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Original Article

EVALUATION OF THE EFFECTS OF *HYPERICUM SCABRUM* L. PLANT FROM ANATOLIA ON SURVIVAL AND FERTILITY PARAMETERS OF *CAENORHABDITIS ELEGANS*

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ABSTRACT

The drugs produced from plant extracts continue to be widely used around the world, although studies have been conducted on the use of certain synthetic and semi-synthetic compounds as medicines. Additionally, it is well known that various parts of plants are commonly used in ethnopharmacology by the public for therapeutic purposes after applying simple processing. Considering the existing plant diversity in the world, scientific data on the biological effects and mechanisms of action of most plant-derived extracts are still not enough; however, interest in this subject is growing day by day. The treatment of diseases using teas, drops, dragees, capsules, syrups, and tablets produced from fresh and dried plant parts (drogs) or their extracted products with therapeutic value is referred to as "phytotherapy". In this study, it was aimed to evaluation of the effects of *Hypericum scabrum* L. plant samples collected in the summer season from Sivas province in Türkiye, by extracting them through different methods and testing them in *Caenorhabditis elegans* culture. Through this study, it has been possible to obtain fundamental data on the in vitro biological activity and efficacy of the plant on *C. elegans* cultures, which can serve as a basis for the use of the plant in medical applications, as well as in the food, pharmaceutical, and cosmetic industries. The plant stems were dried in a way that avoided direct sunlight, and extractions were carried out using ethanol and water extraction methods. The effects of the obtained extracts on the egg yield and survival parameters of the *Caenorhabditis elegans* nematode were examined. The data were analyzed using the SPSS program. It was found that when different doses of plant extracts were used in *C. elegans* cultures, there were significant differences in the growth, development, survival and fertility parameters of the nematodes compared to the control group.

KEYWORDS: Biological activity, Fertility, *Caenorhabditis elegans*, *Hypericum scabrum*, Model organism.

1. Introduction

The rich phytochemical content has facilitated plants' use from ancient times, and many modern medicines are derived from medicinal plants [1]. Many plant species have been traditionally used for centuries, not only as spices to enhance the flavor and aroma of foods, but also in folk medicine in various forms such as teas, ointments, tinctures, and extracts. These preparations have served a wide range of purposes, including pain relief, easing respiratory conditions, soothing digestive issues, and promoting relaxation [2]. *Hypericum scabrum* L. is a perennial herbaceous plant, 30-80 cm high, with yellow flowers. Leaves are elliptic, oblong or linear. It is mostly found in places with arid climates in Anatolia and in Europe, Cyprus, Iran, Northern parts of Africa [3, 4]. *H.scabrum* is a pharmacologically and biologically rich plant used in traditional medicine for the treatment of many diseases. It is seen that *Hypericum* species are used for therapeutic purposes by soaking in olive oil and obtaining oil [5]. Recent studies have revealed several biological activities and potential therapeutic effects of this plant. *H. scabrum* is rich in flavonoids, phenolic compounds and essential oils. These compounds

contribute to the biological activities of the plant. The extracts obtained from the flower and leaf parts of the plant were found to have high antioxidant capacity. This indicates the potential of the plant to scavenge free radicals and reduce oxidative stress. [6]. The aqueous extract of *H. scabrum* showed antidepressant and antihypoxic effects come through nitric oxide pathways [7]. The essential oils of this plant showed cytotoxic effect on colon cancer cell line (HT-29). In addition, its potential to inhibit acetylcholinesterase and butyrylcholinesterase enzymes suggests that it can be used in the treatment of neurodegenerative diseases such as Alzheimer's disease [8]. Essential oils of *H. scabrum* accelerated wound healing and reduced oxidative stress markers in diabetic rats [9]. Various extracts of *H. scabrum* showed antimicrobial activity on microorganisms such as *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* [6]. Furthermore, *H. scabrum* extract prevented synaptic plasticity impairments in rats fed a high-fat diet, which was attributed to its anti-inflammatory and antioxidant effects [10]. *H. scabrum* extract showed anti-leishmanial effect on *Leishmania major* promastigotes [11]. *H. scabrum* extract showed hypolipidemic effect by lowering serum triglyceride, total cholesterol and LDL-cholesterol levels [12]. There may be changes in the content of plants according to the regions where they are grown and this may be reflected in the biological activity values they show.

