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Semi Automated Movement Based Screening of Aqueous Extracts of Cinnamon Leaf, Almond, Beetroot Carrot, Ashwagandha and Tulsi on Caenorhabditis Elegans Model System for Health Beneficial Impact

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The research was conducted at University of Agricultural Sciences Raichur during 2021-22. In this research Caenorhabditis elegans is utilized by the researchers to know the effects of various extracts, drugs, nano particles, xenobiotics, magnetic effects on behaviour, neuronal effects, feeding pathways, apoptosis, ageing related pathways. Experiments were performed to evaluate the traditional herbs (Cinnamon, Almond, Beetroot Carrot, Ashwagandha and Tulsi) consumption for their health beneficial effects by monitoring the movement as an end point. Movement of an organism is considered as the health and age influencing factor. As the movement is well maintained in organism means health is also well maintained. Hence consistent movement is the factor for the healthy life. In the present study the organism movement has been monitored from L4 onwards every day for 30 minute till 21st day of the survival by automated system. The worm movement during this period has been categorised based on activity in three categories from L4 to 4days the activity ranged from 28.0 units to 10 IR units, 5th to 15th day 27.82 to 14.67 and from 16th to 21st day ranged from 17.87 to 14.44 IR units in non exposed N2 wild type worms. There is a gradual increase in the activity has been recorded on the exposure to health beneficial extracts in a single dose of supplementation. Hence, the study concludes that the health supplements can impact on the move ability of worms and maintains consistent movement till 21 days. Further studies are required to establish comprehensive health beneficial effects of these extracts at organism level.

Keywords: Cinnamon; almond; beetroot; carrot; ashwagandha; Tulsi; C. elegans.

1. INTRODUCTION

Caenorhabditis elegans is a multi cellular organism that has many different organs and tissues. *C. elegans* is a model organism that has the potential to bridge the gap between *in vitro* and *in vivo* approaches by virtue of being amenable to high-throughput technologies while providing physiologically relevant data derived from a whole-animal setting. Several features of *C. elegans* make it a powerful tool for the pharmaceutical industry. These include being easy to culture; undergoing rapid reproduction with a short generation time enabling large-scale production of nematodes; small size, which allows assays of more than a hundred worms in a single well of a 96-well plate; transparency, which enables the use of fluorescent markers to study biological processes *in vivo*; and cellular complexity [1].

Some of the research that has been carried out in the areas of neuro, genetic and environmental toxicology, as well as high-throughput experiments with *C. elegans* including genome-wide screening for molecular targets of toxicity and rapid toxicity assessment for new chemicals. An increased role for *C. elegans* in complementing other model systems in toxicological research has been well established [2].

The global nematicide market is expected to continue growing with an increasing demand for synthetic chemical-free organic foods, botanical nematicides are taking the lead as replacements. Consequently, in the recent years, there have been vigorous efforts towards identification of the active secondary metabolites from various plants. These include mostly glucosinolates and their hydrolysis products such as isothiocyanates; flavonoids, alkaloids, limonoids, quassinoids, saponins and more recently probed essential oils, among others. The potency of the identified phytochemicals from the key important plant families and deciphered some of the impediments involved in standardization of the active compounds in addition to the concerns over the safety of the purified compounds to non-target microbial communities [3].

The life span extending herbs Shi Quan Da Bu Tang (SQDB) and Huo Luo Xiao Ling Dan (HLXL) significantly delayed human amyloid beta (Ab)-induced toxicity in transgenic *C. elegans* expressing human Ab. C [4]. Root extract (RE) and its purified ingredients (PI-RE) with a similar composition as in RE obtained from the roots of Ashwagandha (*Withania somnifera Dunal*- WSD)

for lifespan extension in the well-established model system, *C. elegans* [5].

