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Evaluation of the lifespan extension effects of several Turkish medicinal plants in *Caenorhabditis elegans*

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Abstract: Research on longevity is important to both prolong lifespan and support healthy aging. Natural products are widely being utilized and used as new resources for drug molecules. *Caenorhabditis elegans* is an advantageous organism for longevity research and age-related diseases. In this study, we tested a number of plant extracts for their effects on *C. elegans* longevity. In lifespan assays, age-synchronized wild-type *C. elegans* specimens were treated with different concentrations of plant extracts. Plant extracts were prepared as either infusions or decoctions, similar to their traditional utilization. *Hedera helix* L. (Araliaceae) extended lifespan in worms in a concentration-dependent manner. The mean survival rates in the *H. helix*-treated groups were significantly higher, by 23.7% when applied at 1000 µg/mL, 16% when applied at 500 µg/mL, and 16% when applied at 250 µg/mL, compared to the control group. HPLC analysis identified chlorogenic acid as the major component of *H. helix*. *Salvia verticillata* L. (Lamiaceae) and *Myrtus communis* L. (Myrtaceae) treatments resulted in median lifespan extension. Maximum lifespan was extended in worms by *Rubus sanctus* Schreb. (Rosaceae) treatment. This study provided the first evidence demonstrating the possible lifespan-extending effects of a group of Turkish medicinal plants in an in vivo model, *C. elegans*.

Key words: Aging, longevity, medicinal plant, panacea, *Caenorhabditis elegans*, chlorogenic acid, *Hedera helix*, *Salvia verticillata*, *Myrtus communis*, *Rubus sanctus*

1. Introduction

Aging is a process that leads to the functional loss of an organism and ultimately results in death. The medical sciences have long sought to improve quality of life and increase maximum life expectancy (Sinclair, 2005; Baur et al., 2006). Aging research has become important because it is related to not only prolonging life expectancy and healthy aging but also preventing age-related diseases, such as obesity, diabetes, atherosclerosis, cancer, depression, and Alzheimer and Parkinson diseases (Baur et al., 2006). *Caenorhabditis elegans* is an advantageous model organism that is widely used in longevity studies because its genome has been extensively studied, it is easily manipulated under laboratory conditions, and it has a 3-week lifespan. A variety of different genes and pathways have been shown to be involved in the progression of aging in *C. elegans* (Schaffitzel and Hertweck, 2006). The main pathways that have been implicated in aging/longevity include the following: the insulin/IGF-1 like signaling pathway, JNK signaling pathway, oxidative stress pathway, TOR signaling pathway, and mitochondrial signaling pathway. The insulin signaling pathway has been demonstrated to be one of the most widely studied and influential pathways in *C. elegans* aging research (Warner, 2005).

The vast majority of all drugs are obtained from plant sources, and there is a high probability that a drug candidate molecule has been developed from a plant that is used as a medicinal plant. It has previously been reported that blueberry *Vaccinium angustifolium* Aiton (Ericaceae) extended lifespan in *C. elegans* (Wilson et al., 2006), resveratrol increased lifespan in mice (Baur et al., 2006), and n-butanol extracts from *Platycladus orientalis* (L.) Franco (Cupressaceae) and water extracts from *Damnacanthus officinarum* C.C.Huang (Rubiaceae) had lifespan-extending effects in *C. elegans* (Yang et al., 2012; Liu et al., 2013). Extended lifespans were also observed in *C. elegans* treated with products from traditional Chinese medicine (Wang et al., 2014).

Turkey has a rich traditional medicine culture, and many plants are currently used as panacea. We have previously

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documented most of the traditional medicine culture of Turkey within the scope of the "Anatolian Folk Medicine Inventory Study" project (Tabata et al., 1988, 1993, 1994; Sezik et al., 1991, 1992, 2001; Yeşilada et al., 1993a, 1993b, 1995, 1999; Fujita et al., 1995; Honda et al., 1996; Yeşilada and Sezik, 1998). In this study, we analyze the lifespan-extending effects of a group of traditional medicines on the in vivo model *C. elegans*. We have included the following plants in our study, which were previously reported to have many beneficial activities: *Helichrysum plicatum* DC. subsp. *plicatum* (Asteraceae) (Tabata et al., 1988, 1993), *Helichrysum stoechas* (L.) Moench. subsp. *barrelieri* (Ten.) Nyman (Asteraceae) (Sezik et al., 1991, 2001; Tabata et al., 1993), *Myrtus communis* L. (Myrtaceae) (Sepici et al., 2004), *Hedera helix* L. (Araliaceae) (Ugulu et al., 2009), *Plantago major* L. (Plantaginaceae) (Yeşilada et al., 1999), *Paliurus spina-christi* Mill. (Rhamnaceae) (Sezik et al., 1992), *Rubus sanctus* Schreb. (Rosaceae) (Honda et al., 1996; Sezik et al., 1997), *Salvia fruticosa* Mill. (Lamiaceae), *Salvia verticillata* L. (Lamiaceae), *Salvia tomentosa* Mill. (Lamiaceae) (Aşkun et al., 2010), and *Urtica dioica* L. (Urticaceae) (Sezik et al., 1997).

The aim of this study was to investigate the effects of a group of medicinal plant extracts that are believed to be panacea, or remedies for many conditions, on *C. elegans* longevity.

2. Materials and methods

2.1. Nematode strain and cultures

Specimens of the *C. elegans* wild-type N2 (Bristol) strain were obtained from the *Caenorhabditis* Genetics Center of the University of Minnesota. The nematodes were maintained and assayed at 21 °C on Nematode Growth Medium (NGM) agar plates supplemented with *Escherichia coli* OP-50. Following the egg preparation methods described by Lewis and Fleming (1995), which involved treating gravid adults with hypochlorite, embryos were allowed to hatch overnight. Age-synchronized *C. elegans* were distributed on plates as L1 larvae. *E. coli* were grown overnight at 37 °C in Luria broth medium supplemented with 33 µg/mL streptomycin. Heat-killed *E. coli* OP-50 cultures were used in all experiments. To heat-kill the OP-50 cultures, the cells were heated to 65 °C for 30 min and then pelleted and resuspended in 1/10 volume in S Basal supplemented with 10 mM $MgSO_4$.

