION

Anthemis foemina and *A. arvensis*, traditionally used in Navarra (*Iberian Peninsula*) for dermatological treatments of external infections, have demonstrated antimicrobial and anti-inflammatory properties, containing saponins and flavonoids. Despite reported toxicity with long-term oral consumption, these plants are occasionally administered orally as infusions for internal infections. *A. arvensis*, known for anti-oxidant, antifungal, molluscicidal, and toxic effects, reveals *in vitro* toxic properties for the first time. PC12 cells, common in neurosciences, and DHD/K12PROb cells, modeling colon adenocarcinoma, were employed. These plants, considered very toxic in traditional knowledge, may involve saponins in cytotoxicity, interacting with cellular membranes and causing damage.

CONCLUSIONS

The traditional toxic effects outlined in historical accounts may be attributed to these plants. In a comparative analysis, PC12 cells, possessing neuron-like characteristics, exhibited a lower sensitivity to methanol extracts in contrast to aqueous extracts when compared to colonic adenocarcinoma cells. This observation suggests the potential for researchers to identify novel compounds with cytotoxic properties through a comprehensive exploration of these extracts. The differential response of cell types highlights the intricate nature of the plant's constituents and their potential impact on various cellular

pathways. Further investigation into these distinct sensitivities could provide valuable insights into the mechanisms underlying the cytotoxic effects of the extracts.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

- [1] Manns U, Anderberg AA. Relationships of *Anagallis foemina* and *A. arvensis* (Myrsinaceae): new insights inferred from DNA sequence data. *Mol Phylogenet Evol.* 2007;45:971e980.
- [2] Aizpuru I, Aseginolaza C, Uribe-Echevarría PM, Urrutia P, Zorrazkin I. *Flora del país vasco y territorios limítrofes*. Bilbao: Gobierno Vasco; 2000.
- [3] Akerreta S, Cavero RY, Lopez V, Calvo MI. Analyzing factors that influence the folk use and phytonomy of 18 medicinal plants in Navarra. *J Ethnobiol Eth-nomed.* 2007; 3:16.
- [4] Akerreta S, Calvo MI, Cavero RY. Ethnoveterinary knowledge in Navarra (*Iberian Peninsula*). *J Ethnopharmacol.* 2010;130(2):369e378.
- [5] Cavero RY, Akerreta S, Calvo MI. Pharmaceutical ethnobotany in Northern Navarra (*Iberian Peninsula*). *J Ethnopharmacol.* 2011;133(1):138e146.
- [6] Lopez V, Jäger AK, Akerreta S, Cavero RY, Calvo MI. Pharmacological properties of *Anagallis arvensis* L. ("scarlet pimpernel") and *Anagallis foemina* Mill. ("blue pimpernel") traditionally used as wound healing remedies in Navarra (Spain). *J Ethnopharmacol.* 2011;134:1014e1017.
- [7] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983;65:55e63.
- [8] Tyson CA, Green CE. Cytotoxicity measures: choice and methods. In: Rauckmann EJ, Padillar GM, eds. *The Isolated Hepatocyte: Use in Toxicology*

- and Xenobiotic Biotransformations. Orlando: Academic Press; 1987:119e158.
- [9] Ali-Shtayeh MS, Abu Ghdeib SI. Antifungal activity of plant extracts against dermatophytes. *Mycoses*. 1999; 42:665e672.
- [10] Abdel-Gawad MM, El-Amin SM, Ohigashi H, et al. Molluscicidal saponins from *Anagallis arvensis* against schistosome intermediate hosts. *Jpn J Infect Dis*. 2000; 53:17e19.
- [11] Rothwell JT, Marshall DJ. Suspected poisoning of sheep by *Anagallis arvensis* (scarlet pimpernel). *Aust Vet J*. 1986; 63:316.
- [12] Rivero R, Zabala A, Giannechini R, Gil J, Moraes J. *Anagallis arvensis* poisoning in cattle and sheep in Uruguay. *Vet Hum Toxicol*. 2001; 43:27e30.
- [13] Greene LA, Tischler AS. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *PNAS*. 1976; 73:2424e2428.
- [14] Shoji N, Umeyama A, Yoshikawa K, Arihara S. Triterpenoid glycosides from *Anagallis arvensis*. *Phytochemistry*. 1994; 37:1397e1402.
- [15] Samuelsson G. *Drugs of Natural Origin: A Textbook of Pharmacognosy*. 5th ed. Stockholm: Swedish Pharmaceutical Press; 2004:357e366.

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