Evaluation of In-vitro Anthelmintic Activity of Hydroethanolic Extracts of Musa Paradisiaca Linn and Helianthus Annuus Linn

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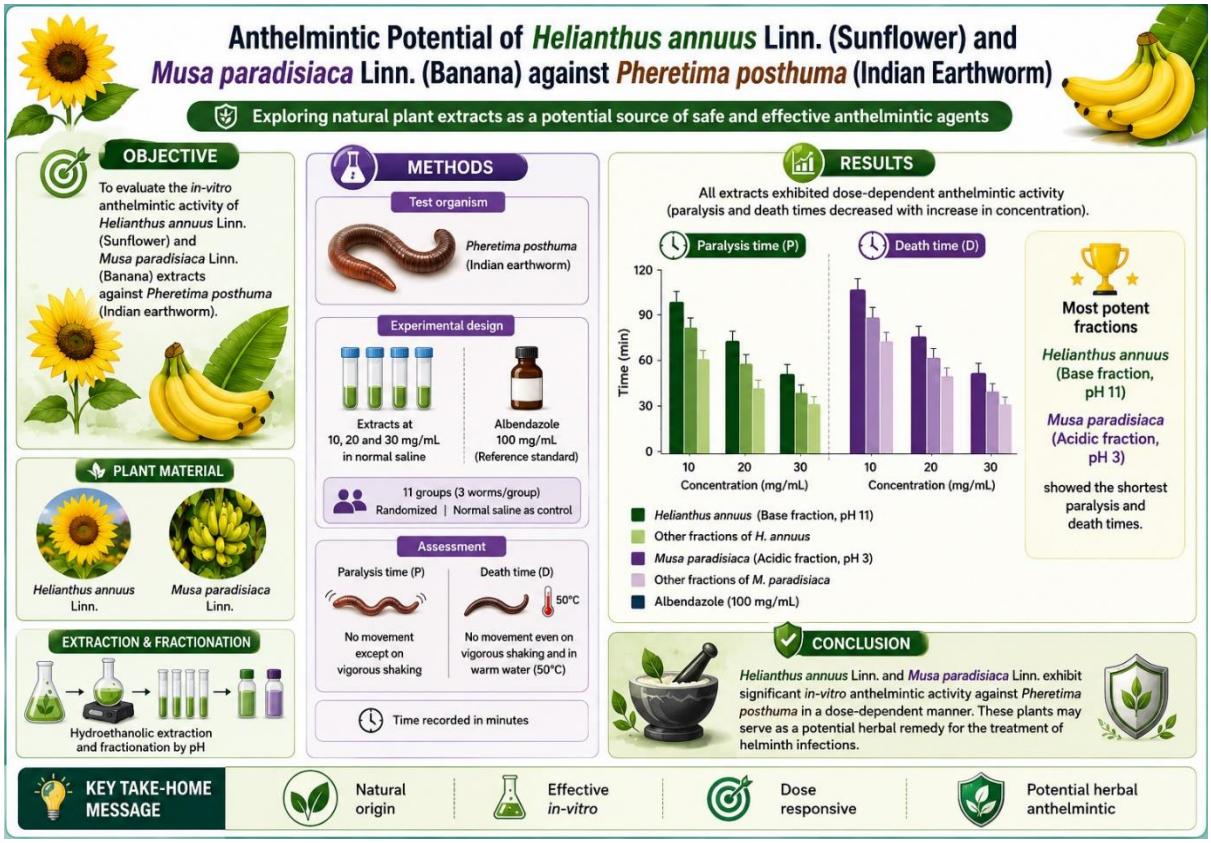
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Abstract

Objective: The present study aimed to evaluate the in vitro anthelmintic activity of Helianthus annuus Linn. (Sunflower) and Musa paradisiaca Linn. (Banana) extracts against Pheretima posthuma (Indian earthworm), a widely accepted model for screening anthelmintic agents. **Methods:** Hydroethanolic extracts of Helianthus annuus and Musa paradisiaca were prepared and fractionated. The extracts were tested at concentrations of 10, 20, and 30 mg/mL in normal saline. Earthworms were randomly allocated into eleven groups, with three worms of similar size in each group. Albendazole (100 mg/mL) served as the reference standard. Anthelmintic activity was assessed by recording the time required for paralysis and death of the worms. Paralysis was considered when no movement was observed except upon vigorous shaking, while death was confirmed by the complete absence of movement even after vigorous shaking and immersion in warm water (50°C). **Results:** All extracts exhibited significant anthelmintic activity, producing paralysis and death of the worms in a concentration-dependent manner. Among the tested fractions, the alkaline fraction of Helianthus annuus (pH 11) and the acidic fraction of Musa paradisiaca (pH 3) demonstrated the greatest potency, showing the shortest paralysis and death times compared with other fractions. **Conclusion:** The findings indicate that Helianthus annuus and Musa paradisiaca possess promising anthelmintic properties and may serve as potential sources of herbal therapeutics for the management of helminth infections. Further studies are warranted to isolate the active constituents and elucidate their mechanisms of action.