Treatment plates contained standard NGM containing the reproductive suppressant 5-fluorodeoxyuridine (FUDR, Sigma) at a final concentration 10 µg/mL. FUDR inhibits cell division and prevents eggs from hatching but does not affect adult lifespans. For the toxicity assays, plant extracts were added to the *E. coli* OP-50 cultures and spread across the surface of the plates to achieve final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.5, 7.5, and 3.75 µg/mL and 0 µg/mL for the control plate.

2.2. Lifespan assay

For the lifespan assays, age-synchronized animals were transferred as young adults to FUDR-containing treatment or control plates (2 days after hatching, day 0 of assay) and retransferred to fresh treatment or control plates every 2 days for the first 4 to 6 days of the assay. In all of the assays, body movement was monitored in the worms by gently probing them with a platinum pick on a daily basis. The fraction of animals that were alive was scored and dead animals were removed from the plates. The number of live and dead animals was recorded to calculate the proportion of live animals for each day, and the values were plotted graphically as a survival curve to calculate the median lifespan. Assays were performed at 21 °C using at least three treatment plates for each plant extract concentration that was studied.

2.3. Plant material

Plants were collected from different regions of Turkey between April and August of 2010. Professor Mecit Vural of the Department of Biology, Faculty of Science and Art, Gazi University, Ankara, Turkey, identified all species. Voucher specimens were stored in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey (GUE). Table 1 includes the names, families, herbarium numbers, used parts, extraction types, extraction yields, and collection sites for each of the plants used in this study.

2.4. Preparation of plant extracts

To prepare the decoctions used in this study, 1 g of air-dried plant material was added to 100 mL of distilled water, and the mixture was slowly boiled for 30 min. The infusions were prepared by pouring 100 mL of boiling water onto 1 g of dried plant material. Extraction continued for 30 min while the solution cooled, and the aqueous extracts were then filtered and finally freeze-dried.

2.5. RP-HPLC

Phenolic compounds of *H. helix* extract were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments, Kyoto, Japan). Qualitative and quantitative analyses were performed with an LC-10ADvp pump, a diode array detector, a CTO-10Avp column heater, an SCL-10Avp system controller, a DGU-14A degasser, and an SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, Columbia, MD, USA). Separation was performed at 30 °C on an Agilent Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm length, 5 µm particle size, Agilent, Santa Clara, CA, USA). The detection UV wavelength was 278 nm. The mobile phases were A: 3.0% acetic acid in distilled water and B: methanol. For analysis, the sample (12 mg) was dissolved in pure water (1 mL) and 20 µL of this solution was injected into the column. The elution

Table 1. Plant names, families, herbarium numbers, extraction types, used parts, and collection sites for the plants that were used in extractions.

Plant name	Plant family	GUE herbarium number	Part used	Extraction type	Extraction yield (%)	Collection site
Helichrysum plicatum DC. subsp. *plicatum*	Asteraceae	2355	Flowers	Infusion	27.66	Ilgaz Mountain, Bolu
Helichrysum stoechas (L.) Moench. subsp. *barrelieri* (Ten.) Nyman	Asteraceae	2353	Flowers	Infusion	22.50	Saint Pierre Church, Antakya
Myrtus communis L.	Myrtaceae	3032	Leaves	Decoction	12.46	Narlıkuyu, Mersin
Hedera helix L.	Araliaceae	2948	Leaves	Decoction	24.26	Tennis Club, Ankara
Plantago major L.	Plantaginaceae	2957	Leaves, flowers	Decoction	17.97	Işık Mountain, Ankara
Paliurus spina-christi Mill.	Rhamnaceae	2955	Fruits	Decoction	31.79	Gülnar Plateau, Mersin
Rubus sanctus Schreb.	Rosaceae	2960	Leaves	Decoction	30.65	İnözü Vadisi Beypazarı, Ankara
Salvia fruticosa Mill.	Lamiaceae	2061	Aerial parts	Decoction	23.42	Kızılağaç Forest, Bodrum
Salvia verticillata L.	Lamiaceae	2963	Aerial parts	Decoction	20.85	Işık Mountain, Ankara
Salvia tomentosa Mill.	Lamiaceae	2962	Aerial parts	Decoction	35.39	Işık Mountain, Ankara
Urtica dioica L.	Urticaceae	2928	Leaves	Decoction	24.63	Işık Mountain, Ankara

gradient was applied at a flow rate of 0.8 mL/min. The composition of the gradient was 93% A/7% B for 0.1 min, 72% A/28% B in 20 min, 75% A/25% B in 8 min, 70% A/30% B in 7 min and the same gradient for 15 min, 67% A/33% B in 10 min, 58% A/42% B in 2 min, 50% A/50% B in 8 min, 30% A/70% B in 3 min, 20% A/80% B in 2 min, and 100% B in 5 min until the end of the run. Phenolic composition of the extract was determined using a modified method of Caponio et al. (1999). Gallic acid, protocatechuic acid, catechin, *p*-hydroxy benzoic acid, chlorogenic acid, caffeic acid, epicatechin, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapinic acid, benzoic acid, *o*-coumaric acid, rutin, hesperidin, rosmarinic acid, eriodictyol, cinnamic acid, quercetin, luteolin, kaempferol, and apigenin (Sigma) were used as standards. Identification and quantitative analysis were done by comparison with standards. The amount of each phenolic compound was expressed as μg/g dry extract. External calibration curves were obtained for each phenolic standard.