Depending on the soil structure and climate of the regions where the plants are located or grown, the amount and type of secondary metabolite components accumulated in their structures may vary slightly according to different regional conditions [13]. Sivas province is located in the middle of Anatolia, has a large surface area, terrestrial vegetation and gypsum soils. [13, 14]. Due to the soil structure and climate characteristics of the plants growing in Sivas, there may be changes in the amount and variety of secondary metabolite components they carry. In this study, it was aimed to determine the effects of *H. scabrum* plant grown in Sivas province on *Caenorhabditis elegans* nematode. *Caenorhabditis elegans* organism is used as an alternative to mammalian laboratory animals [15].

C. elegans is a nematode about 1 mm in length and has a 4-stage life cycle. Under favorable environmental conditions for reproduction, hatched larvae pass through four larval stages, L1, L2, L3 and L4, in just 3 days at 20 °C. When conditions are stressful, such as lack of food, overpopulation density or high temperature, *C. elegans* may enter an alternative third larval stage, L2d, called the dauer stage. The stage ends when conditions improve for the larva to grow further, even if gonad development has stopped at the L2 stage, it is now moving into the L4 stage. *C. elegans* is used analysing biological processes for its close to 70% similarity with the human genome [16]. By making ethanol and water extractions of *H. scabrum* plant, it was possible to show the changes in the survival and reproductive properties of the extracts obtained on *C. elegans* as an experimental animal model.

This study aims to investigate the biological effects of *H. scabrum* L. extracts, obtained through ethanol and water extraction methods, on the model organism *C. elegans* (Figure 1).

2. Materials and Methods

2.1. Preparation of Plant Extracts

H. scabrum was collected from Sivas province in June-August.The aerial part of plant were dried in a cool, shaded area and stored for experimental studies. The ethanol (70%) and water solvents were used in extraction process. The dried plant parts were pulverized using a grinder-homogenizer. The sample, which was ground in a ratio of 1/10 weight to volume, was placed in the relevant solvent and kept in the incubator with a mixer for 48 hours. Occasional shaking was done to ensure the completing maseration. Filtration was then carried out. The water phase extract obtained with water solvent was placed in a lyophilizer for drying and kept for 3-4 days until complete drying. After three days of maceration, the collected ethanol (70%) macerates were concentrated to solvent removal using a rotary evaporator under low heat (40°C) with vacuum [17]. The concentrated and dried plant extracts were taken into dark bottles after the yield calculation and stored at -20°C until use.

2.2. *Caenorhabditis elegans* Culturing

Wild-type (N2) *C. elegans* and *E. coli* OP50 strain were obtained from the *Caenorhabditis* Genetics Center (Minneapolis, USA). Worms were maintained according to Brenner's standart instructions [18]. Obtaining *C. elegans* eggs and synchronous cultures were performed according to the method previously described [16, 19]. 1 L of Nematode Growth Medium (NGM) was prepared and 1 mL of 1 M CaCl2, 1 mL of 1 M MgSO4, 25 mL of 1 M KPO4 buffer, 1 mL of cholesterol solution (5 mg/mL) was added [16, 20, 21]. After autoclaving, NGM medium was poured into petri dishes. Afterwards, *E. coli* OP50 (500 µL) required for the feeding of *C. elegans* was added to the midpoint of the petri dishes [16]. The Petri dish was washed with distilled water to syncronized the *C. elegans*. The solution was transferred to a centrifuge tube and centrifuged at 3400 rpm for 5 min to pellet the released eggs. The precipitate (pellet) was transferred to a petri dish (NGM) containing *E coli* OP50 with a pasteur pipette. The temperature was set at 20 °C in all studies [16, 19, 20, 22]

2.3. Survival Assay

5-Fluoro-2′-deoxyuridine (FUDR) was added to Nematode Growth Medium used in survival analysis. Thus, the number of *C. elegans* in NGM was kept constant in the analyzes and fertility was prevented from affecting survival analyses. Synchronized 20 individual of *C. elegans* (L4) were selected under a stereo microscope (7x). The plant extracts used in this study were taken from the stock solutions with a micropipette and added to the medium so that their final concentrations in the medium were 1mg/mL-100mg/mL [23, 24]. No plant extract was added to the NGMs used as control. Plant extract concentrations were determined using previous studies on plants according to the literature. Fresh *E. coli* OP50 was added to NGM every 3 days. *C. elegans*

individuals that died in the petri dishes were detected by stereo microscopy every day. Experiments were conducted almost 15 days [25]. In the study, all tests were performed independently at 20 °C with 3 replications [16, 19, 20].

2.4. Fertility Assay

All the fertility applications performed according to the previous studies [16, 19]. 15 individuals from the synchronized L4 forms worms were taken under a stereo microscope and transferred to NGMs containing different dose concentrations of plant extracts. Eggs seen in the medium after 36 h were counted with a light microscope. All the tests were performed with 3 replications. 100 eggs were collected from each group and the proportion of live nematodes hatched from the eggs was determined.