Keywords: Helianthus annuus, Musa paradisiaca, Anthelmintic activity, Pheretima posthuma, Hydroethanolic extract, Fractionation, pH-based extraction.

Graphical abstract



Introduction

Helminthiasis is one amongst the diseases that are responsible for intestinal infections. It leads to heavy health loss of an individual. It is seen frequently in the developing nations due to insufficient regulatory protocols or ineffective management techniques. Hence a comprehensive approach is necessary to control the helminthes, which includes use of anthelmintic remedies. It is treated by medications like Albendazole, Piperazine citrate and few other drugs are also available. These drugs expel the parasitic worms and parasites out of the body from the intestine [1]. Few drugs turned out to be toxic and in order to avoid the toxicity caused due to these drugs, use of combinational therapy is preferred; but helminths developed resistance even against the drug combinations, this lead to survey of medicinal plants rather than synthetic drugs for the treatment of Helminthiasis. Many herbs are reported to possess anthelmintic activity and it was observed that saponins were found in major quantities in many extracts. Literature survey revealed that, the saponins present in these extract were responsible for the paralysis of the worms by directly acting on CNS and further leading to death of the worms. [2, 3] Saponins show various pharmacological activities like fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant, immunomodulatory and anthelmintic. [4-11]The results of the present work shows that, the peel extract of Musa Paradisaca contains about 2.86 ± 1.23% of saponins as well as the stem extract of Helianthus annuus contains 7.74% of saponins. [12] and the peel extract of Musa Paradisaca has great amount of flavonoids around 196mg/g Quercetin equivalent.[13] According to the literature survey, flavonoids also possess potent anthelmintic activity.[14] On the basis of previous findings, the plants rich in saponins and flavonoids were selected for the present study. Musa Paradisiaca is also reported for antidiabetic activity, antihypertensive activity, anti-oxidant, anti-atherosclerotic activity, anti-cancer activity, anti-ulcer, anti-depressant [15]. Helianthus annus Linn has also been reported for its antioxidant, anti-inflammatory, analgesic, anti-cancer**,** anti-ulcer, antidiabetic, antimicrobial, and antihypertensive effects [16]. The plant, Helianthus annus Linn is characterized by the presence of various bioactive phytocompounds effective against the helminths. So, these extracts are aimed for its study against the helminths.