The uncovering of instrument crucial human developing require essential device like model animals which can be manhandled in explore office conditions for examining potential mediations that can widen future. The animal giving homology to human will undoubtedly bestow essential framework to individuals and can filled in as an assessment gadget for examining developing miracle [6]. The gerontological research uses an extent of model living things, for instance, *Escherichia coli*, *S. cerevisiae*, *C. elegans*, *D. melanogaster* and *M. musculus* for mulling over the developing wonder [7].

Antioxidant and stress resistance potentials of *B. monnieri* aqueous extract (BMW) using *Caenorhabditis elegans* acts as an antistressor and potent reactive oxygen species scavenger which enhances the survival of the worms in different stress conditions [8]. *B.monnieri* prevents mitochondrial, and oxidative stress in the cultured cells. Furthermore, it can prolong the healthy lifespan of *C.elegans*, indicating that *B.monnieri* the potential for therapeutic and preventative use in neurodegenerative disease [9]

The mamey and carrot carotenoid extracts decreased the oxidative damage in *C. elegans* by 20–40 per cent. Extracts increased the resistance and enhanced the survival of nematodes, and showed better effects than pure β-carotene, probably owing to the complex mixture in the carotenoid extracts [10].

In year 1965, Sydney Brenner introduced free living soil nematode as a model structure with little size of 1mm, short life cycle, innate heritability and direct body gives basic section to focus every single telephone during progression [11]. *C. elegans* an ideal model to screen natural compounds that extend lifespan and investigate their positive health effects. Cherries, tomatoes and ginger are known to have health promoting effects as a means to increase health and healthy ageing. Effect of these foods in isolation, not as a blend of multiple functional foods.

Toxic response behaviour of *Caenorhabditis elegans* by automatic recognition of line movement through an image-processing system identified some sequential line-movements on nematodes that confirmed the toxicological effect on nematode behaviour [12].

Dark field illumination provides high-contrast images of the worms which are acquired by a video camera and fed to a microcomputer which is programed to simultaneously track and record the movements and changes in direction of as many as 25 animals [13]. Novel frustrated total internal reflection (FTIR)-Based Imaging Method for High Throughput Locomotion analysis of large groups of crawling Drosophila larvae using ARENA tracker [14].

WMicroTracker ARENA System captures population level activity data by relying on optical interferometry, which uses a large array of infrared LED microbeams to detect both the movement and position of worms on a culture plate [15].

The present investigation was performed to evaluate the traditional herbs consumed (Cinnamon, Almond, Beetroot Carrot, Ashwagandha and Tulsi) for their health beneficial effects in the model organism *C. elegans* to uncover the impact on activity in terms of health benefits for the 21 days of worm survival by the newly developed technology to monitor the movement based on IR rays tracking the small organism movement.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials and Extract Preparation

2.1.1 Plant materials

Were purchased from the local market: Cinnamon leaf, Almond, Beetroot, Carrot, Ashwagandha and Tulsi and were thoroughly washed with water. Samples were dried under shade and powdered. About 5 g of sample dissolved in 100 ml of sterile double distilled water and blended with electric blender for 10 m to make fine powder and incubated for 72 h with constant shaking conditions and it is filtered through Whatman filter paper No.1.The extracts thus prepared were stored in a refrigerator at 4ºC till further analysis.

2.1.2 Worm collection

The N2 (Wild-type) strain of *Caenorhabditis elegans* was procured from Caenorhabditis Genetics Center (NIH funded National Center for

Research Resources, Minneapolis, MN, USA). The worms were transferred to nematode growth medium (NGM) plate pre-seeded with a lawn of Escherichia coli (strain OP50) and cultured for 3 days at 20ºC until they reached adulthood. The worms were washed off from the plate with cold K-medium (53 mM NaCl,32 mM KCl), pelleted by centrifugation (3000 g, 5 m), washed again twice with cold K-medium, and finally suspended in Kmedium to obtain 30 to 50 nematodes per 10 µl [11].

