

Withania somnifera root extract extends lifespan of *Caenorhabditis elegans*

Ranjeet Kumar¹, Kuldeep Gupta¹, Kopal Saharia¹, Deepak Pradhan² and Jamuna R Subramaniam¹

¹Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, INDIA; ²IXOREAL Biomed Pvt. Ltd., Hyderabad, INDIA

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Corresponding Author:

Jamuna R. Subramaniam, Ph.D
Tel : 91-512-2594042
E-mail : jamuna@iitk.ac.in

ABSTRACT

Background: In the ancient Indian herbal medicine system several ayurvedic preparations are claimed to have longevity enhancing effects. But, so far, no clear scientific evidence has been provided. One among them, is the roots of the plant, commonly known as Ashwagandha (*Withania somnifera* Dunal- WSD), which is supposed to have myriad of beneficial effects including long life. **Purpose:** Here, we evaluated both the root extract (RE) and its purified ingredients (PI-RE) with a similar composition as in RE obtained from the roots of WSD for lifespan extension in the well established model system, *C. elegans*. PI-RE could extend the lifespan of *C. elegans*. **Methods:** We used wild type *C. elegans* (N2) or RB918: *acr-16* (*ok789*); and NL2099: *rrf-3* (*pk1426*) mutant worms and analysed their lifespan assay in Ashwagandha extract spreaded on plates containing Bacterial Lawns. **Results:** Strangely, while there was no effect on the wild type worms, the mutant for the human nicotinic acetylcholine receptor, nAChR, $\alpha 7$ equivalent, *acr-16*, showed around ~20% lifespan extension when treated with PI-RE. **Conclusion:** Thus, we are able to show that one of the age old healthy longlife supplements, Ashwagandha does extend lifespan of *C. elegans*.

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Introduction

Aging is a complex, pleiotrophic phenomenon yet to be fully understood. Ever being youthful (anti-aging) and prolonged healthy living without diseases is a quest from time immemorial. Identification of long-lived mutants in *C. elegans* opened the avenue for healthy lifespan extension research.¹⁻³ The major challenge with lifespan extension studies is the long time requirement (in months and years) in the mammalian systems. This could be circumvented using the versatile, seemingly simple model organism, *C. elegans*, which has a short lifespan (in days), but, fundamental mechanisms and systems amazingly similar to the mammalian system.⁴ Further, the increase in longevity mechanisms identified in *C. elegans* is shown to follow similar pattern in flies,⁵ mice⁶ and humans.^{7,8} The positive correlation between prolonged healthy long life and modulation of insulin/IGF-1 signaling was first reported from *C. elegans*^{2,3} which was later shown in flies and mice.⁹ The Japanese and Jewish centenarians have variants of the IGF-1 receptors.^{7,8} Thus, adding credence to the reduced insulin/IGF-1 signaling's contribution to lifespan extension.¹⁰ This makes the *C. elegans* longevity studies more relevant. Moreover, it is less time consuming and easily amenable.⁴

So far, neither an innocuous longevity supplement nor a therapy to translate this longevity in humans is yet known. Recently, we reported an antihypertensive drug, reserpine, derived from the plant *Rauwolfia serpentina*, which is able to extend *C. elegans* lifespan^{11,12} and alleviate Amyloid β toxicity in *C. Elegans*.^{12,13} Consumption of red wine is suspected to delay aging. Later, resveratrol present in red wine is shown to extend lifespan in *C. elegans*¹⁴ and mice,⁶ and is being used as a nutritional supplement. However, this needs to be consumed in huge amounts (in gms). In the ancient Indian system, various herbal formulations have been used for thousands of years¹⁵⁻¹⁷ and some of them are known to have longevity effect.¹⁸ One among them is Ashwagandha – *Lithuania somnifera* Dunal.¹⁸ The leaf extract of WSD is shown to have anticancer effects.¹⁹ The root extract has been shown to provide antistress¹⁸ and anti-inflammatory effects¹⁹ and protection against Alzheimer's disease²⁰ Ashwa-

gandha has minimal side effects and is in use for thousands of years.^{16,18} This is also implicated to have longevity enhancing effects but not clearly demonstrated.

Here in this report, we evaluated the RE extract prepared from the roots of *Withania somnifera* (Ixoreal Biomed Pvt Ltd, India) and the purified components in it for the effect on lifespan extension in *C. elegans*. We find that indeed the purified fraction can extend *C. elegans* lifespan.