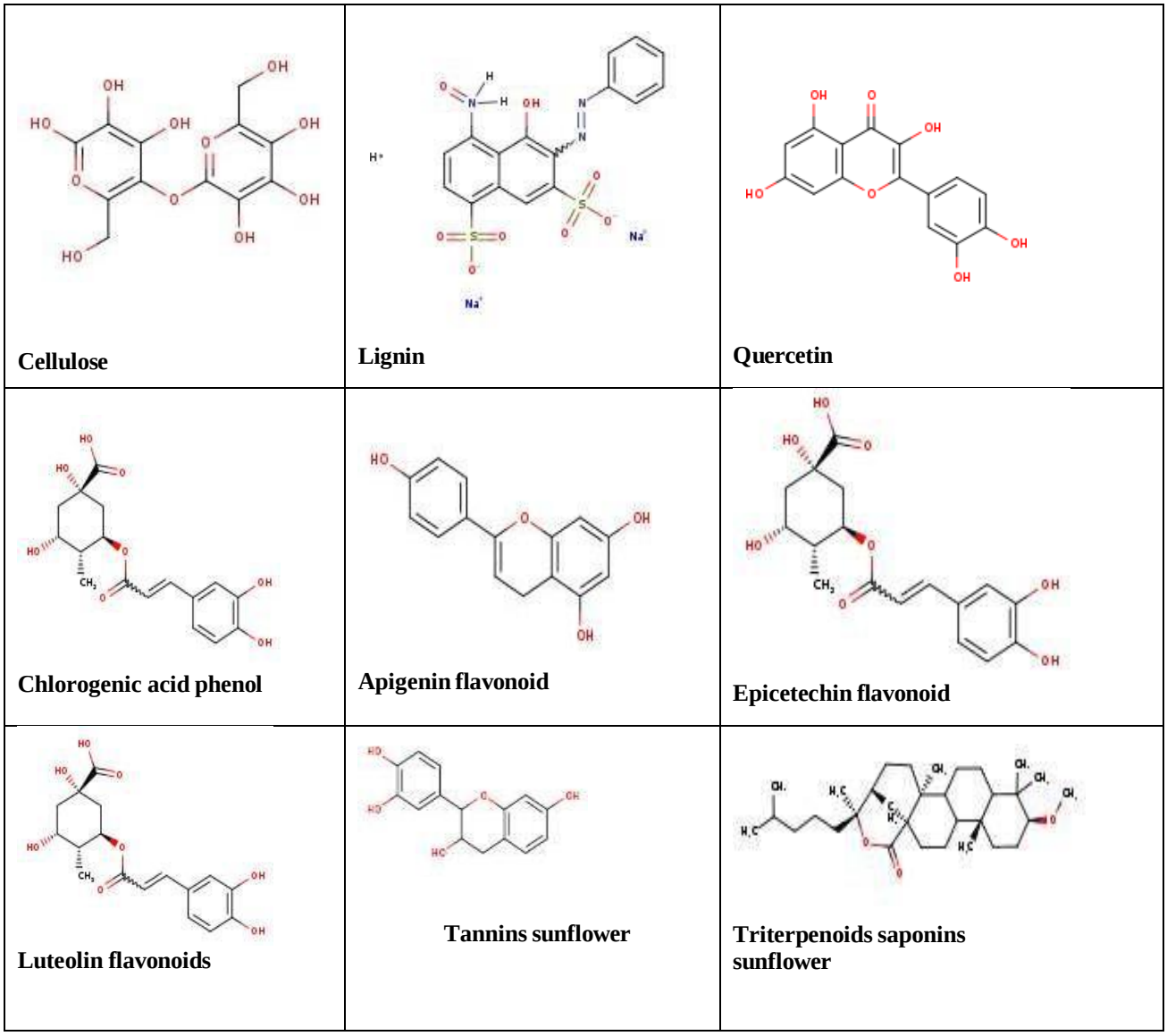


Figure 1: Phytochemicals present in Helianthus annuus Linn**.**

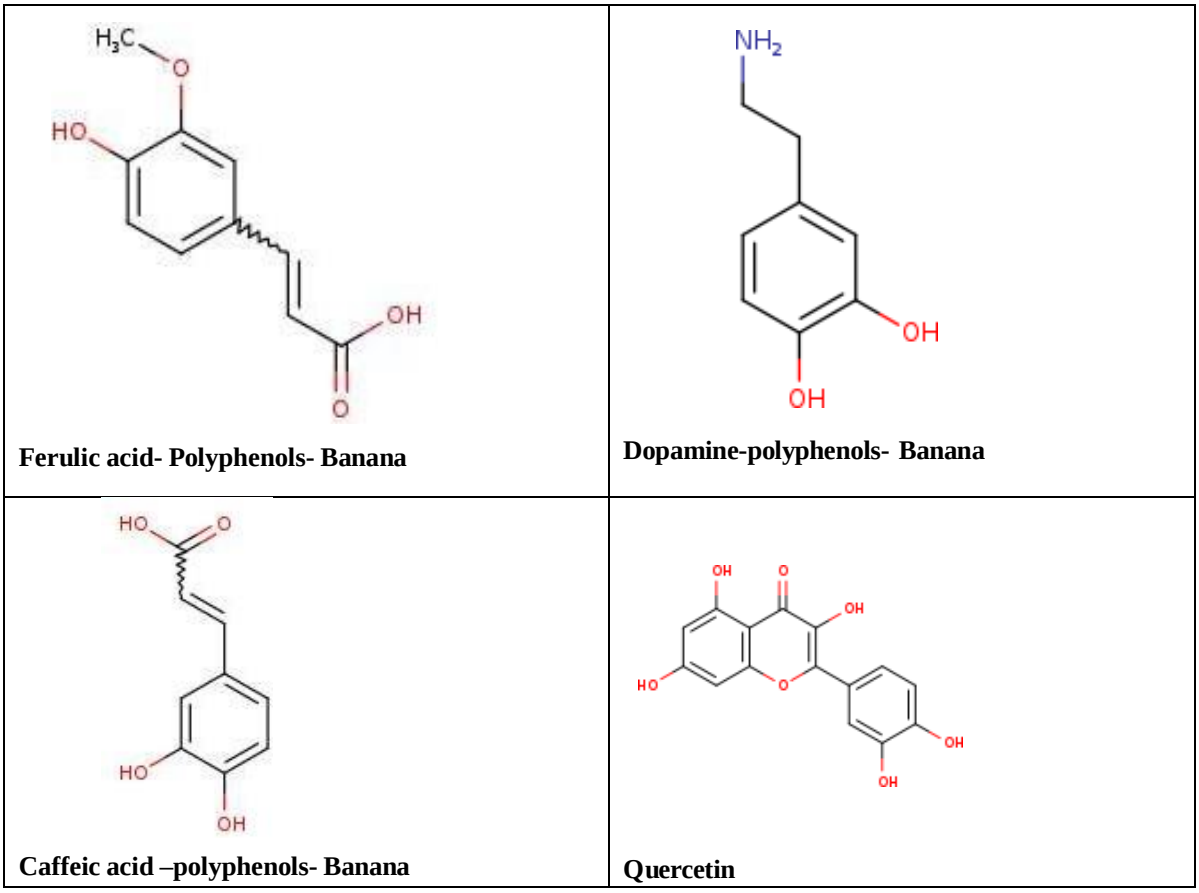


Figure 2: Phytochemicals present in Musa Paradisaca**.**

Reagents and chemicals: All the chemicals used for the extraction process were of analytical grade. Albendazole used for the assay was collected from the local pharmacy store.