2.6. Statistical analysis

All values are expressed as the mean ± standard error (SE) of the mean. Linear regression analyses and correlation coefficients were used to determine the relationship between 2 variables, and differences between treated and untreated animals were calculated using the GraphPad Prism 6 statistical program.

3. Results

In this study, we selected a group of Turkish medicinal plants that are believed to be panacea and were previously reported to have a wide range of beneficial effects, such as antimicrobial, antioxidant, and antidiabetic activities (Aslan et al., 2007; Yousefzadi et al., 2007; Deliorman Orhan et al., 2012) , to investigate their lifespan-extending effects in *C. elegans*.

3.1. Preparation of plant extracts

Extracts were prepared as either infusions or decoctions because these are the methods in which they are traditionally utilized as panacea. The extraction yields that were achieved after freeze-drying are shown in Table 1.

3.2. Lifespan assays

For the longevity assays, age-synchronized worms were transferred as young adults (2 days after hatching) to plates containing FUDR and various concentrations of plant extracts. This was counted as day 0 of the assay. Table 2 shows all of the results of the lifespan assay that were obtained during the study. The number of animals (n) plated for each extract concentration is shown in the same line as the plant extract. Whether it was statistically significant or not, any treatment that extended lifespan is shown with bolded numbers.

Table 2. The effects of medicinal plant extracts on *C. elegans* lifespan. Animals were treated with different concentrations of each plant extract, and the resulting median and maximum lifespan values are shown. The number of animals used in each group is indicated in parentheses. Whether statistically significant or not, any value indicating a lifespan extension is shown with bolded numbers.

Helichrysum plicatum										
	1000 µg/mL (n = 106)	500 µg/mL (n = 95)	250 µg/mL (n = 96)	125 µg/mL (n = 72)	62.5 µg/mL (n = 110)	31.25 µg/mL (n = 106)	15.5 µg/mL (n = 100)	7.5 µg/mL (n = 87)	3.75 µg/mL (n = 100)	Control (n = 100)
Median lifespan (days)	**17.17 ± 1.09**	**17.0 ± 1.0**	**17.83 ± 0.17**	**18.5 ± 0.29**	16.0 ± 0.0	**19.67 ± 0.33**	16.83 ± 1.17	16.0 ± 0.29	**18.67 ± 0.33**	16.33 ± 1.45
Maximum lifespan (days)	**26.0 ± 1.16**	**24.0 ± 0.58**	**27.33 ± 0.67**	**26.0 ± 1.53**	**26.0 ± 0.58**	**27.33 ± 1.86**	**25.33 ± 1.45**	**21.33 ± 0.88**	**26.0 ± 1.0**	**23.33 ± 2.19**
Helichrysum stoechas										
	1000 µg/mL (n = 164)	500 µg/mL (n = 213)	250 µg/mL (n = 196)	125 µg/mL (n = 202)	62.5 µg/mL (n = 213)	31.25 µg/mL (n = 227)	15.5 µg/mL (n = 236)	7.5 µg/mL (n = 214)	3.75 µg/mL (n = 228)	Control (n = 224)
Median lifespan (days)	**22.0 ± 0.0**	**21.33 ± 0.88**	20.33 ± 0.33	22.0 ± 1.0	21.75 ± 0.25	21.0 ± 0.0	20.83 ± 0.44	19.67 ± 0.17	**21.75 ± 0.25**	**20.67 ± 0.88**
Maximum lifespan (days)	**34.5 ± 0.5**	**32.0 ± 2.0**	27.33 ± 0.33	28.0 ± 0.0	**31.5 ± 0.5**	29.0 ± 0.0	30.0 ± 2.0	27.33 ± 0.88	30.0 ± 2.0	**31.0 ± 2.1**
Myrtus communis										
	1000 µg/mL (n = 223)	500 µg/mL (n = 168)	250 µg/mL (n = 133)	125 µg/mL (n = 122)	62.5 µg/mL (n = 169)	31.25 µg/mL (n = 163)	15.5 µg/mL (n = 173)	7.5 µg/mL (n = 164)	3.75 µg/mL (n = 200)	Control (n = 210)
Median lifespan (days)	22.0 ± 0.58	**23.83 ± 1.5**	**23.99 ± 0.19**	**24.17 ± 0.6***	22.67 ± 1.20	**23.67 ± 0.33**	**24.17 ± 0.83**	21.33 ± 1.20	**23.66 ± 0.88**	**21.83 ± 0.33**
Maximum lifespan (days)	33.0 ± 1.0	**35.67 ± 0.88**	**35.33 ± 1.33**	**35.33 ± 1.76**	33.0 ± 1.0	**35.67 ± 0.88**	**36.67 ± 1.33**	34.0 ± 1.16	**36.33 ± 0.33**	**34.66 ± 0.67**
Hedera helix										
	1000 µg/mL (n = 165)	500 µg/mL (n = 260)	250 µg/mL (n = 237)	125 µg/mL (n = 219)	62.5 µg/mL (n = 223)	31.25 µg/mL (n = 249)	15.5 µg/mL (n = 257)	7.5 µg/mL (n = 287)	3.75 µg/mL (n = 270)	Control (n = 295)
Median lifespan (days)	**20.83 ± 0.33****	**19.5 ± 0.87***	**20.0 ± 1.0***	**17.5 ± 1.04**	**14.5 ± 0.5**	**15.75 ± 0.25**	**16.33 ± 0.17**	**16.0 ± 0.0**	**15.33 ± 0.44**	**16.83 ± 0.33**
Maximum lifespan (days)	**33.33 ± 0.67**	31.33 ± 0.67	31.0 ± 0.58	**33.67 ± 0.88**	**33.67 ± 0.88**	**33.33 ± 0.67**	32.33 ± 1.20	32.33 ± 0.67	31.5 ± 1.50	**32 ± 1.5**
Plantago major flowers										
	1000 µg/mL (n = 196)	500 µg/mL (n = 270)	250 µg/mL (n = 272)	125 µg/mL (n = 240)	62.5 µg/mL (n = 233)	31.25 µg/mL (n = 178)	15.5 µg/mL (n = 241)	7.5 µg/mL (n = 222)	3.75 µg/mL (n = 235)	Control (n = 232)
Median lifespan (days)	20.0 ± 2.0	20.67 ± 0.33	20.67 ± 0.33	20.0 ± 0.0	20.89 ± 0.39	20.0 ± 0.0	20.33 ± 0.33	20.5 ± 0.29	20.67 ± 0.33	20.5 ± 0.29
Maximum lifespan (days)	30.0 ± 0.58	29.0 ± 0.0	29.67 ± 0.33	28.67 ± 0.33	29.0 ± 0.58	29.0 ± 0.58	29.67 ± 0.33	30.67 ± 0.33	31.33 ± 0.33	31.0 ± 0.58
Plantago major leaves										
	1000 µg/ml (n = 188)	500 µg/ml (n = 209)	250 µg/ml (n = 238)	125 µg/ml (n = 280)	62.5 µg/ml (n = 238)	31.25 µg/ml (n = 251)	15.5 µg/ml (n = 191)	7.5 µg/ml (n = 215)	3.75 µg/ml (n = 270)	Control (n = 208)
Median Lifespan (day)	15.67 ± 0.6	17.0 ± 1.16	16.5 ± 1.44	15.0 ± 0.76	20.17 ± 0.6	15.0 ± 1.53	16.5 ± 1.04	12.67 ± 0.33	17.67 ± 1.09	18.17 ± 0.17
Maximum Lifespan (day)	26.67 ± 1.33	30.33 ± 0.67	27.67 ± 0.33	27.0 ± 1.0	28.67 ± 0.33	27.33 ± 1.86	27.67 ± 0.33	25.0 ± 3.05	29.67 ± 0.88	29.33 ± 0.67