2.2 Observations were Recorded by Using WMicrotracker ARENA to Know the Effect on Movement of Worms till 21st Day on Exposure to Extracts

Movement quantification in terms of activity units by W Micro tracker ARENA [16]. Breifly, a locomotor activity recording system, W Micro tracker ARENA (Phylum tech, Argentina), detects through infrared microbeam interruptions. Detection of the analog signal is mathematically processed to detect the movement of the small worms in the sensing arena. Synchronized L4 stage worms were used this experiment. The worms were removed from the NGM plates and washed with K-medium thrice. The basal movement was measured for 60 m at 23^{0}C. Using a 24 well plate that contains worms (75-100) in each well with 1.5mlof M9 media with OP-50 (OD at 550 nm adjusted to ± 01 with OP50) and FUDR at a final concentration of (0.6 mM) to avoid hatching of the embryos and the worms have been raised and maintained at 20ºC with shaking conditions were maintained for 21 days. The wells were loaded with 10µl of extract in the 1.0ml of media for the final concentrations of plant extracts with control for the assay period. The survivability was scored every day by gently touching with platinum wire to the worms. The worms which failed to move in response to touch are considered as dead. The ARENA tracker was utilised to monitor the locomotion activity on the daily basis till 21st day of worm survival on exposure. The 24 well plates with flat bottom were fed to the WMicrotracker ARENA and it was set up for 30 m of analysis through computerised software. After 30 min of movement recording the results were recorded in excel sheet which showed the activity of the worms on exposure. The data was expressed as movement activity in Infra red units and converted into percent movement [16].

3. RESULTS AND DISCUSSION

The present investigation was envisaged to study the effect of six plant extracts *In vitro* against *Caenorhabditis elegans*. Impact of plant extracts on activity in *Caenorhabditis elegans* by using WMicroTracker ARENA.

This experiment was performed by using the six aqueous extracts and the activity of movement was recorded by using WMicroTracker ARENA. This instrument quantifies movement and survival of worms in plant extracts. The results of the extracts was analysed based on IR units were the instrument tracks the worm movement based on infrared ra ys (IR) in terms of IR units. The IR units are measured every day for the 30 m at a fixed time of the day and the IR units in terms of movement were averaged for the movement determination for all the samples under study.

In cinnamon leaf the highest and lowest activity was recorded in day 1 (11.64 activity/m) and day 21 (17.93 activity/m) (Fig. 1) as cinnamon contains the biologically active chemical constituents such as trans-cinnamaldehyde, cinnamyl acetate, 3-phenylpropionaldehyde, coumarin and linalool which are involved in antidiabetic activity, antioxidant effect, neurological disorder and anti-inflammatory activity [17].

On exposure to Almondthe highest and lowest activity was recorded in day 1 (11.27 activity/m) and day 21 (27.28 activity/m), because almonds are a source of Proanthocyanidins (Epicatechin and catechin), hydrolyzable tannins, anthocyanidins, Proanthocyanidins which reduces the risk of metabolic syndrome, Antioxidant activity and pancreatic lipase inhibition [18].

On feeding of Beetroot the highest and lowest activity in day 1 (11.09 activity/m) and day 21 (25.29 activity/m)as beet roots contain betalamic acid, betaxanthins and betacyanins which helps in regulating blood pressure [19].

On feeding of Carrotthe highest and lowest activity in day 1 (14.45 activity/m) and day 21 (19.38 activity/m) as carrots contain acidic oligosaccharides which are able to block the adherence of various entero pathogenic

microorganisms to Hep -2 cells and human intestinal mucosa [20].

On exposure to Ashwagandha the highest activity was recorded in day 8 and day 14 (39.73 activity/m) and the lowest was recorded on 21st day that that was (4.3 activity/m). Ashwagandha contains the biologically active chemical constituents such as Withanosides, Withanolides, Withaferin A and Withanolide A. Studies indicate ashwagandha possesses Antioxidant, anti-diabetic and Reduction in cell viability of cervical cancer [21].