Plant Materials:
Helianthus annuus Linn flowers, stems and leaves were procured from the regional flower markets of Pune. Musa Paradisaca fruits, leaves and flowers were collected from the localized farms. The herbarium of both the plants were authenticated by the Botany Department of Savitribai Phule Pune University with Voucher number mms1653 and mms1652 respectively.

Preparation of Extracts:
The fresh flowers of Helianthus annuus Linn were collected from the local markets of Pune. The stems of Helianthus annuus Linn were cut into small pieces and kept for maceration in hydroethanolic solution (Ethanol: Water – 80:20) for about 3 days (72-hours). It was ground and the marc was again macerated for another 3 days (72-hours) in the same hydroethanolic solution. After completion of 3-days, the mixture was filtered and the solvent was evaporated at room temperature to obtain the crude extract.
Musa Paradisaca fruits were collected from the local markets of Pune. The peels were dried and grind into fine powder. Musa Paradisaca peel powder was macerated into the beaker by addition of ethanol till the powder is soaked for 2-days. The extract was then filtered using filter paper and the solvent was evaporated.

Quantitative estimation of total phenolic content:
The total phenolic content of Helianthus annuus Linn stem extract and Musa Paradisaca peels extract was determined by using Folin Ciocalteu reagent. Gallic acid was considered as standard phenolic compound. Different concentration (10-100µg/ml) of Gallic acid were prepared in ethanol. Test sample solution of each extract was prepared in ethanol (100µg/ml). 0.5 ml of increasing concentrations of Gallic acid/test sample was mixed in 2ml of Folin Ciocalteu reagent followed by 4ml of sodium carbonate solution. The absorbance was taken at 765 nm by keeping ethanol as blank. To find the regression line standard curve of Gallic acid was taken at different concentrations. The TPC of extract was obtained from the calibration curve of standard Gallic acid and expressed as mg/ml or µg/ml Gallic acid equivalent (GAE). [17, 18]

Quantitative estimation of total flavonoid content:
The total flavonoid content was determined by UV-spectroscopy method. Rutin solution was utilized as standard in different concentrations (10- 100ug/ml) in ethanol. Calibration curve of rutin was taken as a standard. Ethanolic samples of extract were prepared (100µg/ml). 0.5 ml of diluted solution of samples were mixed in 2 ml distilled water followed by addition of 0.15 ml $NaNO_2$ solution. After the period of 6 minutes, 0.15 ml of $AlCl_3$ solution was added and allowed to stand for another 6 minutes. 2ml NaOH solution added to the above mixture and kept

undisturbed for 15 minutes. The absorbance was measured at 510 nm with water as blank by using ultraviolet spectrophotometer. The absorbance of extract was measured by the regression line of standard curve rutin. TPC is expressed as value of rutin equivalent (RE) mg RE/g extract. [19]

pH differential extraction process:
2-grams of the crude extract was weighed, treated with 5% HCL, and boiled over a water bath to dissolve the solids in order to isolate the saponins from the extract. Following filtering, the liquid mixture was extracted with chloroform (CHCl3), allowing the organic and aqueous phases to be separated. The procedure is repeated for 2-3 times. After mixing the organic phases, anhydrous sodium sulfate (Na2So4) was added and the extract was treated as an acid fraction. 2-alkaline extracts with pH values of 8 and 11 were synthesized by treating the residual aqueous phase with 20% sodium hydroxide (NaOH) in order to prepare the basic fraction. [20]

Collection of earthworms:
The Indian earthworm Pheretima Posthuma (Annelida) of approximately 8cm was collected from College of Agriculture, Shivajinagar, located in Pune and manufacturing plant at post Jalna. The adhering dirt and soil were removed by cleaning the worms with clean tap water.