Table 2. (Continued).

Paliurus spina-christi										
	1000 µg/mL (n = 187)	500 µg/mL (n = 260)	250 µg/mL (n = 165)	125 µg/mL (n = 163)	62.5 µg/mL (n = 218)	31.25 µg/mL (n = 200)	15.5 µg/mL (n = 195)	7.5 µg/mL (n = 225)	3.75 µg/mL (n = 180)	Control (n = 204)
Median lifespan (days)	22.83 ± 0.73	23.0 ± 0.58	22.33 ± 0.33	22.0 ± 0.5	22.67 ± 0.17	22.83 ± 0.73	23.0 ± 0.58	22.83 ± 0.17	22.33 ± 0.88	22.83 ± 0.93
Maximum lifespan (days)	33.67 ± 1.45	33.76 ± 0.88	31.67 ± 2.33	32.33 ± 2.4	34.67 ± 1.2	33.67 ± 1.45	33.67 ± 0.88	37.67 ± 0.33	36.33 ± 0.67	34.0 ± 2.0
Rubus sanctus										
	1000 µg/mL (n = 223)	500 µg/mL (n = 247)	250 µg/mL (n = 262)	125 µg/mL (n = 245)	62.5 µg/mL (n = 248)	31.25 µg/mL (n = 217)	15.5 µg/mL (n = 205)	7.5 µg/mL (n = 209)	3.75 µg/mL (n = 220)	Control (n = 191)
Median lifespan (days)	**22.0 ± 1.16**	**21.67 ± 1.45**	**21.83 ± 0.83**	**22.5 ± 1.04**	**21.16 ± 1.48**	**21.61 ± 0.20**	**21.0 ± 0.58**	**22.75 ± 0.25**	**23.0 ± 1.16**	**20.83 ± 0.17**
Maximum lifespan (days)	32.33 ± 1.20	**34.67 ± 0.33**	**36.33 ± 0.33****	**35.0 ± 0.58**	**35.0 ± 0.0**	**34.67 ± 0.33**	**34.67 ± 0.33**	**35.0 ± 0.0**	33.33 ± 1.20	**33.33 ± 0.33**
Salvia fruticosa										
	1000 µg/mL (n = 147)	500 µg/mL (n = 205)	250 µg/mL (n = 205)	125 µg/mL (n = 189)	62.5 µg/mL (n = 211)	31.25 µg/mL (n = 150)	15.5 µg/mL (n = 205)	7.5 µg/mL (n = 193)	3.75 µg/mL (n = 174)	Control (n = 222)
Median lifespan (days)	23.67 ± 0.33	23.33 ± 0.44	**27.83 ± 1.01**	**27.28 ± 0.49**	**27.0 ± 0.29**	**27.25 ± 1.25**	23.33 ± 1.45	25.67 ± 1.33	24.67 ± 0.33	**25 ± 1.05**
Maximum lifespan (days)	35.33 ± 0.33	34.0 ± 1.0	38.0 ± 0.58	36.67 ± 0.33	36.67 ± 0.33	35.67 ± 0.88	35.33 ± 1.20	35.0 ± 1.16	34.0 ± 0.58	37.67 ± 0.67
Salvia verticillata										
	1000 µg/mL (n = 192)	500 µg/mL (n = 189)	250 µg/mL (n = 188)	125 µg/mL (n = 221)	62.5 µg/mL (n = 175)	31.25 µg/mL (n = 205)	15.5 µg/mL (n = 161)	7.5 µg/mL (n = 215)	3.75 µg/mL (n = 147)	Control (n = 207)
Median lifespan (days)	**23.83 ± 0.88****	19.0 ± 0.58	18.5 ± 0.29	19.83 ± 0.93	18.33 ± 0.33	17.83 ± 0.60	17.17 ± 0.17	18.83 ± 0.17	16.17 ± 0.60	**18.5 ± 0.29**
Maximum lifespan (days)	**32.67 ± 0.33**	**32.0 ± 0.58**	**32.67 ± 0.33**	**31.33 ± 0.33**	30.67 ± 0.33	30.67 ± 0.67	29.33 ± 0.33	31.33 ± 0.33	24.5 ± 1.5	**30.0 ± 0.58**
Salvia tomentosa										
	1000 µg/mL (n = 275)	500 µg/mL (n = 239)	250 µg/mL (n = 255)	125 µg/mL (n = 200)	62.5 µg/mL (n = 227)	31.25 µg/mL (n = 222)	15.5 µg/mL (n = 197)	7.5 µg/mL (n = 143)	3.75 µg/mL (n = 197)	Control (n = 195)
Median lifespan (days)	21.83 ± 1.48	18.83 ± 0.44	19.25 ± 1.75	18.17 ± 1.17	14.25 ± 1.75	21.83 ± 1.44	23.0 ± 0.58	21.33 ± 0.33	22.33 ± 0.88	23.0 ± 1.04
Maximum lifespan (days)	30.0 ± 1.0	32.33 ± 0.67	32.0 ± 0.58	30.33 ± 0.67	31.0 ± 1.16	30.0 ± 1.0	30.67 ± 1.20	32.0 ± 0.58	32.33 ± 0.33	32.0 ± 0.58
Urtica dioica										
	1000 µg/mL (n = 187)	500 µg/mL (n = 260)	250 µg/mL (n = 152)	125 µg/mL (n = 172)	62.5 µg/mL (n = 209)	31.25 µg/mL (n = 188)	15.5 µg/mL (n = 197)	7.5 µg/mL (n = 221)	3.75 µg/mL (n = 197)	Control (n = 195)
Median lifespan (days)	**24.33 ± 0.33**	**24.0 ± 0.29**	22.33 ± 0.33	22.11 ± 0.39	22.67 ± 0.17	22.83 ± 0.50	23.0 ± 0.58	22.83 ± 0.17	22.33 ± 0.88	**22.83 ± 0.93**
Maximum lifespan (days)	**34 ± 1.53**	**35.0 ± 1.0**	32.0 ± 2.65	30.0 ± 1.0	34.67 ± 1.20	33.67 ± 1.45	33.67 ± 0.88	**37.67 ± 0.33**	**36.33 ± 0.67**	**33.33 ± 1.33**

