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Antioxidant and anti-aging potential of Juniper berry (*Juniperus communis* L.) essential oil in *Caenorhabditis elegans* model system

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ABSTRACT

Plant and plant products including essential oils are globally acclaimed for their medicinal and therapeutic values. It's an age-old practice, owing to their safety and protective effects against reactive oxygen species (ROS)/oxidative stresses. Aging is a global challenge and needs proper attention to check on its causative factors. The increased ROS production/oxidative stress is one of the major contributing factors endorses aging and age-related disorders. To this end, the present study was designed to explore the *in vivo*-antioxidant and anti-aging potentials of Juniper berry essential oil (JBEO) by using *Caenorhabditis elegans* as a model organism. Present work investigated the impact of different doses (0, 10, 50, 100 ppm) on lifespan and healthspan of *C. elegans*. The present study revealed that lower dose (10 ppm) was highly effective and enhanced the lifespan of *C. elegans* by 18.54% as compared to the control. Similarly, same concentration, i.e. 10 ppm was also showing potential against various oxidative and thermal stresses. The JBEO treated worms showed 30.40% more survival under thermal stress as compared to control. Besides the increased survival percent of worms, the elevated expression of SOD-3 (39.49%) and GST-4 (25.13%) was also observed, indicating oxidative stress resistance in worms. The screening studies on mutants of *C. elegans* for prediction of mechanism demonstrated the involvement of major conserved transcription factors *(DAF-16, SKN-1, and HSF-1)* which coordinates in the stress-induced transcription and extends longevity. Overall, here we unrevealed potentials of JBEO in anti-stressor activities and lifespan extension in *C. elegans*. Thus, in future, more studies on JBEO will pave paths for commercialization of essential oils in the formulations of antiaging products.

1. Introduction

Essential oils (EO) are plant-based economically important secondary metabolites. They have low molecular weight compounds extracted by steam distillation, hydrodistillation or by solvent extractions for agriculture-based industries (Nakatsu et al., 2000; Raut and Karuppayil, 2014). These secondary metabolites are usually found in oil ducts, resin ducts, glands or trichomes (glandular hairs) of the plants (Baser and Demirci, 2007). EO's are usually vital components or constituents used as flavoring agents in food products, drinks, perfumeries, pharmaceuticals and cosmetics (Raut and Karuppayil, 2014). The EO's have been reported to bear marvelous antioxidant and antimicrobial properties (Bais et al., 2014), which opens a wider arena for exploration of its therapeutic potentials to severe human diseases including aging and age-related disorders (Tiwari et al., 2017). The juniper berries obtained from the medicinally important herb *Juniperus communis* L. is traditionally well known as a powerful detoxifier and immune system booster. They have a long history of usage as a folk medicine to treat several opportunistic infections and for flavoring purposes (Hoferl et al., 2014; Bais et al., 2014). Juniper berries are most commonly used in natural remedies for a sore throat, respiratory infections, fatigue, muscle aches, and arthritis. It also helps in soothing skin flair-ups, boosts the immune system, and helps with insomnia and digestion activities. It has also been reported (Bulgaria) that plant stems were used to prevent both short- and long-term illnesses. The Juniper berry essential oils (JBEO's) extracted from juniper berries have been explored and established for its *in-vitro* antioxidant and anti-radical activities which are mostly dependent on the oil components nature and on concentrations (Ruberto and Baratta, 2000; Misharina et al., 2009; Wei and Shibamoto, 2007; Emami et al., 2007; Hoferl et al., 2014).

Aging and age-related disorders are globally acclaimed problems. Aging is a natural phenomenon that occurs in every living organism

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including humans which, progressively deteriorates almost all biological functions and leads to death. It has been considered as a hallmark, (major risk factor) which makes humans prone to several diseases including cancer, diabetes, neurodegenerative disorders, etc. (Niccoli and Partridge, 2012). One of the major contributing factors responsible for the aging and age-related disorders are Reactive oxygen species (ROS) generated during metabolism. ROS are accountable to damage the major biological macromolecules including DNA, lipids, and protein and cause various diseases (Phulara et al., 2015). The current scenario presents an increased prevalence of aging populations and age-related diseases thus, drags the attention of researchers for prevention and cure. The recent decades plant-based drugs and secondary metabolites (essential oils) have gained momentum and much attention for the medicinal applications in aging and age-related ailments (Wilson et al., 2006; Yu et al., 2010; Lautenschlager et al., 2012; Yang et al., 2012; Binic et al., 2013; Phulara et al., 2015).

Caenorhabditis elegans, a free-living nematode is a living test tube and promising model organism in which aging and ROS associated studies can be performed in very short duration (Villatoro-Pulido et al., 2012; Tiwari et al., 2014; Phulara et al., 2015; Shukla et al., 2016). *C. elegans* have been bestowed with awesome features viz., rapid life cycle, short lifespan, well-established genetic pathways, human homologous and many more which makes it more suitable for the aging and age-related studies (Kenyon, 2010; Kaletta and Hengartner, 2006; Kim, 2007). Owing to its above potentials, it has been considered as an ideal model system for live animal high-throughput drug screening. Now there are many identified novel plant-based therapeutics for aging and age-related disorders which have been explored using this model system (Shukla et al., 2012a,b; Villatoro-Pulido et al., 2012; Lucanic et al., 2013; Argyropoulou et al., 2013; Akhoon et al., 2016). Therefore we have used *C. elegans* model organism to evaluate the *in-vivo* antioxidant and longevity-promoting properties of JBEO. The present study sheds an insight into both health and lifespan-extending effects of economically important Juniper berry oil (JBEO) using *C. elegans* model system.

