Antinematodal Potential of Leaf Extracts of *Adiantum Raddianum* and *Artemisia Absinthium* with the Model Organism Caenorhabditis elegans

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ABSTRACT:

Nematodal diseases are treated with a number of drugs. However, because of their toxicity and the resistance that worms developed, it is important that we have to produce highly effective drugs that are both safe and effective. The purpose of this research was to exploit the potential of nematocidal effects of Adiantum Raddianum (A. Raddianum) and Artemisia Absinthium (A. Absinthium) against Model Organism Caenorhabditis elegans. worldwide parasitic nematodes can cause significant morbidity and mortality in humans and livestock. selected medicinal plants, Adiantum Raddianum (A. Raddianum) and Artemisia Absinthium (A. Absinthium) Antinematodal potential at the cellular level has been studied for the preparation of the extract, distilled water, methanol, and n-hexane were used. Antinematodal, anti-egg hatching, Fluorescence microscopy and Gene expressions of selected genes gst-4 and hsp-6 were used in RT-PCR. The extracts of A. Absinthium methanol (LD50 = 3.0 mg/mL) and A. Raddianum D. water (LD50 = 4.80mg/mL) potent Antinematodal effect. Strong anti-egg hatching activity at (1mg/mL). Adiantum Raddianum methanol (LD50 = 3.12mg/ml), Artemisia absinthium methanol (LD50= 3.00mg/ml) had a strong effect on by *C. elegans* egg laying activity, resulting in a 50% reduction of egg laying when exposed to plant compounds. The methanol extracts of A. Absinthium and A. Raddianum had a potent apoptotic effect on the intestines, muscles, uterus (eggs), and gonads of c. elegans. Nematicidal effect of plants due to phytochemicals. Both plant extracts tested in tables 1.10 and 1.11 showed **statistically significant** effects ($p < 0.05$, often much lower) on at both stress-related gene in *C. elegans*. Genes gst-4 and hsp-6 shows strong expression during quantitative RT-PCR after treated with plant compounds. Our research recommended that both plants could be used as alternative treatments to newly developed, broad-spectrum Antinematodal drugs.

1. INTRODUCTION.

The human health is severely affected by parasitic nematodes, it also effects the animal and plant productions due to this effect decreases the worldwide economic growth [1]. Around the world, Parasitic Nematode significantly increase morbidity and mortality rates in human population [2] 4,000 different species of nematodes effect plants [3]. WHO reported, approximately two billion individuals are infected with parasitic worms. People in poor nations are particularly vulnerable to nematode parasites (roundworms) infections [4]. Many alternative plant-based treatments are available in the market to treat plant-parasitic nematodes [5]. The majority of natural and pharmaceutical industries use medicinal plants as a source of drugs. Out of the 250,000 species of higher plants on Earth, approximately 80,000 are medicinal plants [6]. Pharmaceutical industries in Pakistan used around 600 species of medicinal plants [7]. Biologically active phytochemicals with high activity and less toxicity are obtained from medicinal plants [8]. A very huge number of secondary metabolites present in Medicinal plants, it consists of glycosides, sesquiterpenoids, isothiocyanates, alkaloids, steroids, polyacetylenes, diterpenoids, triterpenoids, lipids, quassinoids, simple and complex phenolics, glucosinolates, and various other classes of plants contains tannins, flavonoids, and polyphenols [9]. Secondary metabolites present in plants having a strong nematicidal effect [10]. Almost all the information available yet about the physiology, anatomy and genetics of nematodes has been derived from *C. elegans*. short life cycle, low cost, transparent body, simple, rapid growing, and sequenced genome [11]. In medicinal research for human and veterinary parasites, C. elegans is an excellent model organism. The nematodes and trematodes cause helminth infections in humans and livestock animals [12]. Filariasis, Strongyloidiasis, River blindness, and other diseases are caused by nematodes. The three most often used medications to treat nematodes are albendazole, levamisole, and piperazine [13]. It is the only new anthelmintic medication approved for human use in the past thirty years [14]. Because of the continuous usage of these drugs, nematodes have become resistant, which leads to crop loss and human diseases. Adiantum Raddianum and Artemisia absinthium contains secondary metabolites having nematicidal properties that showed outstanding results [15]. In my research the antinematodal potential of the selected plant extracts were used. To study the effect of potential plant extracts on Morphology, cellular changes and reproduction of C. elegans. To analyze the expression of selected genes in response to different plant extracts. To analyze the phytochemical constituent of most active plant extracts through GC-MS analysis.

2. Material and Methods.

Instrument and chemicals.

All chemicals, including solvents and reagents, were purchased from Sigma-Aldrich USA, (WIZ BioSolutions Korea and GeneON). The following equipment was used in the study, the rotary evaporator (SD-RE 52A China), and plants grinding machine (HR40B-China), a bright field microscope (IRMECO), the fluorescent microscopy (B-510FL, Italy), the small centrifuges (5425-R Hamburg, Germany).

Strains of Bacteria and C. elegans.

C. elegans N2 strain, E. coli (OP50) bacterial strains were used, and they purchased from the University of Minnesota Caenorhabditis Genetic Centre in the United States.

Medicinal plants Collection and Extract preparations.

A. Raddianum and A. Absinthium Plants were collected in the District Mansehara of Khyber Pakhtunkhwa, Pakistan) and Muzaffarabad Division (Azad Jammu and Kashmir, Pakistan). A Thomas Willey milling machine was used to grind the leaves of both medicinal plants into a fine powder after they had been shade-dried. The extracts were made using three different solvents N-hexane, Methanol, distilled water (D-water). 2L of each solvent were used to soak 500 grams of dried leaf powder up to 15 days a room temperature. After 15 days, extracts were dry by using rotary evaporator, and stored for future use. Filtration was done using filter paper (Whatman no. 42, 125 mm). All extracts were diluted in DMSO.

GC-MS Analysis.

Plant Compounds that were volatile or semi-volatile were found using gas chromatography–mass spectrometry analysis. The standard methodology established by Makkar et al. (2018).

Nematicidal Characterizations.

Antinematodal Activity.

The antinematodal activity of *Adiantum Raddianum* and *Artemisia Absinthium* leaf extracts was investigated using *Caenorhabditis elegans* as the model organism. A nematode media (100 µL of nematode growth medium containing 30 to 50 *C. elegans*) was added to each well of a sterile 96-well plate. Various concentrations of the leaf extracts (1.25, 2.5, 5, and 10 mg/mL) were then introduced into the wells. The plates were incubated at 20 °C for 24 hours. After the incubation period, nematode survival was assessed. Viability was determined by observing worm mobility under a microscope worm that failed to respond the stimulation with a fine needle were considered dead.

Anti-egg Hatching Activity.

Eggs isolation from C. elegans.

The Escherichia coli (OP50) strain was used to seed an NGM plate with the N2 strain of *C. elegan*. The nematodes were washed off of the agar plates using NGM buffer and then put into a 50mL conical tube. Centrifuged the worms twice for two minutes at 1000 rpm, and then again while placed in the NGM buffer. A single 15 mL tube holding up to 0.5 mL of gravid worms was treated for five minutes. with 5 mL of a solution of hypochlorite (3.75 mL sterile water, 1 mL household bleach, and 250 µ L 10 M NaOH). Only eggs were remained when all C. elegans were dissolved. The eggs were centrifuged three times at 1000 rpm, 1 minute using NGM buffer, and they were again suspended in 10 milliliters of NGM buffer. Test were conducted using eggs to find out the anti-egg hatching activity of the leaves extracts of A. Raddianum and A. Absinthium.