Values are expressed as the mean ± standard error of the mean.
** P < 0.01; * P < 0.05 significant from the control animals.

According to the results obtained during the lifespan assays, none of the concentrations of *Salvia tomentosa*, *Plantago major*, and *Paliurus spina-christi* extracts had a lifespan-extending effect in the animals, but some doses of these extracts shortened the median and/or maximum lifespan by 5%–10% compared to control worms. Since we did not observe this effect for all doses, these data are not enough to show that these extracts were toxic to the worms. Furthermore, we excluded a few of the extracts from the study after our initial screenings, which were toxic to the worms at all of the applied concentrations. Some of the remaining extracts that were included in the study resulted in longer lifespans than the control. We calculated the P-values for the significance of the values that indicated a lifespan-extending effect (Table 2).

Some plant extracts, such as *S. fruticosa* and *U. dioica*, nonsignificantly extended lifespan in *C. elegans* when applied at various concentrations (Table 2).

H. plicatum and *H. stoechas* extracts were not toxic to worms, and their lifespan-extending effects were not statistically significant. These effects were observed both in median and maximum lifespan.

Median lifespan was extended in the *M. communis*-treated worms. In this group, while the control worms had a median lifespan of 21.83 days (SE = 0.33, n = 210), a mean lifespan of 24.17 days (SE = 0.6, n = 122, $P < 0.05$) was achieved in the worms treated with 125 µg/mL *M. communis* extract, providing these worms a 10.7% median lifespan extension. An increase was also observed in the maximum lifespan of the *M. communis*-treated worms, but this difference was not statistically significant (Table 2).

Maximum lifespan was extended in worms by *R. sanctus*. In the *R. sanctus* assay, the control worms had a maximum lifespan of 33.3 days (SE = 0.33, n = 191), whereas treatment with 250 µg/mL *R. sanctus* resulted in a maximum lifespan of 36.33 days (SE = 0.33, n = 262, $P < 0.01$), indicating a 10% increase in maximum lifespan. Although this difference was not statistically significant, an increase in median lifespan was observed in these worms treated with all concentrations of *R. sanctus*.

In the *H. helix* group, whereas the control worms had a median lifespan of 16.83 days (SE = 0.33, n = 295), the median lifespan in the worms treated with 1000 µg/mL *H. helix* plant extract increased to up to 20.83 days (SE = 0.33, n = 165, $P < 0.01$). An increase in the median lifespan was also observed in the animals treated with the 500 µg/mL (19.5 ± 0.87 days, n = 260, $P < 0.05$) and 250 µg/mL (20 ± 1 days, n = 237, $P < 0.01$) concentrations of this extract (Table 2). These results indicate that *H. helix* significantly extends lifespan in nematodes in a concentration-dependent manner. The mean survival rates in the *H. helix*-treated groups were significantly higher, by 23.7% when applied at 1000 µg/mL, 16% when applied at 500 µg/mL, and 16% when applied at 250 µg/mL, than the values in the control group (Figures 1a–1c).