Evaluation of Anthelmintic activity:
To perform the assay, crude extracts, acid and base (pH 8 and pH 11 solutions) fractions of both plants were used. Both extracts and acid and base fractions were weighed and dissolved in sterile saline solution and 5% dimethyl sulfoxide solution to prepare required concentrations 10, 20 and 40 mg/ml for crude extract and 100mg/ml for both acid and base fractions of each plant. Albendazole was used as positive control in different concentrations for crude and fractionated extracts (10 mg/ml for crude extracts and 100mg/ml for both acid and base fractions). For performing assay, three worms of approximately same size (previously washed with sterile saline to eliminate the dirt and other residue) were used for each concentration. Worms were placed into petri plate along with the different solutions of crude extracts and acid and base fractions of both the plants. Along with these, additional petri plate was used for positive control i.e. Albendazole and solution of sterile saline and 5% dimethyl sulfoxide was used as negative control. For all concentrations, time for paralysis and death was recorded (in minutes). The worms were considered paralyzed if they showed no movement, and then after a minute, the worms were placed in water of 50°C temperature to confirm its death. If movement persisted then it was put back again into the solution. Readings were recorded three times for each concentration. [21]

Statistical Analysis:
The data were processed according to the Shapiro-Wilk test to determine if they were normally distributed, followed by one way ANOVA Tukey's multiple comparison test using Graph Pad Prism software version 6.0 for data analysis. $P < 0.05$ was considered statistically significant.

Results

A) Percentage yield:
The percentage yield of crude Musa Paradisaca peel powder was found to be **15-17%**. The percentage yield of Helianthus annuus Linn stem was found to be 20-22%.

B) Total Phenolic content and Total Flavonoid content:
Total phenolic content:

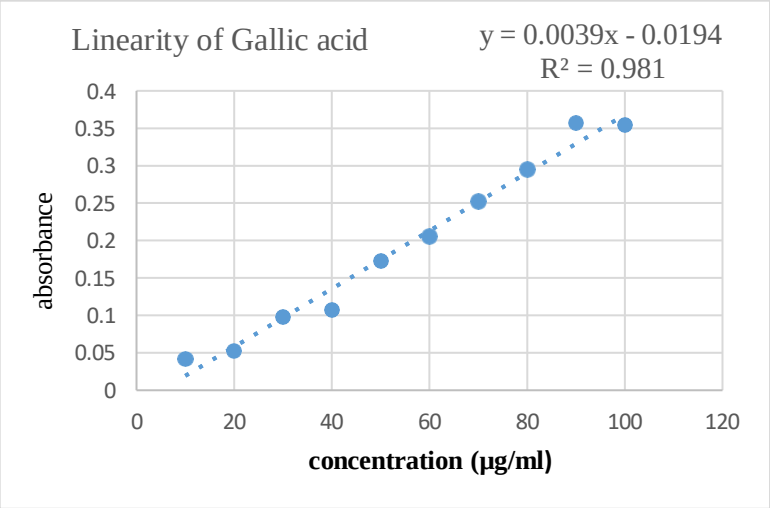


Figure-3: Linearity of Gallic acid

x represents quantity/concentration of phenol which is obtained from the equation: y=0.0039x-0.0194, where y is absorbance of samples and R^2=0.981.
Results are expressed in mean ± SD (n=3), SD: Standard deviation.

Table 1: Total Phenolic Content

Extract	Total Phenolic content mg GAE/g extract
Musa Paradisiaca (Ethanolic extract)	5.41
Helianthus annuus Linn (Ethanolic extract)	72.84

Total Flavonoid content:

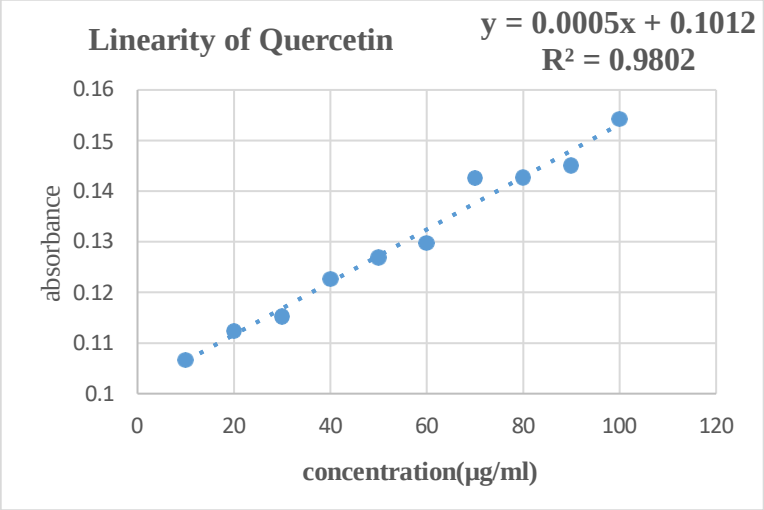


Figure 4: Linearity of Quercetin

x represents quantity/concentration of flavonoids which is obtained from the equation: y=0.0005x+0.1012, where y=absorbance of samples and R^2=0.9802.
Results are expressed in mean ± SD (n=3), SD: Standard deviation.