Plant extracts effect on C. elegan Egg hatching.

In order to investigate the effect of plant extracts of A. Raddianum and A. Absinthium, Using a sterile 96-well plate, different leaf extract concentrations (1.25, 2.5, 05, and 10 mg/mL) were used. Each well contained 100 µ L of the NGM buffer and contained 40–80 C. elegans eggs and different concentrations of leaf extracts of A. Absinthium and A. Raddianum. The only substances present in a control sample were egg and NGM buffer. Plates were set up for 24 hours at 20 °C. Following the incubation time, after incubation under microscope number of eggs and larvae were counted [16].

Effect of Plants Compounds on C. elegans Egg Laying.

In order to study the egg-laying defect, worms were placed on NGM-containing plant extract agar plates. A few of the adult hermaphrodite worm were placed in to fresh agar NGM plates and given 03 h to lay eggs in order to grow age-synchronized worms. Adult hermaphrodites were removed after egg laying, and the NGM plates kept at 20°C to enable the eggs to develop into larva and adult hermaphrodites. 10 adult hermaphrodites with age synchronized were placed in new NGM plates with their LD50 values of Adiantum Raddianum (LD50 = 3.12 mg/ml methanol) and Artemisia Absinthium (LD50 = 3.0 mg/ml methanol). At 20°C, each plate was incubated. After counting the number of eggs on each plate, the worms were moved to new NGM plates with the exact same concentration every 24 hours. 05 consecutive days had been given to counting eggs and moving worms to clean plates. Same time in a day under microscope counted the numbers of eggs.

Fluorescence Microscopy.

The apoptotic effect of extracts of A. Raddianum and A. Absinthium were analyses by using fluorescence microscopy.

Affected C. elegans staining with AO.

Plant extracts of A. Absinthium and A. Raddianum were used to treat C. elegans (at LD50 concentrations). AO dye was used to the dead C. elegans after it had been incubated for 24 hours. From the AO stock solution (10 mg/ mL), 2 µ L was added in 01 mL S-medium, then 500 µ L of AO was added to C. elegans and incubated for 1 h at 37 °C in dark. After 1 h, C. elegans was fixed on a slide and washed with NGM buffer to prepare for fluorescence microscopy (OPTIKA B-510FL Italy) [17].

RNA extraction.

Newly cultured agar plates containing adult *C. elegans* were treated with leaf extracts of *Adiantum Raddianum* and *Artemisia Absinthium* at a concentration of 1.25 mg/mL. The worms were incubated with the extracts for 3 hours. After incubation, the worms were washed three times with NGM buffer to remove any remaining bacteria. The motile *C. elegans* were then collected into centrifuge tubes, and total RNA was extracted using the Triazole method. The concentration of RNA was determined by taking 0.5ml tightly packed volume of mix population of C. elegans in Eppendorf tube after washing with M9 buffer. After washing 1-1.5µg/µL of RNA was extracted through triazole method (Cornell university, 2020) [18].

cDNA Synthesis.

cDNA synthesis was performed using the WizScript High-Capacity cDNA Synthesis Kit. A 10 µL cDNA master mix—comprising 10X buffer, 20X dNTP mix, random hexamers, WizScript RTase, RNase inhibitor, and RNase-free water—was added to each PCR tube containing mRNA. The thermal cycling program for cDNA synthesis included: annealing at 25 °C for 10 minutes, extension at 37 °C for 2 hours, enzyme inactivation at 85 °C for 15 minutes, followed by an indefinite hold at 4 °C. (Wizbiosolutions, Korea).

Quantitative RT-PCR.

WizPure™ qPCR Master (SYBR) with ROX Dye kit (Wizbiosolutions, Korea) was used to perform the RT-PCR analysis. A 20µL reaction mixture was used for RT-PCR assays. This mixture contained nuclease-free H_2O, qPCR Master (SYBR), reverse and forward primers, and 18µL of q RT-PCR master mix. To the mixture, 2µL of template cDNA was also added. An instrument known as the Quantitative RT-PCR was used to conduct the q RT-PCR. After being sealed, the labelled PCR 96-well plate was put inside the q RT-PCR apparatus. The 96-well plate-specific codes for the RT-PCR program were added to the apparatus. In order to perform the quantitative analysis of the samples, the q RT-PCR machine was finally operated. The polymerase was first activated at 95°C for 10 minutes, and then the template was denaturated for 15 seconds at the same temperature. Annealing and elongation were completed for 60 seconds at 60°C. a melting curve analysis was carried out following the completion of 40 cycles by progressively raising the temperature from 60°C to 95°C. Finally, check that the completed, unique final product is present. Use CT values for each selected gene. Next, input these expression data for various desired genes into an Excel sheet to produce the related graphs.

RESULTS AND DISCUSSION.

Statistical Analysis.

Graphing was performed using Origin (version 8). Mean and standard deviation were calculated using Statistic (version 8.1). Gene expression analysis was carried out using Prism GraphPad (version 9).

Antinematodal activity.

Antinematodal potential of Adiantum Raddianum.

To analyses the antinematodal properties of leaf extracts of A. absinthium and A. Raddianum. The model organism used was *C. elegans.* The antinematodal properties of A. Raddianum (leaf extracts) were observed in this study. C. elegans were exposed to n-hexane, distilled water, and methanol extracts made from A. Raddianum leaves at different concentrations of 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, and 10 mg/mL were used Comparing the three extracts to the control groups, there was a noticeable increase in activity. all extracts were dissolved in ≤ 1% DMSO for the negative control. The n-hexane and methanol extracts of A. Raddianum leaves killed 100±7.54% and 95.33±5.85% of C. elegans at a concentration of 10 mg/mL, respectively, whereas the distilled water extract killed 88±11.13% of C. elegans. The n-hexane, methanol, and distilled water extracts killed 84±9.53%, 80±9%, and 78±7.93% of C. elegans at a concentration of 5 mg/mL, respectively. The n-hexane, methanol, and distilled water extracts killed 73.33±7.02%, 75.21±8.50%, and 77±9.01% of C. elegans at a concentration of 2.5 mg/mL, respectively. The methanol, n-hexane and distilled water extracts killed 60.33±7.02%, 55.33±5.033%, and 53.66±8.08% of C. elegans at a concentration of 1.25 mg/mL, respectively. For every fraction (extract), the negative control Negative Control (≤ 1% DMSO) showed 4±4.0%, 4±1.52%, 2±1% and 1±1.3 potential. The current investigation analyzes important results about the A. Raddianum extracts. When tested against C. elegans, every extract showed impressive antinematodal activity. As shown in Table. 1.1.

Table. 1.1: Nematicidal activity of extracts in A. Raddianum leaves at different concentrations in percentage.