2. Materials and methods

2.1. Juniper berry oil

Juniper berry essential Oil was purchased from the Fragrance & Flavors Development Centre, Kannauj, Uttar Pradesh, India (Autonomous body of Ministry of Micro, Small and Medium Enterprises, Govt. of India).

2.2. Gas chromatography-mass spectrometry (GC–MS) analysis

The qualitative and quantitative analysis of active components of Juniper berry oil was performed by following protocols established by Kumar et al. (2016) using TRACE GC ULTRA coupled (Thermo Fisher) with DSQ II Mass Spectrometer. A constant flow of 1 mL/min of carrier gas (Helium) was maintained throughout the whole analysis process. The injector temperature of the instrument was kept at 220 °C while oven temperature was started from 50 °C, (hold time 5.0 min) to 250 °C with the ramp of 4 °C/min (hold time 5 min). 1 µL sample (injection volume) was injected in a split mode (1:50) method. The ion source temperature was set at 220 °C, and transfer line temperature was at 300 °C. The electron impact mode was used for sample ionization (voltage of 70 eV) and the mass range varied from *m/z* 50–650 amu. Identification of individual compounds was carried out by comparison of their mass spectra with those of the internal reference mass spectra library (NIST/Wiley) or with authentic compounds (Kumar et al., 2016). Standards were procured from Fragrance & Flavor creation lab, Fragrance & Flavors Development Centre, Govt. of India Autonomous body (NABL accredited based on iso17025-2005). The GC–MS of standards and samples were performed in triplicates. The relative amount of individual components is based on peak area obtained by the detector response (Kumar et al., 2016).

2.3. Antioxidant activity (in vitro) of JBEO

2.3.1. DPPH assay

DPPH assay is usually regarded as a reaction of hydrogen atom transfer. It (an *in vitro* test) was used to determine the ability of JBEO to act as hydrogen atom donors. The DPPH scavenging effect was studied by following the methods of Mensor et al. (2001). One mL 0.3 m methanolic DPPH solution was added to 2.5 mL of the ethanolic juniper berry oil dilutions (different concentrations). The sample was kept at room temperature in the dark, and after 30 min the optical density of the samples, and the blank was measured at 517 nm by using UV/Visible spectrophotometer (Niranjan et al., 2009).

2.3.2. Determination of total phenolic content (TPC)

The amount of total phenolic contents in the JBEO was determined calorimetrically with the Folin-Ciocalteu (FC) reagent, here 500 µL sample (mixed with FC in 1:1 ratio) and one mL sodium carbonate were mixed in the dark and incubated for 30 min till color development occurs. The absorbance reading was performed at 720 nm by UV/visible spectrophotometer (Bae et al., 1996). Phenolic contents of the sample were calculated by the standard curve for Gallic acid. The results were expressed as Gallic acid equivalents (GAE)/g of the plant materials.

2.3.3. Determination of total flavonoid content (TFC)

For the determination of the amount of total flavonoid contents in the oil calorimetric methods were used. Here 500 µL of sample, 1.5 mL sodium nitrite (5%), 10 µL aluminum chloride (10%) and One mL sodium hydroxide (1N) were mixed. After incubation, an orange color was found to be developed, and the absorbance was measured at 415 nm by UV/visible spectrophotometer (Manita and Harjinder, 2012). Flavonoid contents of the sample were calculated by the standard curve.

2.4. C. elegans strains and maintenance

C. elegans strains N2 Bristol *(Wild-type); TK22: mev-1(kn1); PS3551:hsf-1(sy441)*, GR1307:daf-16(mgDf50), TJ356:zIs356 (daf-16:gfp + rol-6), EU31:skn-1(zu135), used in this study were procured from Caenorhabditis Genetics Center, University of Minnesota, MN, USA.All strains were grown and maintained on nematode growth medium (NGM) agar plates seeded with *E.coli* OP50 at 20 °C (Brenner, 1974). For experiment, the synchronous populations were obtained by using bleaching method (sodium hypochlorite treatment of adults (Lithgow et al., 1995).

2.5. Toxicity assay

Toxicity of JBEO's in *C. elegans* was examined by 24-h lethality test using 10, 50, 100, 250, 500 and 1000 ppm concentrations. Worms (adult day 2) were exposed to JBEO's for 24 h at 20 °C in 24-well tissue culture plates, and the number of live/dead worms was recorded after every hour.

2.6. Lifespan analysis

The lifespan assay was performed as described by Shukla et al. (2012a,b) and Tiwari et al. (2014). Age-synchronized L1 worms (Sulston and Hodgkin, 1988) were treated with the different concentrations of JBEO (0, 10, 50 and 100 ppm) till they reached their L4 stage. The pretreated (n = 100) L4 worms were further transferred to fresh NGM plates having different concentrations of JBEO (0, 10, 50 and 100). The plates having no supplementation (0) were kept as control plates (Tiwari et al., 2014). To ensure the proper