2.5. Determination of Physical Growth of Nematodes

Fifteen *C. elegans* eggs were selected under light microscope and transferred to NGM medium containing different doses of plant extracts. Petri dishes were kept in an incubator at 20 °C. After 10 days, pictures of *C. elegans* individuals in the medium were taken with a stereo microscope camera and the length measurements of the individuals were determined using Image Focus Plus V2 camera measurement program.

2.6. Statistical Procedure

In this study data were analysed using GraphPad Prism and SPSS 23.00 statistics programme.

3. Results

The yield of ethyl alcohol (70%) extract of *H. scabrum* was 3.5% and the yield of water extract was 2.2%.

3.1. Effects of *H. scabrum* Extracts on the Survival Percentage of Nematodes

The results of the effect of different doses of water and ethanol extracts of *H. scabrum* on the survival of *C. elegans* nematodes for 10 days are shown in Table 1.

Table 1. Effect of different doses of *H. scabrum* extracts on the survival rate of *C. elegans*. **(EEE: Effect of Ethanol Extract MEAN±SE; EWE: Effect of Water Extract MEAN±SE; *Values with the same letter in the same column are insignificant at P>0.05 level. MEAN±SE: Mean±Standard Error.)**

Doses	Number of individuals	% Survival of *C. elegans*	
		EEE*	EWE*
Control	20	90.9±3.5[a]	89.5±4.2[a]
1 mg/mL	20	90.48±3.8[a]	90.8±4.4[a]
10 mg/mL	20	91.2±4.9[a]	90.9±4.7[a]
100mg/mL	20	91.6±4.8[a]	91.1±6.5[a]

There was no significant difference between the control group and the application doses of plant ethanol and water extracts (P>0.05) (Table 1). For 10 days, there were no statistically significant differences in the number of nematode individuals surviving in the experimental and control group petri dishes.

3.2. Effects of *H. scabrum* Plant Extracts on *C. elegans* Egg Production

The results showing the effects of different doses of ethanol and water extracts of the plant on egg production in *C. elegans* are shown in Table 2. While the lowest percentage of nematodes hatched from eggs was 70.2% in the groups where ethanol extracts were applied, this ratio increased in parallel with the dose increase. As a result of statistical evaluations, it was determined that the difference between the control group and the treatment groups was significant. When the groups were compared among themselves, statistically significant differences were found (P<0.05). On the other hand, the lowest rate of 68.9% was observed in the groups where water extracts of the plant were applied. Although the rate of hatchlings increased in the groups where different doses of plant water extracts were applied, the difference between the groups was found to be statistically insignificant.

Table 2. Effect of *H. scabrum* plant extracts on egg production. **(EEE: Effect of Ethanol Extract MEAN±Se; EWE: Effect of Water Extract MEAN±Se; *Values with the same letter in the same column are insignificant at P>0.05 level. MEAN±Se: Mean±Standard Error.)**

Doses	Quantity of eggs counted	% Egg Yield of *C. elegans*	
		EEE*	EWE*
Control	100	69.2±6.6[a]	68.9±6.4[a]
1 mg/mL	100	70.2±7.8[a]	68.9±5.9[a]
10 mg/mL	100	70.4±6.1[a]	68.9±6.8[a]
100 mg/mL	100	71.6±6.7[b]	69.6±6.4[a]

3.3. Effects of Plant Extracts on the Length of *C. elegans* Nematode

Table 3 shows the differences in the length values of *C. elegans* living in NGMs with constant temperature and same nutritional conditions with different doses of water and ethanol extracts of *H. scabrum* plant. At the end of 10 days, it was observed that the extracts increased the growth of *C. elegans* nematodes compared to the control group (p<0.05) (Table 3) (Figure 1).

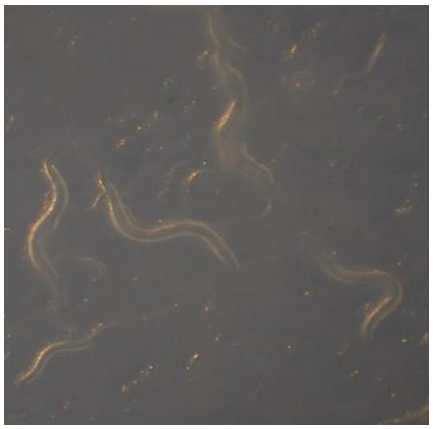

Fig 1. Nematodes that continuing to live in the petri dish for 10 days.