Concentration	N-hexane	Methanol	D- water	Negative Control (≤ 1% DMSO)
10 mg/mL	100±7.54	95.33±5.85	88±11.13	4±4.0
05 mg/mL	84±9.53	80±9	78±7.93	4±1.52
2.5 mg/mL	73.33±7.02	75.21±8.50	77±9.01	2±1
1.25mg/mL	60.33±7.02	55.33±5.033	53.66±8.08	1±1.3

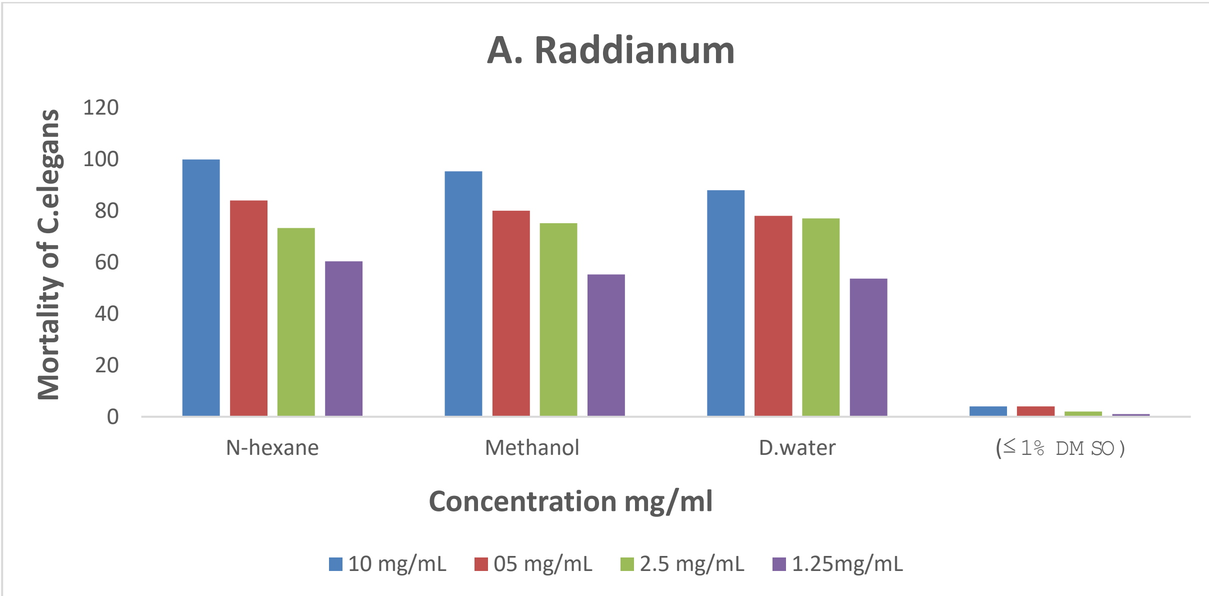


Figure 1.1: Antinematodal potential of Adiantum Raddianum

Antinematodal potential of Artemisia Absinthium.

The antinematodal potential of A. Absinthium leaf extracts were investigated in this study. C. elegans were exposed to n-hexane, methanol, and D. water extracts of A. Absinthium at 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, and 10 mg/mL were the selected concentrations. Comparing the three A. Absinthium extracts to the controls (negative control = 1% DMSO), all three displayed high activity as shown in Table 1.2 and Figure 1.2.

The n-hexane extract killed 100.33±7.02% of C. elegans at a concentration of 10 mg/mL, while the methanol and distilled water extracts killed 98.33±2.081% and 90.66±7.094% of C. elegans, respectively. The n-hexane extract killed 84.33±5.033% of C. elegans at a concentration of 5 mg/mL, whereas the methanol and distilled water extracts killed 77.66±3.78% and 80±5.56% of C. elegans, respectively. The n-hexane, methanol, and distilled water extracts killed 74.33±7.37%, 62±3.60%, and 60±9.53% of C. elegans at a concentration of 2.5 mg/mL, respectively. The n-hexane, methanol, and distilled water extracts killed 55.33±5.0322%, 44.66±5.033%, and 47.33±3.51% of C. elegans at a concentration of 1.25 mg/mL, respectively. The negative control showed % potential of 1±1.7%, 2±1.52%, 2±1.1%, and 1±1 respectively. As shown in Table 1.2.

Table 1.2: Nematicidal activity of leave extracts of A. Absinthium in %age at different concentration

Concentration	N-hexane	Methanol	D- water	Negative Control (≤ 1% DMSO)
10 mg/mL	100.33±7.02	98.33±2.081	90.66±7.094	1±1.7
05 mg/mL	84.33±5.033	77.66±3.78	80±5.56	2±1.52
2.5 mg/mL	74.33±7.32	62±3.60	60±9.53	2±1.1
1.25mg/mL	55.33±5.0322	44.66±5.033	47.33±3.51	1±1

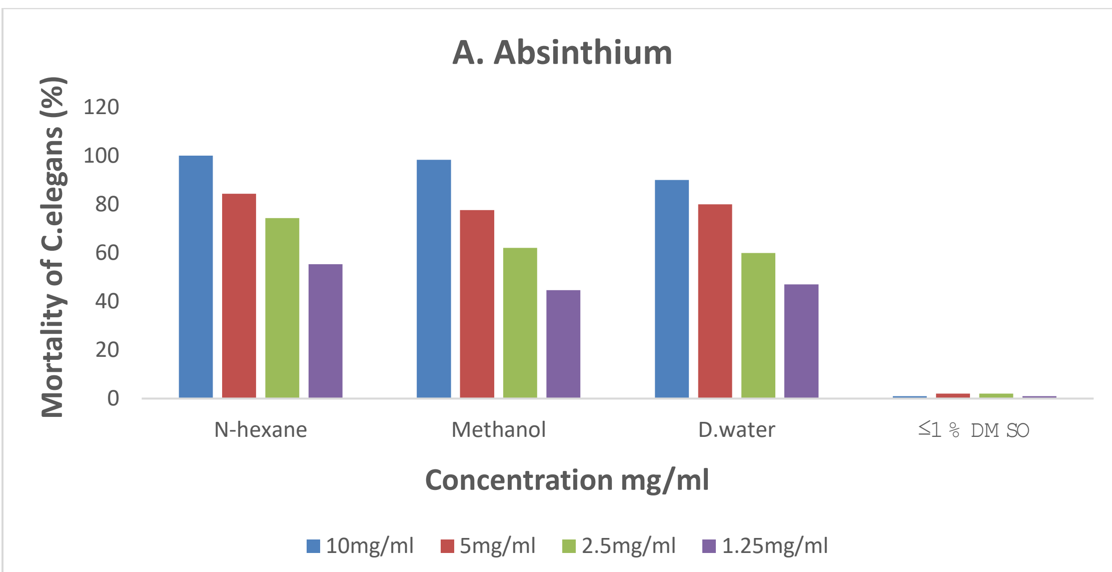


Figure 1.2: Antinematodal potential of A. Absinthium

Effect of plant compounds on C. elegans egg hatching.

Leaf extracts from A. Raddianum and A. absinthium, each at a 1 mg/mL, were investigated against C. elegans eggs together with a negative control (≤1% DMSO) and the unhatched eggs were 50±5, 55.33±3.06, 48.67±10.40, and 4±1.53 in the A. Raddianum methanol, n-hexane, distilled water extracts, and the control sample, respectively. Over 50% of the eggs in the D-water and methanol extracts were unhatched (EC50). The unhatched eggs were observed to be 50.33±2, 57±5.0, 62.34±10.21, 4±4, and in A. Absinthium methanol, n-hexane, D-water, and the control sample, respectively. Over 50% of the eggs in the methanol and n-hexane extracts were unhatched (EC50) [17]. As shown in Table. 1.3.