In the *S. verticillata*-treated worms, the control worms had a median lifespan of 18.5 days (SE = 0.29, n = 150), whereas worms treated with the highest concentration (1000 µg/mL) of *S. verticillata* had an average lifespan of 23 days (SE = 0.88, n = 150, $P < 0.01$), resulting in a 19.5% median lifespan extension (Table 2; Figure 1d). Although this difference was not statistically significant, we found that the worms treated with a higher concentration did have longer lifespans than those that were observed in the control animals.

3.3. RP-HPLC analysis

HPLC analysis was performed for the most significant lifespan-extending activity-providing extract, *H. helix*. Table 3 shows the amounts of phenolic compounds of *H. helix* decoction identified by RP-HPLC and the chromatogram profiles are reported in Figure 2. The major component present in *H. helix* extract was chlorogenic acid (6634.9 ± 174.0 µg/g dry extract), followed by rutin (3008.2 ± 120.9 µg/g dry extract) and protocatechuic acid (2371.9 ± 66.2 µg/g dry extract), respectively (Table 3). Other minor components were apigenin, gallic acid, caffeic acid, *p*-coumaric acid, and *o*-coumaric acid.

4. Discussion

Aging is considered as a process that involves gradual tissue decline, resulting in diseases and a deterioration in cellular and organismal functions (Kenyon, 2010). The commonly used model organism *C. elegans* is useful for exploring aging processes and age-related diseases. It is an advantageous organism because it has a rapid life cycle, is easy to cultivate, and has a well-researched genome. Furthermore, the high homology between *C. elegans* and human sequences at the protein level allows researchers to study the molecular processes underlying aging in *C. elegans* (Abbas and Wink, 2010). In this study, we used *C. elegans* to test the lifespan-extending effects of several medicinal plants that are used as panacea. These extracts are each applied to treat specific medical problems and they each have different activities, as described below. The plant extracts were prepared either by infusion or decoction, similar to their traditional utilization. The results of available studies on chemical compositions and our own results are also discussed below with the results of our longevity assay.

P. major leaves and flowers had no effect on lifespan in *C. elegans* according to our results. Earlier studies of chemical constituents reported the presence of phenylpropanoids, flavonoids, iridoid glycosides, and terpenoids in *P. major* (Nhiem et al., 2011).

Because the insulin-signaling pathway is one of the most well-studied and important pathways in *C. elegans* aging research, *H. plicatum* and *H. stoechas* were included

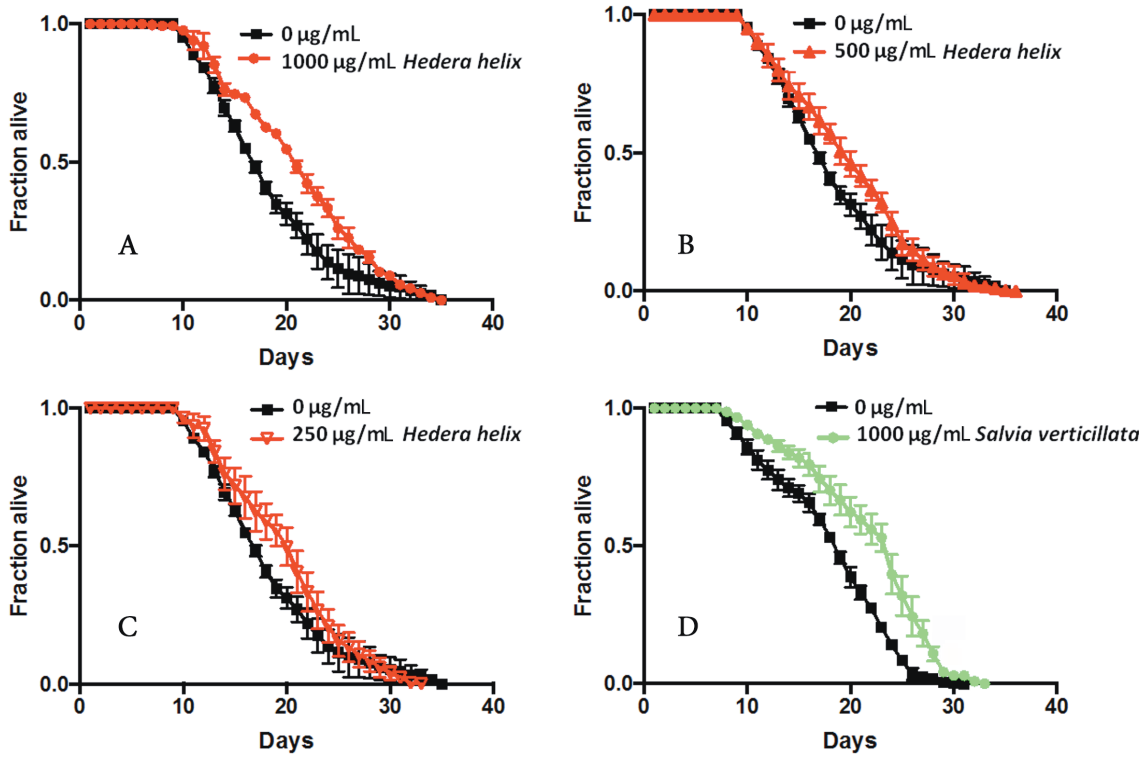

Figure 1. Effects of *H. helix* and *S. verticillata* on *C. elegans* longevity. Extension of median lifespan is observed with all concentrations.

Table 3. Retention times (Rt), calibration curves, amounts, correlation coefficients (R^2), LOD, and LOQ values of phenolic compounds of *H. helix* decoction identified by RP-HPLC.