Methods

C. elegans strains

The *C. elegans* wild type (N2) or RB918: *acr-16* (*ok789*); and NL2099: *rrf-3* (*pk1426*) mutant worms were grown on NGM plates with OP50 *E. coli* as food at 20°C following standard protocols.²²

Preparation of Ashwagandha root extract (RE) containing plates: We followed the protocol of spreading of the RE and purified ingredients on the OP50 bacterial lawn containing NGM plates. We diluted Ashwagandha extract-RE (IXOREAL, Hyderabad, India) of various fractions: i) suspension ii) ~80% water soluble fraction iii) ~60% water soluble fraction and iv) ethanol solubilised mixture of purified components in the same ratio as in RE. The required concentration dilution was done in M9 buffer and spread on the plates containing bacterial lawn with two controls i)- no vehicle and ii) with ethanol as the vehicle.

Lifespan assays: Approximately 30–40 embryos were transferred to 35 mm plate with *E. coli* OP50 bacterial lawn in NGM agar with or without the compound and maintained at 25°C. The lifespan assay was carried out as described earlier¹¹ with minor modifications. Once the worms reach the adult stage, we shift them every day to fresh plates till the end of reproduction. After that we transfer them to fresh plates every two or three days. The lifespan was counted starting L4 stage which was considered as 0. The worms which crawled off the plates were excluded. The worms were considered dead when they did not move at all to prodding with the platinum wire. We carried out the initial screening once

or twice and the positive fraction assay was repeated at least two to three times. Survival Graph and statistical analyses were done using Sigmaplot 10.0. and SigmaStat 3.5.

Results and Discussion

Purified ingredients (PI) mixture of Root extract (RE) extends lifespan in C. elegans

We screened *C. elegans* for lifespan extension following the published protocol.¹¹ Briefly, we treated the worms chronically

with various RE and its fractions from embryo stage till death. We have screened crude (Fig. 1) and water soluble fractions of Ashwagandha (sample 1–80% water soluble and sample 2–60% water soluble) (Fig. 2) and the purified mixture with the composition (Fig. 2) as in PI-RE (Fig. 3). We carried out a dose response analysis (Fig. 3A) and found that PI-RE at a concentration of 100 ng/ml is able to extend the lifespan in the human nicotinic acetylcholine receptor $\alpha 7$ equivalent mutant, *acr-16*, but not in the wild type or another mutant which shows enhanced RNAi, *rff-3*, suggesting RE can extend *C. elegans* lifespan in a specific