Table 2: Total Flavonoid Content

Extract	Total flavonoid content mg QE/g extract
Musa Paradisiaca (Ethanolic extract)	117
Helianthus annuus Linn (Ethanolic extract)	87

C) Preliminary phytochemical screening-

Table 3: Phytochemicals screening of Musa Paradisaca

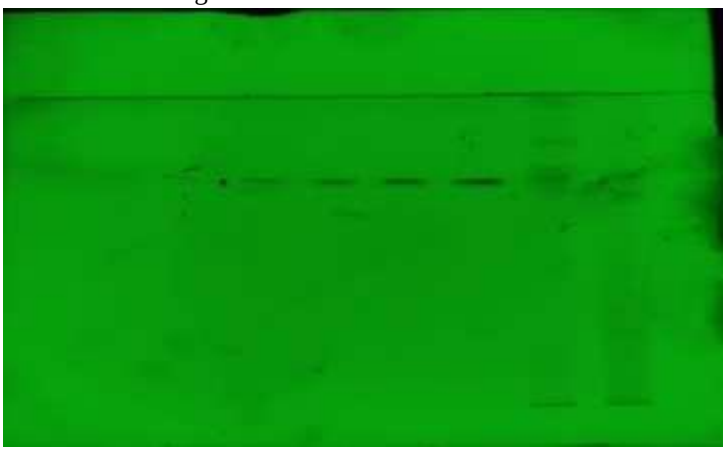
Secondary metabolites	**Musa paradisiaca**
Foam test(saponins)	+
Tannins	+
Phenols	+
Flavonoids	+

Table 4: Phytochemicals screening of Helianthus annuus Linn

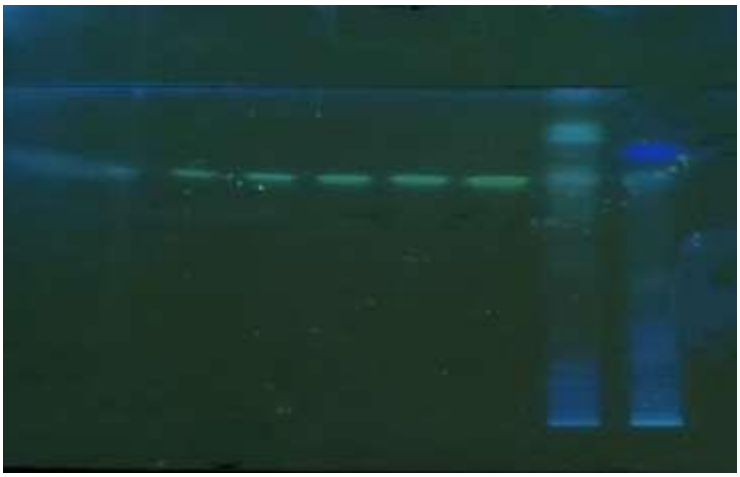
Secondary metabolites	**Helianthus annuus Linn**
Foam test(saponins)	+
Tannins	+
Phenols	+
Flavonoids	+

D) HPTLC Fingerprinting-
MOBILE PHASE:-
Toluene: Methanol: Ethyl acetate: Formic acid- 5:2:4:0.5
Fingerprinting is a useful tool for the quality control of herbal products and HPTLC fingerprinting provides an objective source to compare and identify substances HPTLC fingerprint of Musa paradisiaca peel extract and Helianthus annuus stem extract (A) under UV 254 and (B) under 366nm. In Figure 5, Track 1 to 6 represents standard Quercetin. Track 7 represents banana peel extract, Track 8 represents Sunflower stem extract. The best fingerprint for Musa Paradisiaca and Helianthus annuus represented by Fig.7 and Fig. 8 when viewed under 366nm. Table 6 and 7 shows the fingerprint that can be used for comparison based on Rf values for this plate. HPTLC 3D chromatogram showing separation of compounds with Rf values and peaks presented in Figure 9. Total 5 different compounds were found in Musa Paradisaca peel extract by identifying in different peaks as in Figure 7. After comparing the Rf values to the known standards, Musa Paradisaca 0.74 Rf value confirms the

presence of Quercetin, Catechin and Rutin were reflected at 0.63 and 0.27 RF values, respectively and more such flavonoids were found out and unknown compounds are seen. Total 8 different compounds were found in Helianthus annuus Linn stem extract by identifying in different peaks as in Figure 8. After comparing the Rf values to known standards 0.74 Rf value confirms the Quercetin and some unknown compounds are also detected 0.18 Rf value confirms presence of Chlorogenic acid.