Table 1. 3: Effect of A. Raddianum leaves extracts on C. elegans egg hatching

A. Raddianum	**Unhatched Eggs** **(% Age)**
Methanol	50±5
N-hexane	55.33±3.06
D. water	48.67±10.40
Negative Control **≤1 % DMSO**	4±1.53

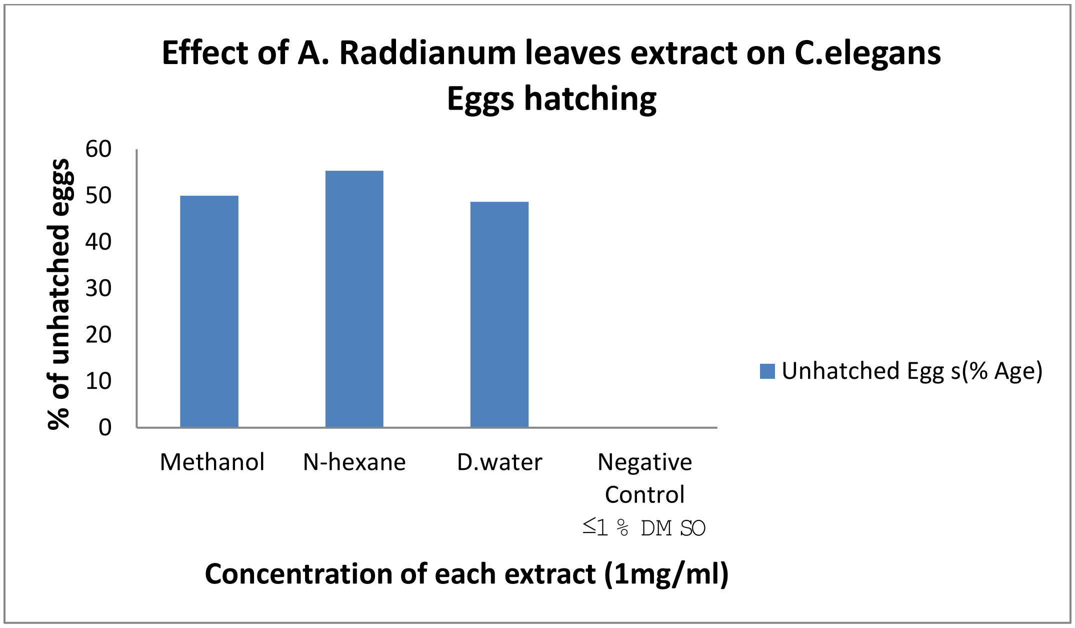


Figure 1.3: Effect of A. Raddianum leaves extracts on C. elegans egg hatching

Table 1.4: Effect of A. Absinthium leaves extracts on C. elegans egg hatching

A. Absinthium	**Unhatched Eggs (% Age)**
Methanol	50.33±2
N-hexane	57±5.0
D. water	62.34±10.21
Negative Control ≤1 % DMSO	4±4

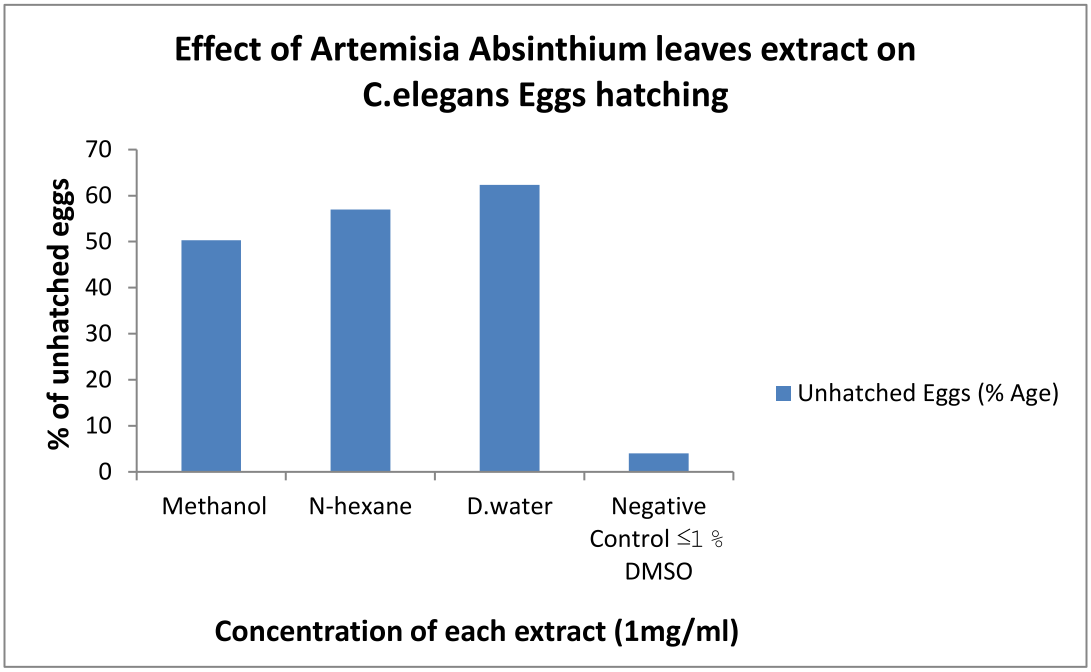


Figure 1.4: Effect of A. absinthium leaves extracts on C. elegans egg hatching

Effects of plant compounds on C. elegans egg laying.

Effects of Plant Compounds on C. elegans Egg-Laying. Hermaphrodite C. elegans is capable of self-fertilization. C. elegans produced sperms in larval (L4) stage and retaining it in the spermatheca [19]. The worms shift to oocyte production during their last moult before becoming adults. After becoming ovulated, after maturing, the oocytes travel to spermathecae, to mate with sperm. The mature hermaphrodite's uterus contains the fertilized eggs. A young adult hermaphrodite's uterus normally contains 10–15 eggs (source: http://www.wormbook.org/toc_neurobiobehavior.html). We investigated the effect of plant compounds Adiantum Raddianum methanol (LD50 = 3.12mg/mL), Artemisia absinthium methanol (LD50 = 3.0mg/mL). The adult (L4) stage worms were allowed to lay their eggs on NGM plates that contained plant Compounds. Over the five days, C. elegans egg-laying behaviors was observed on NGM plates containing plant compounds at **LD_{50}** concentrations. After a 24-hours of incubation, Counted the egg laying in NGM plates under microscope everyday same time. the plant compound effect the egg-laying behaviors of C. elegans. As a result, 50% drop in egg-laying at plant-based compounds in NGM plates. As shown in Table. 1.5.

Table 1.5: Egg laying of (LD_{50}) A. Raddianum and A. Absinthium leaf extracts

Plant Compounds	Day 1 Numbers Egg Laying	Day 2 Numbers Egg laying	Day 3 Numbers Egg Laying	Day 4 Numbers Egg laying	Day 5 Numbers Egg laying	⩽ 1 % DMSO
Adiantum Raddianum LD_{50} (3.12mg/ml)	45±2.0	40±3.1	31±2.9	26±4.0	18±3.6	3±2.1
Artemisia Absinthium LD_{50} (3.0mg/ml)	48±2	39±5	33±4.1	28±5.3	19±4.7	6±3.0