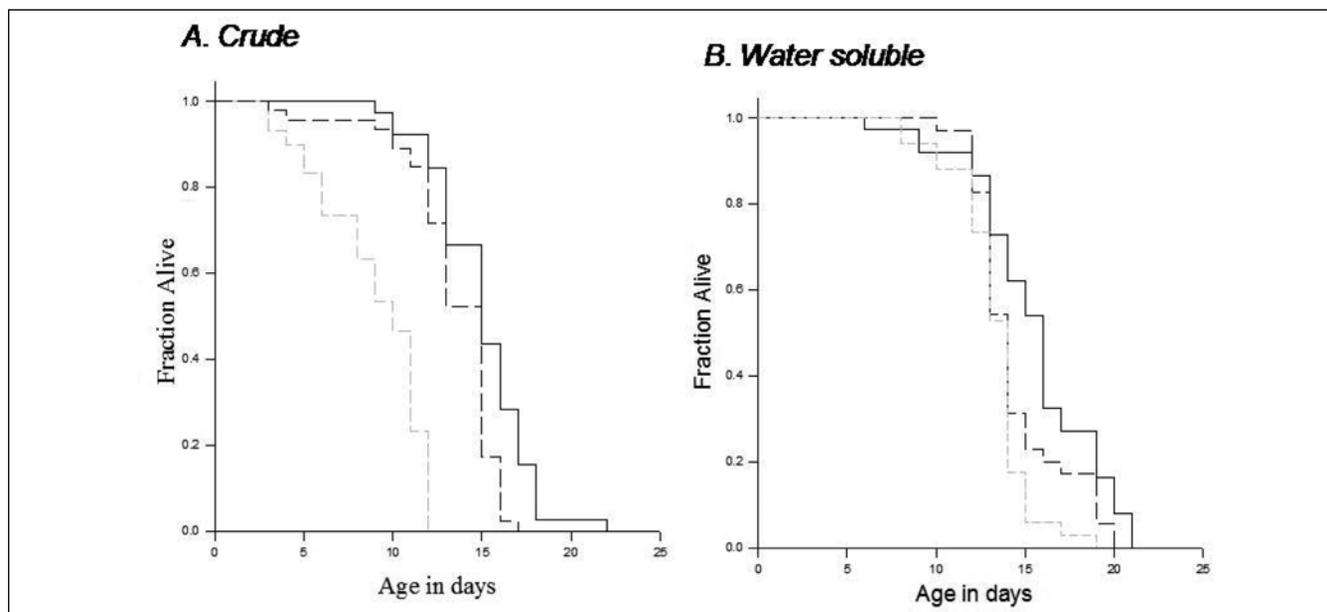


Fig. 1: Evaluation of RE for lifespan extension of *acr-16* mutant of *C. elegans*. A. Crude extract : Black line- NGM- (N = 112); Black dash- RE – 100 µg/ml (N = 131); Grey dash - and 1 mg/ml (N = 77). B. Water soluble: Black line- NGM- (N = 37); Black dash- RE (80% water soluble) – 100 µg/ml (N = 35); Grey dash RE (80% water soluble) – 100 µg/ml (N = 34).

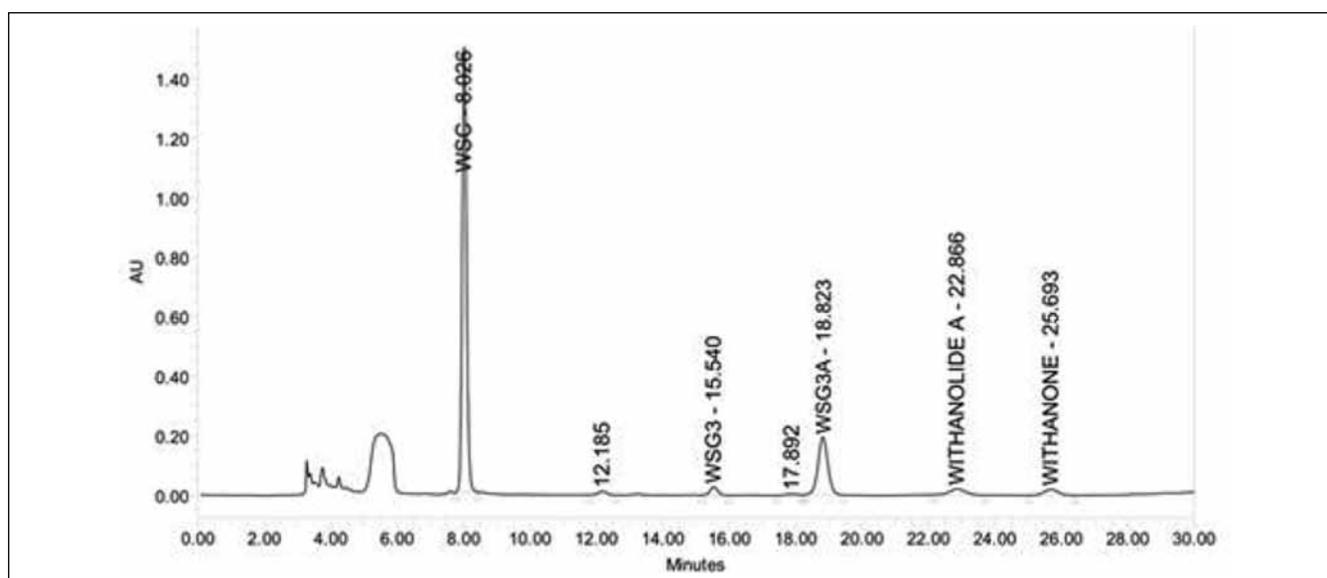


Fig. 2: Active compounds of RE – HPLC trace.