(a)



(b)

Figure 5: HPTLC finger printing of extract of peels of Musa Paradisiaca and Helianthus annuus Linn; under UV light. Solvent system used- Toluene: Methanol: Ethyl acetate: Formic acid (5:2:4:0.5). (a) Under UV 254, (b) Under UV 365nm

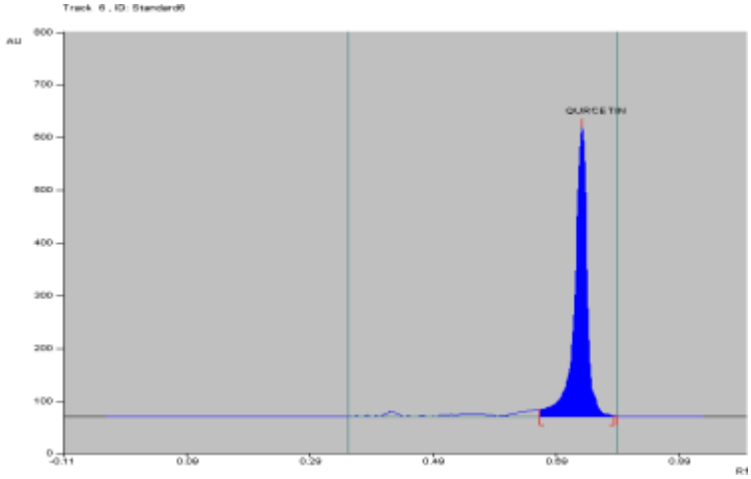


Figure 6: Standard.

Table 5: Standard.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.66 Rf	12.5 AU	0.73 Rf	550.9 AU	100.00 %	0.78 Rf	1.1 AU	9189.5 AU	100.00 %	QUERCETIN

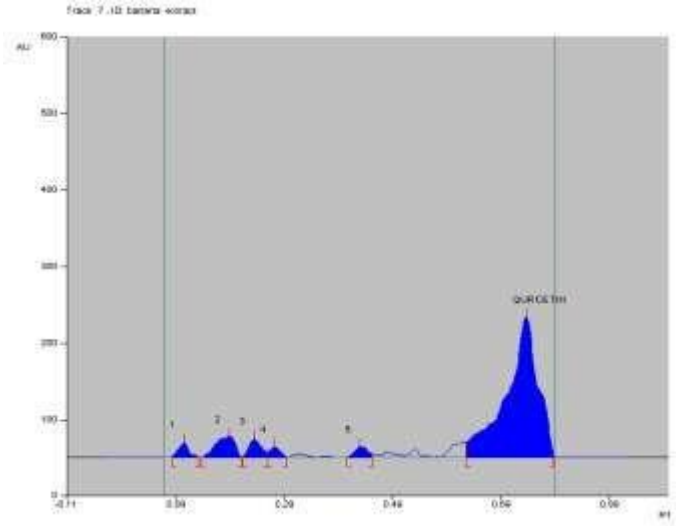


Figure 7: Banana.

Table 6: Banana extract.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	0.4 AU	0.11 Rf	19.9 AU	7.02 %	0.13 Rf	0.2 AU	309.3 AU	2.80 %	unknown *
2	0.14 Rf	0.1 AU	0.19 Rf	27.7 AU	9.74 %	0.21 Rf	0.4 AU	833.9 AU	7.55 %	unknown *
3	0.21 Rf	0.9 AU	0.23 Rf	24.8 AU	8.73 %	0.26 Rf	6.7 AU	454.6 AU	4.12 %	unknown *
4	0.26 Rf	6.9 AU	0.27 Rf	13.7 AU	4.83 %	0.29 Rf	0.3 AU	222.4 AU	2.01 %	unknown *
5	0.41 Rf	0.3 AU	0.43 Rf	14.0 AU	4.94 %	0.45 Rf	5.2 AU	290.5 AU	2.63 %	unknown *
6	0.63 Rf	19.9 AU	0.74 Rf	183.9 AU	64.75 %	0.79 Rf	3.0 AU	8928.7 AU	80.86 %	QURCETIN

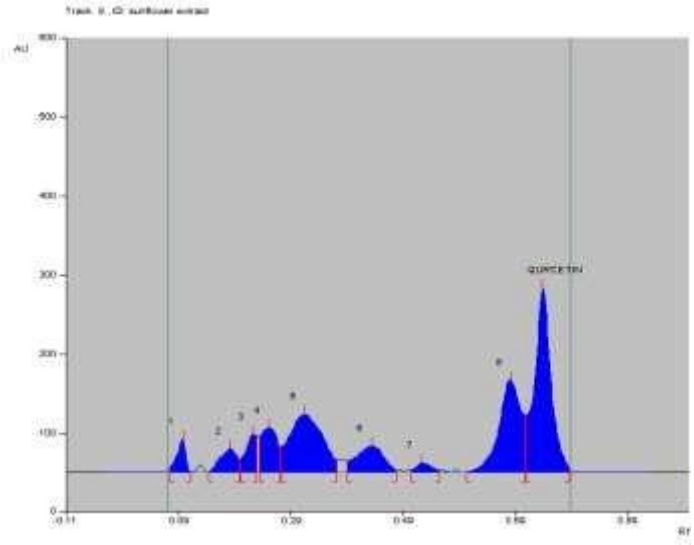


Figure 8: Sunflower.