Table 3. Effects of plant extracts on the length of nematodes.

	Doses	Number of individuals	*Mean*	*Standard error*	*Minimum*	*Maximum*
Ethanol Extract	Control	15	69.3µm	4.2	68.9 µm	77.6 µm
	1 mg/mL	15	71.5 µm	4.6	69.8 µm	79.3 µm
	10 mg/mL	15	76.5 µm	4.8	69.1 µm	80.6 µm
	100 mg/mL	15	85.4 µm	4.1	79.6 µm	88.4 µm
Water Extract	Control	15	69.3µm	4.2	68.9 µm	77.6 µm
	1 mg/mL	15	71.8 µm	3.9	70.2 µm	80.1 µm
	10 mg/mL	15	78.9 µm	3.8	71.1 µm	83.2 µm
	100 mg/mL	15	90.3 µm	4.5	82.1 µm	98.7 µm

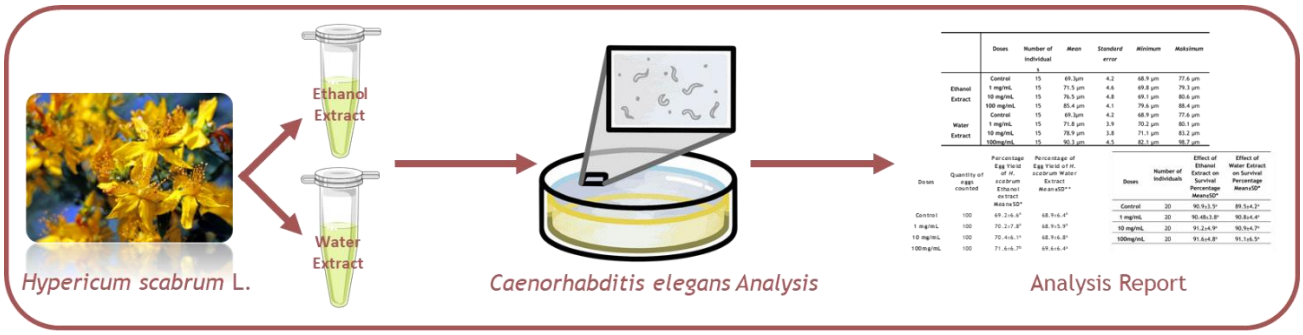

Fig 1. Workflow. This figure was created in part with bioicons.com. microtube-closed-translucent icon by Servier https://smart.servier.com/ is licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/. on_Nematode_Growth_Medium_petri_plate icon by DBCLS https://togotv.dbcls.jp/en/pics.html is licensed under CC-BY 4.0 Unported https://creativecommons.org/licenses/by/4.0/.

4. Discussion

According to our study results, it has been presented that ethanol (70%) and water extracts of the *H. scabrum* plant may be effective high doses (100 mg/mL) on the egg production and growth-development rates of *C. elegans* individuals. The differences were not observed in survival results between experimental and control groups or dose groups. The evaluation of some effects and potential of *H. scabrum* plant samples on *C. elegans* nematode as an experimental animal model was studied for the first time. In this study, the bioavailability potency of *Hypericum* plant extracts was appraised.

The plant was selected based on its historical and ethnopharmacological use as anti-inflammatory activity, anti-microbial activity and wound healing activity etc. *Hypericum* species have been in traditional medicine since the antiquity [26]. In Türkiye, traditionally *Hypericum* species are used for their antispazmolytic, antiseptic, tranquilisan, antiinflammatory, antiviral, antimicrobial, and antitumor properties and wound binding properties [3]. In one previous study [26], it was seen that 50 different compounds were present in leaf extracts of *H. scabrum*. Highest amounts among these compounds were determined as cis-vaccenic acid, palmitic acid, and octa-decanoic acid, alpha-pinene,

oleic acid. In many different studies made in different countries, the major component of essential oil content of *H. scabrum* plant was found as Alpha-Pinene [26, 27]. It is within possible ratio that the same plant would obtain different amount of chemical composition and chemical pattern in another studies due to the geographical distributions and extraction methods [28]. In this way, It is intended to evaluate the therapeutic nature of the *Hypericum* plant which is generally used ethnobotanically on the nematode *C. elegans* which is an unexpensive and user friendly system for drug screening. So, the effect of the treatment of plant extracts on the nematode was observed using the light microscope. The most active extract of *H. scabrum* plant on development and growth values and egg producing activity values of nematode was the ethanolic extract. Survival ratios as percentages were highly similar between study groups following the ethanolic extract exposures of 10 and 100 mg/mL doses.