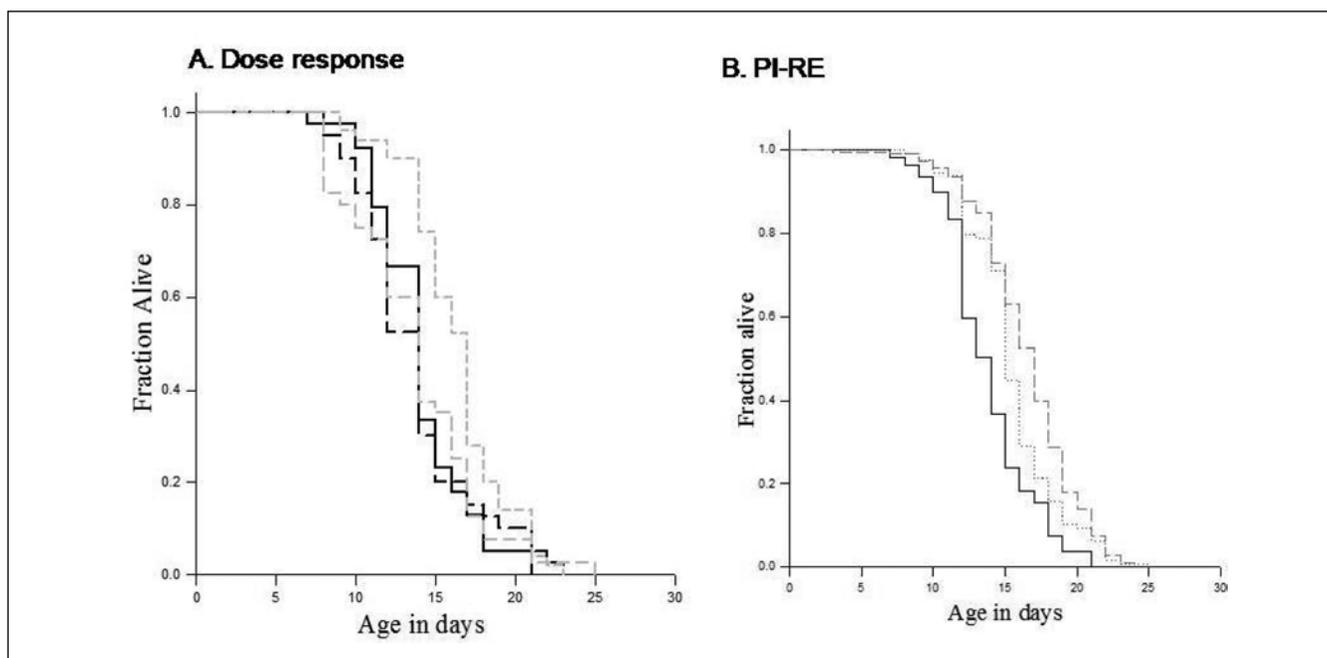


Fig. 3: Evaluation of purified compounds mixture-PI-RE for lifespan extension of *acr-16* mutant of *C. elegans*. A. Dose response : Black line- NGM- (N = 39); Black dash- PI-RE – 5 ng/ml (N = 40); Grey long dash - 10 ng/ml (N = 40); Grey short dash – 100 ng/ml (N = 50). B. PI-RE: Black line- NGM- (N = 109); Grey dash- Ethanol (N = 127) PI-RE 100 ng/ml (N = 213).

Table 1: PI-RE treatment extends *C.elegans* (*acr-16* mutant) lifespan

Sample	Median Lifespan + Std. Dev
NGM	14 ± 3 (109)
NGM + ethanol	15 ± 1.3 (127)
NGM + PI-KSM- 100 ng/ml	17 ± 3.14 (213)

background. The PI-RE treated and the control worm's median lifespans are shown in Table. 1. PI-RE showed 21.4% lifespan extension compared to the control (NGM medium) and ~14% in comparison to the vehicle (ethanol).

It is intriguing that PI-RE did not show lifespan extension in the wildtype worms or the enhanced RNAi mutant, *rrf-3*, but extended the lifespan of the nAChR, $\alpha 7$, subunit mutant, *acr-16*. Despite the reports that ashwagandha is given for healthy long life in ayurvedic medicine, there is no concrete evidence for the same. Here we show that Ashwagandha can indeed extend the lifespan of *C. elegans* adding credence to this report.

Various supplements and drugs are being evaluated for induction of lifespan extension in worms. Once identified in the worms, they can/are further screened in the mammalian model-mice. The search to find an ideal drug/compound, which could extend lifespan, is ongoing. Polyphenols in blueberry²² and resveratrol¹⁴ originally identified in the skin of the black grapes and reserpine from the plant *Raulwolfia serpentina* can extend *C. elegans* lifespan. Now, we find that another ayurvedically used herbal plant, Ashwagandha can extend lifespan (Fig. 3B). Interestingly, the vehicle ethanol *per se* is able to cause

marginal lifespan extension. In terms of drugs, the antiepileptic drug, trimethadione,²³ the antidepressant mianserin²⁴ and the antihypertensive drug, reserpine, extends the worm lifespan.¹¹ In addition, Rapamycin is shown to extend lifespan in worms and mice.²⁵ However the side effects need to be taken into consideration. Hence, it will be worthwhile to evaluate KSM-66 for longevity effects on higher animal model.

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