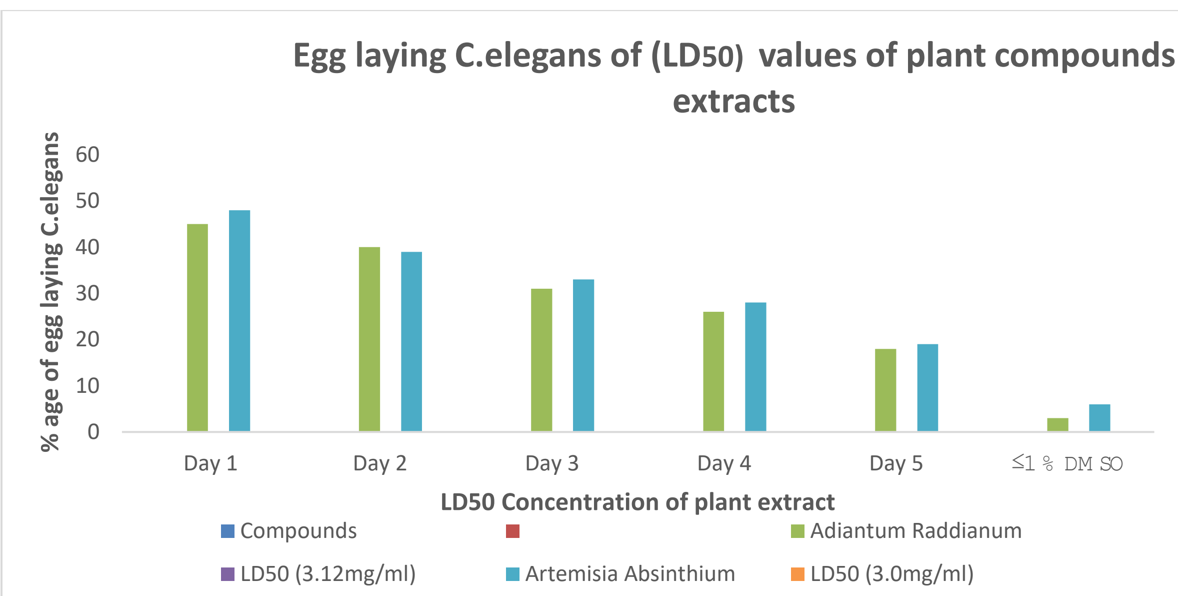


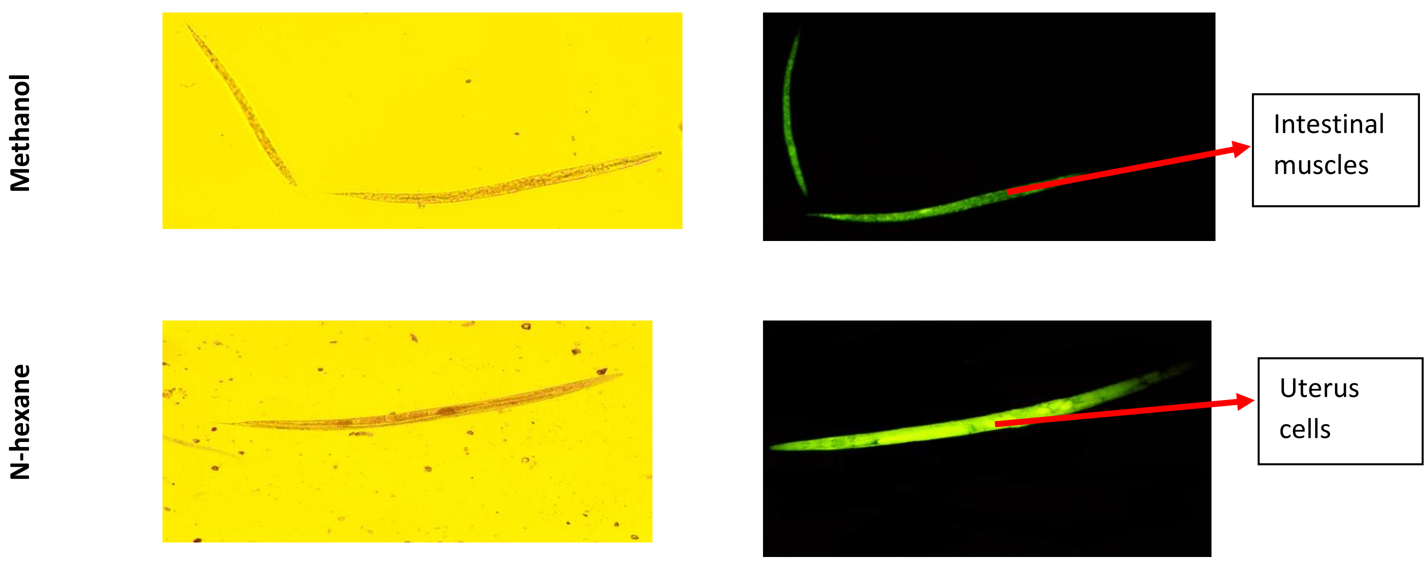
Figure 1.5: Egg laying of (LD50) A. Raddianum and A. Absinthium leaf extracts

Fluorescence Microscopy.

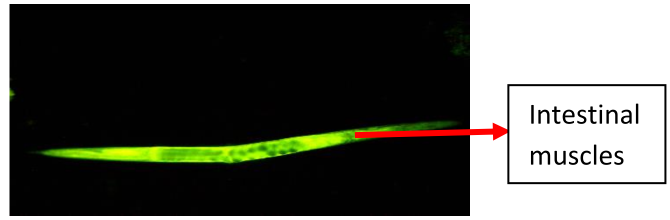
When study the apoptotic effect of C. elegans with leaf extract treatments of A. Raddianum and A. absinthium, fluorescence microscopy is a highly effective method. Intestinal cells treated with methanol extract display green fluorescence, which indicates that the intestinal muscles had apoptotic effect. Only gonadal cells display green fluorescence in the extract of n-hexane the green color displays in uterus. It is important to remember that apoptosis, which results in cell death, happens in the regions that glow green or yellowish-green. Every extract indicated an important level of apoptosis. In the uterus, muscle, and intestine cells, the methanol and distilled water extracts showed a green glow within the gonadal cells, the fluorescence was yellowish-green. It indicates that only intestine, muscular, and uterine cells undergo apoptosis. Only the uterus emits green fluorescence from the N-hexane extract. Both the distilled water and methanol extracts of A. absinthium exhibited substantial levels of apoptosis. Leaf extracts of A. Raddianum and A. Absinthium include a number of active substances (primary and secondary metabolites) that cause apoptosis in C. elegans developing organs, resulting in cell death.

1.6 Fluorescence images of Adiantum Raddianum (N-hexane, methanol, and D. water)

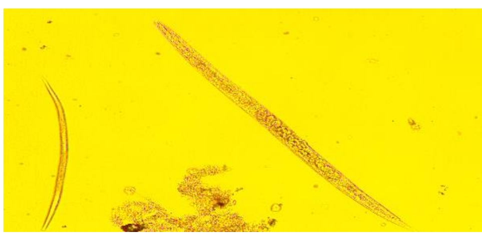
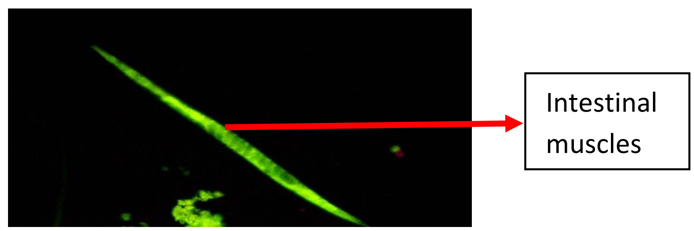
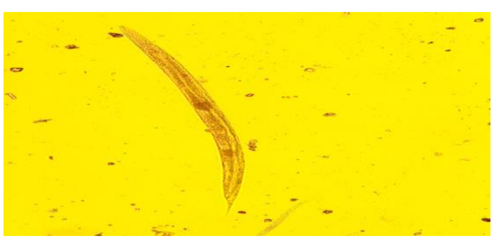
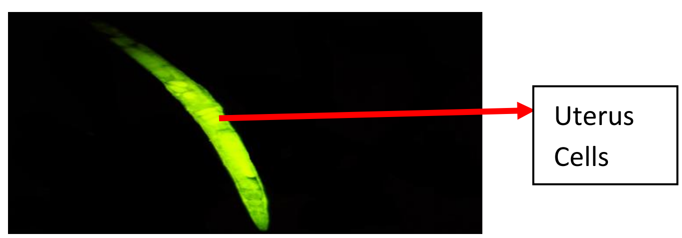
Extract plants	**Bright field**	**Fluorescence**