On exposure totulsi the highest activity was recorded in day 6 (31.36 activity/m) and the lowest as recorded on 26th day that was 4.4 activity/m and the worms have not survived on 27 day of observations (Fig. 1). The major active constituent of tulsi is glutathione that protects the bio markers of organ damage, oxidative stress, antioxidant enzymes, mitochondrial respiratory chain enzymes [22].

The selected once enhancing or supporting the worms to complete its equivalent to having measurable movement has been selected for the study *i.e* cinnamon leaf, almond, beet root, carrot, ashwagandha and tulsi were determined by ARENA TRACKER. All the six samples showed the movability till 21 days. Comparison of the activity can be classified in to four blocks based on days and activity from day 1 to day 5 the activity ranged from 11.00 to 30.09 activity/m (Table 1). Similarly day 6 to day 14 activity ranged from 33.27 to 44.91 activity/m (Table 2) followed by day 15 to day 21 the activity ranged from 44.91 to 20.00 activity/m from day (Table 3). Similar evidence has been reported by Hahm et al*,* [23] who observed that maximum velocity of worm with mutations in daf-2(e1370) insulin/IGF-1 signalling

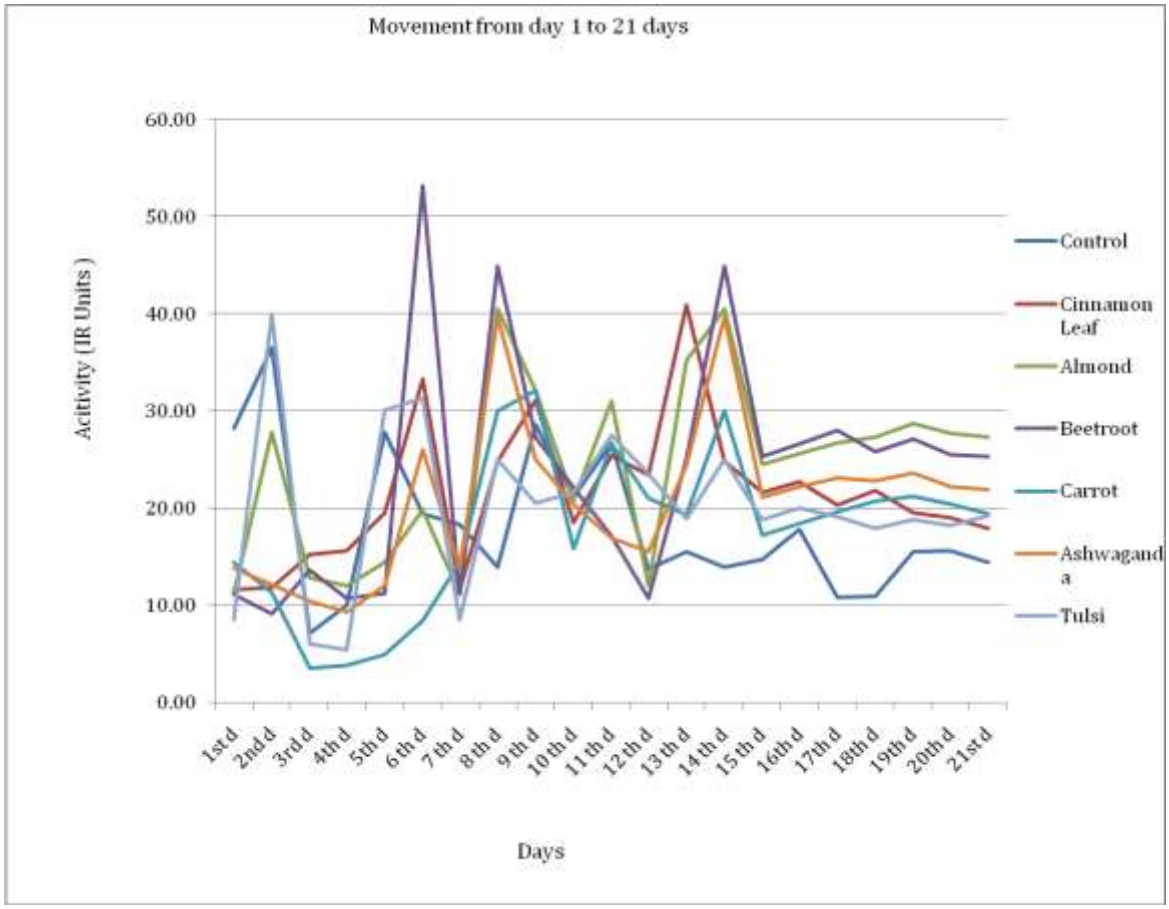

Fig. 1. Arena Tracker activity count of worms and extracts from L4 stage onwards till 21st day of survival

Table 1. The arena tracker based activity (IR units) of worms on treatment with extracts for four days from L4 stage onwards

S. No	Sample Names	1st day	2nd day	3rd day	4th day
1	Control	28.18	36.55	7.09	10.00
2	Cinnamon Leaf	11.64	11.82	15.27	15.64
3	Almond	11.27	27.82	12.91	12.09
4	Beetroot	11.09	9.09	13.55	10.73
5	Carrot	14.45	11.36	3.55	3.82
6	Ashwagandha	13.82	12.18	10.45	9.36
7	Tulsi	8.55	39.91	6.09	5.45

Table 2. The Arena trackeer based activity (IR units) of worms on treatment with extracts from 5th to 15th day

Samples Name	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day