Analyte	Rt (min)	Standard curve	µg/g dry extract*	R^2	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	5.1	y = 59121.2x + 1361.3	278.3 ± 7.4	0.99957	0.06	0.19
Protocatechuic acid	8.8	y = 37511.7x + 1644.9	2371.9 ± 66.2	0.99987	0.05	0.16
Chlorogenic acid	15.0	y = 29014.81x + 84.1	6634.9 ± 174.0	0.99941	0.20	0.60
Caffeic acid	17.8	y = 76114.15x + 600.8	70.2 ± 2.6	0.99974	0.05	0.14
p-Coumaric acid	25.0	y = 231321.3x + 1822	36.1 ± 0.5	0.99988	0.01	0.04
o-Coumaric acid	41.1	y = 222424.8x + 71.9	31.4 ± 0.2	0.99998	0.004	0.010
Rutin	44.1	y = 25592.9x + 6.9	3008.2 ± 120.9	0.99978	0.15	0.45
Apigenin	77.4	y = 54136.41x + 1761	318.5 ± 20.6	0.99993	0.02	0.07

LOD: Limit of detection.
LOQ: Limit of quantification.
*Values are expressed as µg/g sample ± standard deviation.

in this study as panacea that have antidiabetic activities (Aslan et al., 2007). Although these plants were observed to provide lifespan-extending effects in *C. elegans*, their effects were not statistically significant. The chemical composition of *H. plicatum* has been previously reported, and these plants have been shown to be rich in volatile oils, phenolic terpenes, acetophenones, fatty acids, flavonoids, and related phenolic compounds (Öztürk et al., 2014).

U. dioica was previously reported to have antidiabetic and antioxidant activities, and it has reportedly been used

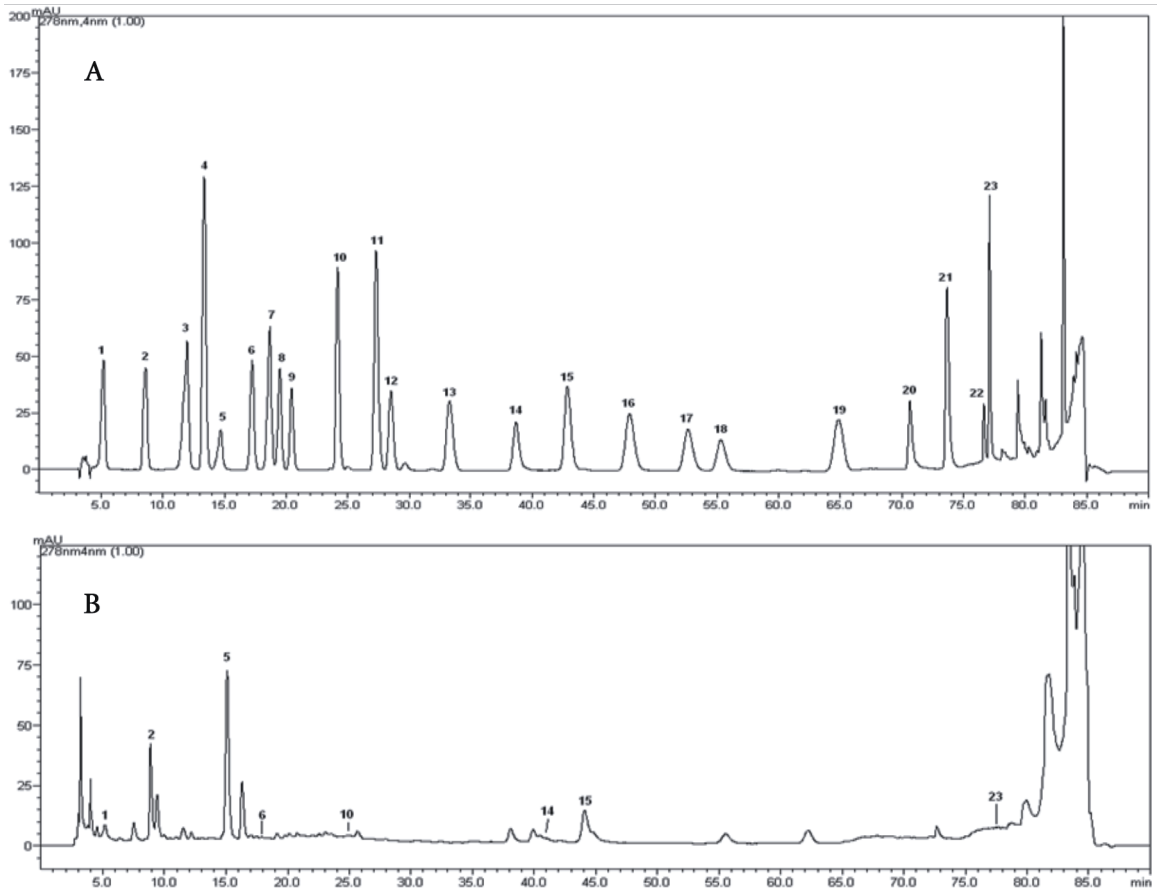

Figure 2. Chromatogram of standard phenolic compounds (A) and *H. helix* decoction extract (B). 1: Gallic acid, 2: protocatechuic acid, 3: catechin, 4: *p*-hydroxy benzoic acid, 5: chlorogenic acid, 6: caffeic acid, 7: epicatechin, 8: syringic acid, 9: vanillin, 10: *p*-coumaric acid, 11: ferulic acid, 12: sinapinic acid, 13: benzoic acid, 14: *o*-coumaric acid, 15: rutin, 16: hesperidin, 17: rosmarinic acid, 18: eriodictyol, 19: cinnamic acid, 20: quercetin, 21: luteolin, 22: kaempferol, 23: apigenin.

against colds (Sezik et al., 1997). Studies of its chemical contents demonstrated the presence of phenolic acids, flavonoids, and anthocyanins in *U. dioica* (Pinelli et al., 2008). Our results showed that it nonsignificantly extended lifespan in *C. elegans* when applied at various concentrations.