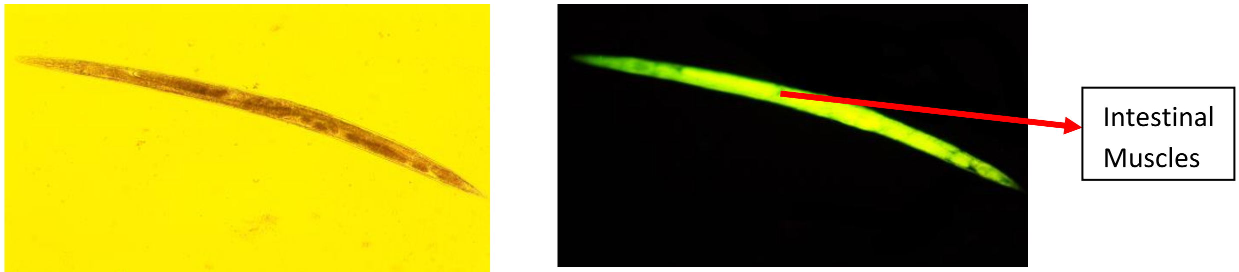
D. water



1.7 Fluorescence images of Artemisia absinthium (N-hexane, methanol, and distilled water)

Plant Extract	Bright field	Fluorescence
Methanol		
N-hexane		

D. water



Gas chromatography-Mass spectrometry (GC-MS) Analysis.

N-hexane extracts of sample were analyzed through GC-MS (Agilent Technologies, USA, and GC/MS 5975 C). For the analysis of volatile compounds, the following parameter were used.

To detect volatile compounds, a non-polar capillary column was utilized. The instrument was initially set to a temperature of 60 °C for two minutes, then programmed to increase at a rate of 40 °C per minute until reaching a final detector temperature of 240 °C. The GC-MS detector was maintained at this final temperature of 240 °C. A 1 µl sample was injected into the GC-MS system using helium as the carrier gas.

The eluted and separated volatile compounds were identified based on the electronic signals generated by each compound. Each compound exhibited a unique retention time (RT). Compounds present in higher concentrations produced stronger electronic signals, as detected by the system. These signals were processed by the GC-MS software to generate a chromatogram representing the volatile compounds.

In the chromatogram, the retention time is represented on the horizontal axis, while the signal intensity is shown on the vertical axis. As the compounds elute from the GC column, they enter the electron ionization detector—also known as the mass spectrometry (MS) detector—where they are fragmented into smaller pieces by a stream of electrons. These fragments are converted into charged ions with specific mass-to-charge (m/z) ratios. The resulting signals generate a chromatogram, which is standardized based on the m/z ratios, serving as a unique molecular fingerprint for compound identification. The oven temperature is maintained at 100 °C during the process.

The volatile compounds present in the extract were analyzed and identified by comparing their mass spectra with reference data from the National Institute of Standards and Technology (NIST) library database.

GC-MS analysis of A. Raddianum.

The GC-MS analysis of A. Raddianum (Pteridaceae) n-hexane, GC-MS analysis was carried out using a regular perken Elmer auto system XL GC-MS analyzer.

Compound identification was performed based on retention time and mass spectrum interpretation, using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 reference spectra of known compounds.

GC-MS analysis of the n-hexane extract of *Adiantum raddianum* was conducted to identify various essential constituents that may contribute to antioxidant activity and the inhibition of foodborne pathogenic bacteria, as well as exhibit antifungal, antinematodal, and antibacterial potential. This bioactivity is attributed to the presence of various polyphenolic compounds. A total of 10 chemical compounds were identified through the GC-MS analysis. chemical constituents with their retention time (RT) and area concentration (%) are presented in Table 1.9. The chromatogram of GC-MS is shown in the figure.1.8.

The five most abundant compounds were Hexadecenoic acid, methyl ester (RT 24.745), methyl 1,3-(3,5-ditert-butyl 1-4 hydroxyphenyl) propanoate (RT 25.097), 1,4- methanoazulen-7 (1H)-one, octahydro-1,5,5,8a-tetramethyl- (RT 26.847), 9-12, Octadecanoic acid, methyl ester (RT 27.971) and 7-hexadecanoic acid, methyl ester, (Z)- (RT 28.090). Chromatogram of the chemical constituents and their peaks point from extract ion has been given in *Adiantum Raddianum* Figure.2 having a strong nematicidal activities, according to the GC-MS analysis [20].

Figure 1.8: GC-MS analysis of leaves extract of A. Raddianum

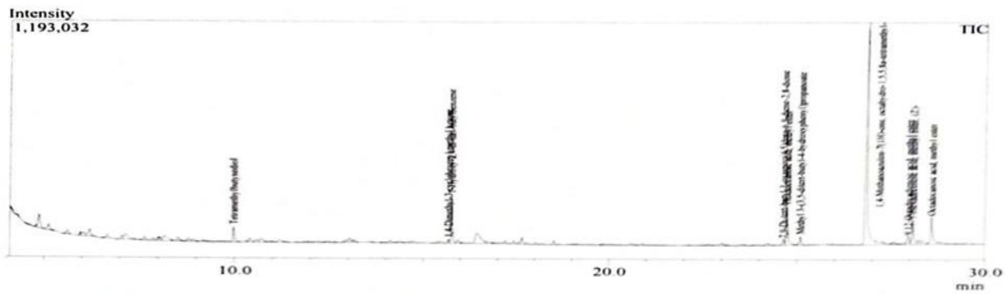
GCMS REPORT

MEDICINAL BOTANIC CENTRE
PCSIR LABS. COMPLEX PESHAWAR

7/24/2023 11:22:01

Sample Information

Analyzed by :Dr. Muhammad Akram
Analyzed : 5/17/2023 9:31:26 AM
Adeel Yunus 02- At-AN 17-05-23
Method Area Normalization
Injection Volume : 1.00
D:\Data files post run GCMS\FAMES Std Mix\02.qgm



QUANTITATIVE RESULT TABLE

ID#	Name	R.Time	Area	Conc. (%)
1	Tetramethylbutynediol	9.976	41805	5.79
2	1,4-Dimethyl-3-cyclohexenyl methyl ketone	15.682	10387	1.44
3	1-Hydroxy-2,4-di-tert-butylbenzene	15.772	216364	29.94
4	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	24.649	7524	1.04
5	Hexadecanoic acid, methyl ester	24.745	123374	17.07
6	Methyl 3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoate	25.097	13050	1.81
7	1,4-Methanoazulen-7(1H)-one, octahydro-1,5,5,8a-tetramethyl-	26.847	197636	27.35
8	9,12-Octadecadienoic acid, methyl ester	27.971	10068	1.39
9	7-Hexadecenoic acid, methyl ester, (Z)-	28.090	28128	3.89
10	Octadecanoic acid, methyl ester	28.586	74261	10.28

Table 1.9: GC-MS analysis of leaf extracts of A. Raddianum

S. No	Compounds in *A. Raddianum*	R-Time	Area	Conc (%)
1	Tetramethylbutynediol	9.976	41805	5.79
2	1,4-dimethyl-3-cyclohexenyl methyl ketone	15.682	10387	1.44
3	1-hydroxy-2,4-di-tert-butylbenzene	15.772	216364	29.94
4	7,9-di-tertbutyl-I-oxaspiro (4,5) deca-6,9-diene-2,8-dione	24.649	7524	1.04
5	Hexadecenoic acid-methyl ester	24.745	123374	17.07
6	Methyl 3-(3,5-ditert-butyl-4-hydroxyphenyl) Propanoate	25.097	13050	1.81
7	1,4-methanoazulene-7(IH) one actahydro-1,5,5,8a-tetramethyl	26.847	197636	27.35

8	9,12-octadecadienoic acid, methyl ester	27.971	10068	1.39
9	7-hexadecanoic acid, methyl ester, (Z)-	28.090	28128	3.89
10	Octadecanoic acid, methyl ester	28.586	74261	10.28

GC- MS Analysis of Artemisia Absinthium.