As an established model organism, *C. elegans* is highly favored for investigations of diverse properties of plant extracts, which include various research indicating lifespan extension, neuroprotection, metabolic improvements, and improved stress tolerance. As an example, a study has showed that seed extracts of Ginko Biloba plant improved lifespan, motility, reproduction while also reducing the oxidative stress by prevention of lipofuscin accumulation. These effects have been linked to regulation of lipid metabolism and autophagy pathways [29]. Extracts of traditional Anatolian medicinal plants *Hedera helix*, *Salvia verticillata*, *Myrtus communis* and *Rubus sanctus* prolonged the life span of C. *elegans*. Especially *H. helix* showed anti-aging effect thanks to its chlorogenic acid content [30]. The leaf methanolic extract of *Caesalpinia mimosoides* alleviated signs of aging and increased oxidative stress resistance by reducing lipofuscin accumulation in *C. elegans* [31]. It has been shown that both lifespan and motily of *C. elegans* were improved by *Cuscuta chinensis* and *Eucommia ulmoides* plant extracts, which was attributed to antioxidant properties of these two traditional Chinese medicinal plants. In addition, *Styphnolobium japonicum* fruit extract showed neuroprotective effects by reducing oxidative stress in *C. elegans* [32]. It has been shown that flavonoids of licorice improved the survival of *C. elegans* exposed to high oxidative stress by enhancing SOD and CAT enzyme activities. On the other hand, ethanolic extracts from *Peganum harmala* seeds have been shown to impair lifespan, motility, and growth of *C. elegans* due to toxicity. This indicates the potential toxicity of the plant. On the other hand, the extract of *Sanghuangporus sanghuang* fungus prolonged the life span and healthspan of *C. elegans*. These effects were mediated through DAF-16/SIR-2.1 pathways [33] .

All in all, these studies on *C. elegans* reveal the effects of plant extracts on biological processes such as aging, oxidative stress, neuroprotection and metabolic regulation. This model organism is a valuable tool to rapidly and effectively evaluate the biological activities of plant-based compounds. As a matter of fact, in this study, the growth-enhancing effect of *H. scabrum* on *C. elegans* and its effects on important characteristics such as life span and egg production were found to be quite good.

Thus, plant extracts are known to contain hundreds of compounds and it is very important for pharmacognostic studies to understand which of these compounds cause biological activities of real therapeutic quality. However, it is not feasible in terms of economic and time management to initiate advanced pharmacognosic experimental processes without the preliminary studies of all plants indiscriminately. As a result of this study, it can be said that the extracts of *Hypericum scabrum* plant, which were investigated as a result of this study, can guide detailed studies with their in vitro activity values. As a result of the data obtained from our study; various effects of *H. scabrum* plant on *C. elegans* experimental animal model have been demonstrated and it has been shown that it may contribute to the literature in the development of new natural resources, new drugs and therapeutic agents for the prevention of various diseases. However, it is recommended that further research should be carried out to study the other therapeutic activities of these extracts with more detailed and comprehensive experiments by applying more advanced and comprehensive techniques, including in vivo experiments.

In studies conducted with raw plant extracts, it is not possible to determine exactly which molecules the activity comes from or the actual amount of activity. In this context, it would be more appropriate to conduct phytochemical analysis on individual active compounds for precise assestments.

Dosage and exposure evaluations are required to be conducted on different model organisms. Due to limited studies in this area, tests with *C. elegans* are crucial.

5. Conclusions

The findings obtained as a result of the analysis on *C. elegans*, which is similar to the human genome, contribute to the development of new products in the fields of medicine, cosmetics and food. In addition, the fact that it is a study aimed at raising awareness in terms of health provides originality.

In this study, the statistically important changes were determined by adding some extracts of *H. scabrum* plant to *C. elegans* cultures. It is possible to contribute to the universal knowledge at a basic level and it is considered to be a preliminary study. In the future it can be a guide other studies in this field. Even though this study provides important findings about potential uses of the *H. scabrum*, there is a need for comprehensive clinical research for this plant to be used in medical practice.

Author Contributions:

Supervision, T.D. and S.D.D; conceptualization, T.D. and S.D.D; methodology, T.D. and S.D.D; Experimental analysis, M.K., data collection, M.K. and S.D.D, investigation, Ş.K. and S.D.D; formal analysis, and writing, Ş.K. and S.D.D; project administration, Ş.K.; the original draft, Ş.K.; review and editing, Ş.K., T.D., S.D.D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

The authors declare that there is no conflict of interest regarding the publication of this article.

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