Other than *U. dioica*, *H. plicatum*, and *H. stoechas*, all of the plant extracts that were included in this study were previously reported to have antimicrobial activities. *S. fruticosa* was found to be rich in flavonoids, catechins, diterpenoids, and volatile oils (Askun et al., 2009), and it nonsignificantly extended lifespan in *C. elegans* when applied at various concentrations in our study. *Salvia* species are being used against cold in traditional medicine (Aşkun et al., 2010).

According to our data, *H. helix*, *S. verticillata*, *M. communis*, and *R. sanctus* extended lifespan and thereby counteracted aging in *C. elegans*. These lifespan-extending extracts were previously reported to have antimicrobial activity (Yousefzadi et al., 2007; Deliorman Orhan et al., 2012). The traditional use of *H. helix*, *S. fruticosa*, and *R. sanctus* is to combat colds (Askun et al., 2009; Sezik et al., 1997; Ugulu et al., 2009). Earlier studies reported that *M. communis* extracts contained volatile substances, terpenoids, triterpene, flavonoids, tannins, and fatty acids (Asgarpanah and Ariamanesh, 2015) and *R. sanctus* extracts contained flavonoids and phenolic acid derivatives (Süntar et al., 2011). *H. helix* extracts were previously reported to contain phenolic acids, flavonoids, and saponins (Trute and Nahrstedt, 1997).

We analyzed the phenolic components of *H. helix* decoction extract by RP-HPLC analysis and identified chlorogenic acid followed by rutin, protocatechuic acid, apigenin, and gallic acid as main components. Chlorogenic acid exhibits many beneficial bioactivities, such as antibacterial, antioxidant, antiinflammatory,

antihypertension, antiobesity, and neuroprotective effects. In concordance with our results, chlorogenic acid was recently found to extend the lifespan of *C. elegans* by up to 20.1%, delay the age-related decline of body movement, and improve stress resistance (Zheng et al., 2017). The authors performed subsequent genetic analysis with worm mutants and revealed that chlorogenic acid extends the lifespan of *C. elegans* mainly through DAF-16 in the insulin/IGF-1 signaling pathway. In other organisms, oral administration of chlorogenic acid (10 mg/kg) for 6 weeks in STZ-induced diabetic rats markedly decreased blood glucose levels (Park et al., 2009). Another study reported dose-dependent antidiabetic activity for the 70% aqueous ethanolic extract of *Morus alba* leaves in type II diabetic rats (Hunyadi et al., 2012). As a result of that HPLC analysis, chlorogenic acid and rutin were found to be the main compounds of the extract. The authors suggested that these compounds could be responsible for the antidiabetic effect of mulberry leaves. Several researchers reported that chlorogenic acid suppresses the N-nitrosating reaction and inhibits glucose 6-phosphatase, lipid peroxidation, and formation of nitric oxide and hydroxyl free radicals. AMP-activated protein kinase (AMPK) activity can also affect lifespan in lower organisms. Chlorogenic acid was reported to regulate glucose and lipid metabolism via the activation of AMPK (Ong et al., 2013). These data suggest another pathway for the lifespan-extending effect of chlorogenic acid.

Reactive oxygen species (ROS) and decreased antioxidant defense play a vital role in the pathophysiology of aging and thermal stress is one of the main reasons why an organism may produce ROS. Kampkotter et al. (2007) showed that rutin reduced the accumulation of ROS when *C. elegans* was exposed to thermal stress. Kim et al. (2014) analyzed the effects of protocatechuic acid isolated from *Veronica peregrina* on lifespan and stress tolerance in *C. elegans*. They found that protocatechuic acid extends lifespan of wild-type worms in a dose-dependent manner and elevated their tolerance against heat, osmotic shock, and oxidative stress, suggesting that protocatechuic acid could enhance health-span as well as lifespan.

Several studies demonstrated that phytochemicals belonging to many different classes, including phenolic compounds, terpenes, polysulfides, organosulfur compounds, indole compounds, modified purines, quinones, and polyamines, have longevity-extending effects (Leonov et al., 2015). Although the plants tested in this study are rich in terpenoids and phenolic compounds, one should keep in mind that lifespan-extending effects may be due to the synergistic effects of individual compounds in the plant extracts. Also, in order to identify which bioactive compound(s) are responsible for the lifespan-extending activities, bioactivity-guided isolation studies should be conducted in the most effective plant extracts.

According to a literature review, we believe that this is the first study to demonstrate that some Turkish medicinal plant extracts, including those derived from *H. helix*, *S. verticillata*, *M. communis*, and *R. sanctus*, extend lifespan in *C. elegans.* Although more detailed molecular biological analyses should be carried out to identify the active substances and pathways that are responsible for their lifespan-extending effects, the data obtained in this study provide support for the medicinal uses of these plants. The plants selected for this study are known as panacea, and many reports by us and others have described their antibacterial, antidiabetic, and antioxidant activities (Sezik et al., 1997; Aslan et al., 2007; Yousefzadi et al., 2007; Askun et al., 2009; Ugulu et al., 2009; Deliorman Orhan et al., 2012). In this study, we have demonstrated that these medicinal plants, which have different beneficial activities, can display lifespan-extending effects in an in vivo model as well, and these findings partially justify the belief in "cure-all drugs" as panacea. As expected, not all of the extracts were effective at extending lifespan. However, we provide the first documented evidence showing that four of these medicinal plant extracts can extend lifespan in *C. elegans*.

In conclusion, in this study, the effects of a group of Turkish medicinal plants that are used as panacea were tested to determine their effects on *C. elegans* longevity for the first time. The results demonstrated that four of these plant extracts significantly extended lifespan in treated animals. These results are valuable not only because they contribute to research on healthy aging but also because they provide support for the medicinal uses of plants. Further analyses should be performed to investigate the mechanisms underlying the activities of the most promising extracts and to isolate their active compounds that contribute to lifespan extension.

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