The GC-MS analysis of *A. Absinthium* (Asteraceae) n-hexane. GC-MS analysis was carried out using a regular perken Elmer auto system XL GC-MS analyzer.

Compound identification was based on retention time and mass spectrum interpretation, using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 spectral patterns of known compounds.

GC-MS analysis of the n-hexane extract was performed to identify various essential constituents that may contribute to antioxidant activity and exhibit potential in controlling foodborne pathogenic bacteria, as well as displaying antifungal, antinematodal, and antibacterial properties. These bioactivities are attributed to the presence of diverse polyphenolic compounds. A total of 12 chemical compounds were identified from the GC-MS analysis of. The *Artemisia Absinthum* chemical constituents with their retention time (RT) and area concentration (%) are presented in Table 1.9. The chromatogram of GC-MS is shown in the figure.1.9.

The five most abundant compounds were Methyl tetradecanoate (RT 20.572), Hexadecanoic acid, methyl-ester (RT 27.981), Methyl 3-(3,5-ditert-butyl-4-hydroxyphenyl) propanoate (RT 25.106), Spathulenol (RT 17.440) and Octadecanoic acid, methyl ester- (RT 28.594). Chromatogram of the chemical constituents and their peaks point from extract ion has been given in *Artemisia Absinthum*.

Figure 1.9: GC-MS analysis of leaf extracts of A. Absinthium

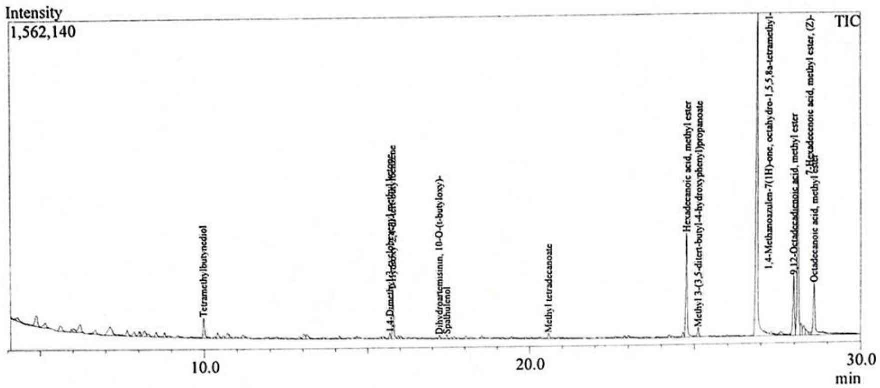
GCMS REPORT

MEDICINAL BOTANIC CENTRE
PCSIR LABS. COMPLEX PESHAWAR

7/24/2023 11:21:53

Sample Information

Analyzed by :Dr. Muhammad Akram
Analyzed : 5/17/2023 10:08:35 AM
Adeel Yunus 03- Az-IN 17-05-23
Method Area Normalization
Injection Volume : 1.00
D:\Data files post run GCMS\FAMES Std Mix\03.qgm



QUANTITATIVE RESULT TABLE

ID#	Name	R.Time	Area	Conc. (%)
1	Tetramethylbutynediol	9.980	48022	3.85
2	1,4-Dimethyl-3-cyclohexenyl methyl ketone	15.682	13323	1.07
3	1-Hydroxy-2,4-di-tert-butylbenzene	15.773	196095	15.73
4	Dihydroartemisinin, 10-O-(t-butyloxy)-	17.216	4837	0.39
5	Spathulenol	17.440	4348	0.35
6	Methyl tetradecanoate	20.572	11562	0.93
7	Hexadecanoic acid, methyl ester	24.752	285944	22.94
8	Methyl 3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoate	25.106	12793	1.03
9	1,4-Methanoazulen-7(1H)-one, octahydro-1,5,5,8a-tetramethyl-	26.868	288952	23.19
10	9,12-Octadecadienoic acid, methyl ester	27.981	74095	5.95
11	7-Hexadecenoic acid, methyl ester, (Z)-	28.099	184628	14.81
12	Octadecanoic acid, methyl ester	28.594	121656	9.76

Table 1.9: GC-MS analysis of leaf extracts of A. Absinthium

Sr. No	Name	R-Time	Area	Concentration
01	Tetramethylbutynediol	9.980	48022	3.85
02	1,4 Dimethyl-3-cyclohexenyl methyl ketones	15.682	13323	1.07
03	1-hydroxy-2,4-di tert butyl-benzene	15.773	196095	15.73
04	Dihydroartemisinin 10-0-(t-butyloxy)-	17.216	4837	0.35
05	spathulenol	17.440	4348	0.35
06	Methyl-tetradecanoate	20.572	11562	0.93
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08	Methyl 3-(3,5-ditert-butyl-4-hydroxyphenyl) propanoate	25.106	12793	1.03
09	1,4-methanozaulen-7 (IH)-one, octahydr-1,5,5,8a-tetramethyl-	26.868	288952	23.19
10	9,12-octadecadienoic acid, methyl ester	27.981	74095	5.95
11	7-Hexadecanoic acid, methyl ester, (Z)-	28.099	184628	14.81
12	Octadecanoic acid-methyl ester	28.594	121656	9.72

Gene expression study of C. elegans genes treated with plant extracts.

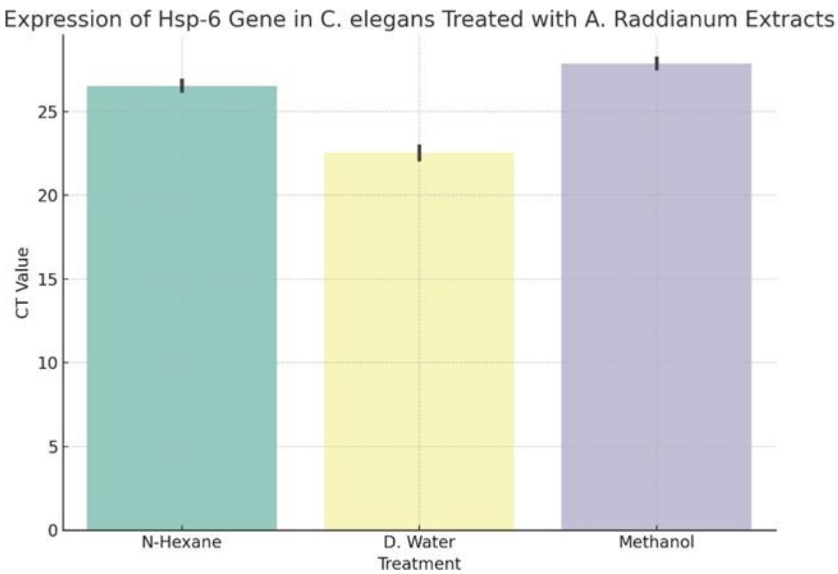
In this study, the expression of genes in both healthy and affected C. elegans were studied with quantitative Real-Time PCR (q RT-PCR). In 3 hours of incubation at 20 ^{o}C, expression analysis of two C. elegans gene transcripts were performed to find out the biochemical pathways and molecular functions that were affected by plant extracts at (1 mg/mL). These genes were selected due to their function in the stress response. Selected genes were hsp-6 and gst-4. These genes are connected to important molecular processes and metabolic pathways, such as heat shock stress, mitochondrial stress, and oxidative stress.