Control	27.82	19.45	18.36	13.91	28.64	21.11	26.73	13.66	15.50	13.91	14.67
Cinnamon Leaf	19.55	33.27	11.64	24.82	31.09	18.57	25.45	23.46	40.90	24.82	21.66
Almond	14.45	19.82	11.27	40.45	32.09	20.82	31.00	12.09	35.18	40.45	24.52
Beetroot	11.18	53.18	11.09	44.91	27.27	22.11	17.09	10.73	25.09	44.91	25.30
Carrot	4.91	8.45	14.45	30.00	32.09	15.81	26.64	20.99	19.27	30.00	17.21
Ashwaganda	12.09	26.09	13.82	39.73	25.00	20.45	16.99	15.56	24.45	39.73	21.14
Tulsi	30.09	31.36	8.55	25.00	20.54	21.66	27.55	23.57	18.98	25.00	18.79

Table 3. The arena trackeer based activity (IR units) of worms on treatment with extracts from 16th to 21st day

S. No	Sample Name	16th day	17th day	18th day	19th day	20th day	21stday
1	Control	17.85	10.82	10.91	15.48	15.62	14.44
2	Cinnamon Leaf	22.75	20.34	21.79	19.51	19.02	17.93
3	Almond	25.65	26.67	27.29	28.75	27.68	27.28
4	Beetroot	26.62	28.03	25.74	27.07	25.45	25.29
5	Carrot	18.42	19.65	20.67	21.23	20.44	19.38
6	Ashwaganda	22.21	23.13	22.86	23.68	22.23	21.97
7	Tulsi	20.01	19.09	17.97	18.83	18.27	19.24

Related evidence has been reported [5]. several ayurvedic preparations are claimed to have longevity enhancing effects. 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 were there was no effect on the wild type worms, the mutant for the human nicotinic acetylcholine receptor, nAchR, α7 equivalent, acr-16, showed around ~20 per cent activity when treated with its purified ingredients.

4. CONCLUSION

Plant extracts tested in the present investigation were found effective in conserving the worm movement from L4 stage onwards till the 15 days and the period and movement was conserved better than the control non exposed worms to extracts. This establishes that the movement is a key factor and can be maintained by the health supplements evaluated. Further study is in progress to understand the health beneficial effects of these extracts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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