Gene expression of Adiantum Raddianum.

Quantitative RT-PCR analysis of C. elegans stress genes treated with A. Raddianum leaves extracts. The CT value of gst-4 gene is 25.961 treated with A. Raddianum (N-hexane) and CT values of HSP-6 gene A. Raddianum (N-hexane) 26.482, (Distilled. water) 22.786, (Methanol) 27.728 Respectively.

Table 1.10: Quantitative RT-PCR analysis of C. elegans stress genes treated with A. Raddianum leaves extracts. (a) gst-4, (b) Hsp-6 genes and CT values are

Sr. No	Genes	N-hexane extract 1mg/mL (CT value)	D. Water Extract 1mg/mL (CT value)	Methanol Extract 1mg/mL (CT value)
1	Gst-4	25.961	0.0	0.0
2	Hsp-6	26.482	22.786	27.728
3	(Control, no plant extract, only ⩽ 1% DMSO	0.0	0.0	0.0



t-tests and p-values for the CT values of the Hsp-6 gene in *C. elegans* treated with A. Raddianum leaf extracts

t-test Results

Comparison	**t-Statistic**	**p-Value**
N-Hexane vs D. Water	**11.315**	**0.0005**
N-Hexane vs Methanol	**-4.294**	**0.0127**
D. Water vs Methanol	**-15.124**	**0.0002**

Explanation of Results:

- p-values < 0.05 indicate statistically significant differences in gene expression between the groups.
- The D. Water extract significantly lowered CT values compared to both N-Hexane and Methanol, suggesting stronger Hsp-6 expression (since lower CT means higher expression).
- The Methanol extract caused the least expression of Hsp-6 (highest CT value).

Biological Interpretation:

- D. Water extract seems most potent in inducing stress response in *C. elegans* as shown by the strong expression of Hsp-6.

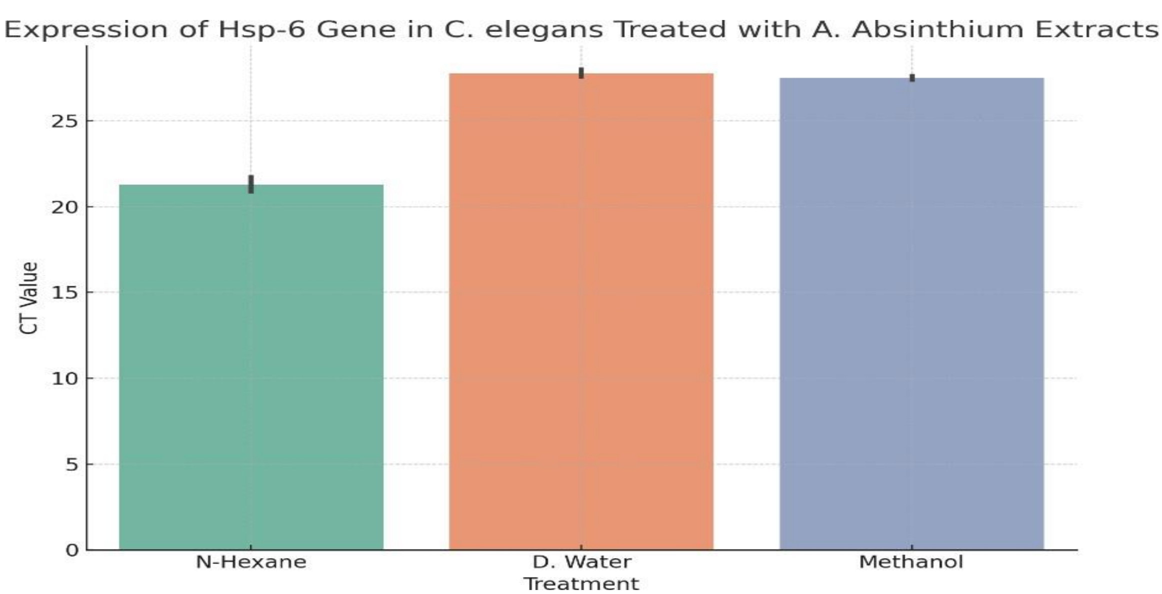
- N-Hexane and Methanol extracts induce relatively weaker expression.

Gene expression of Artemisia Absinthium.

qRT-PCR Analysis of *C. elegans* Stress Genes Induced by *A. absinthium* Leaf Extracts. The CT value of gst-4 gene is 25.601 treated with A. Absinthium (N-hexane) and CT values of HSP-6 gene A. Absinthium (N-hexane) 21.378, (Distilled. water) 27.733, (Methanol) 27.713 Respectively.

Table 1.11: Quantitative RT-PCR analysis of C. elegans stress genes treated with A. Absinthium leaves extracts. (a) gst-4, (b) hsp-6 genes CT values are

Sr. No	Genes	N- hexane Extract 1mg/mL CT values	D. water Extract 1mg/mL CT Values	Methanol Extract 1mg/mL CT values
1	Gst-4	25.601	0.0	0.0
2	Hsp-6	21.378	27.733	27.713
3	(Control, no plant extract, only ⩽ 1% DMSO	0.0	0.0	0.0



t-test Results:

Comparison	T-Statistic	P-Value
N-Hexane vs D. Water	**-19.098**	**0.0004 Significant**
N-Hexane vs Methanol	**-19.611**	**0.0013 Significant**
D. Water vs Methanol	**1.626**	**0.1980 Not Significant**

Biological Interpretation:

- N-Hexane extract significantly lowered CT values compared to both D. Water and Methanol → implies higher Hsp-6 expression, suggesting a stronger stress response.
- No significant difference between D. Water and Methanol indicates similar expression levels from these extracts.
- N-Hexane is likely the most potent extract in inducing the Hsp-6 stress gene in *C. elegans*.

CONCLUSIONS.

The complete results of my study showed that the methanol and N-hexane extracts of A. Raddianum, A. Absinthium, have strong Antinematodal activity. Extract from both plants showed substantial anti-egg hatching activity at the concentration of 1 mg/mL. When treated to plant compounds in NGM medium, Adiantum Raddianum methanol (LD50 = 3.12 mg/ml) and Artemisia absinthium methanol (LD50 = 3.00 mg/ml) had a strong effect on C. elegans egg laying, as a result 50% reduction rate of egg laying is achieved. Florescence microscopy identified the apoptotic effect on intestinal cells, muscle cells, and the uterus. Both plant extracts tested in tables 1.10 and 1.11 showed **statistically significant** effects ($p < 0.05$, often much lower) on at both stress-related gene in *C. elegans.* quantitative RT-PCR analysis studied treated C. elegans expressed the gst-4 and hsp-6 genes, both genes were expressed during stress conditions of heat shock proteins in mitochondria and gst-4, an indicator of oxidative stress gene in mitochondria. Finally, we are recommended that the both plant compounds having a strong nematicidal activity used as alternative medicine to treat nematodal infections.

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