Table 7: Sunflower extract.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.07 Rf	6.7 AU	0.10 Rf	42.3 AU	6.55 %	0.11 Rf	0.0 AU	537.2 AU	2.95 %	unknown *
2	0.14 Rf	0.3 AU	0.18 Rf	29.6 AU	4.61 %	0.20 Rf	16.5 AU	740.1 AU	4.07 %	unknown *
3	0.20 Rf	16.6 AU	0.22 Rf	47.9 AU	7.42 %	0.23 Rf	46.2 AU	768.4 AU	4.22 %	unknown *
4	0.23 Rf	45.7 AU	0.25 Rf	56.5 AU	8.75 %	0.27 Rf	32.4 AU	1369.8 AU	7.53 %	unknown *
5	0.27 Rf	32.4 AU	0.31 Rf	73.4 AU	11.37 %	0.37 Rf	15.5 AU	3620.2 AU	19.90 %	unknown *
6	0.39 Rf	14.3 AU	0.43 Rf	33.4 AU	5.18 %	0.48 Rf	2.4 AU	1353.5 AU	7.44 %	unknown *
7	0.50 Rf	2.3 AU	0.52 Rf	11.7 AU	1.82 %	0.55 Rf	2.1 AU	262.9 AU	1.45 %	unknown *
8	0.60 Rf	0.4 AU	0.68 Rf	117.4 AU	18.18 %	0.71 Rf	72.8 AU	3841.2 AU	21.12 %	unknown *
9	0.71 Rf	73.1 AU	0.74 Rf	233.3 AU	36.12 %	0.78 Rf	7.2 AU	5887.9 AU	31.32 %	QURCETIN

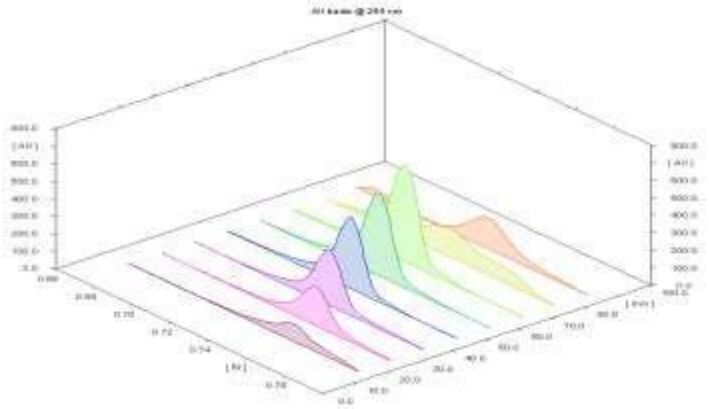


Figure 9: 3d spectra for Quercetin+ Banana extract+ Sunflower extract

Results

Crude extracts of Helianthus annuus Linn-

Treatment with the crude extract of Helianthus annuus Linn of 10mg/kg concentration showed significant ($p<0.001$) increase in the paralysis time to 42.67±1.45. The increase was found to be 412%. Treatment with crude extract of 20mg/kg concentration showed significant ($p<0.01$) increase in the paralysis time to 25.67±3.18. The increase was found to be 208%. Treatment with crude extract of 40mg/kg concentration showed significant ($p<0.05$) increase in the paralysis time to 10.67±0.66. The increase was found to be 28.04%. Treatment with the crude extract of 10mg/kg concentration showed significant ($p<0.001$) increase in the death time to 51.67±3.28. The increase was found to be 474.1%. Treatment with crude extract of 20mg/kg concentration showed significant ($p<0.01$) increase in the death time to 36.00±3.05. The increase was found to be 300%. Treatment with crude extract of 40mg/kg concentration showed significant ($p<0.05$) increase in the death time to 11.00±2.64. The increase was found to be 22.22%.

Acid and Basic fractions Helianthus annuus Linn-

There was no paralysis and death time in the saline treated group. Treatment with the standard drug Albendazole (1mg/ml) showed paralysis time as 5.00±1.00 minutes. Treatment with Helianthus annuus Linn acid fraction showed non-significant increase in the paralysis time. The increase was found to be 46.6% as compared to the standard group. Treatment with fraction (pH 8) showed significant ($p<0.001$) increase in the paralysis time to 41.00±2.08 minutes and the increase was found to be 720%. Treatment with fraction (pH 11) showed non-significant increase in the paralysis time to 6.00±0.52 minutes. The increase was found to be 20%. Treatment with acid fraction showed non-significant increase in the death time. The increase was found to be 150% as compared to the standard group. Treatment with fraction (pH 8) showed significant ($p<0.001$) increase in the death time to 44.33±6.98 and the increase was found to be 731%. Treatment with fraction (pH 11) showed non-significant increase in the death time to 6.00±0.52 minutes. The increase was found to be 50.09%.

Crude extract of Musa Paradisaca-

Treatment with the crude extract of Musa Paradisaca of 10mg/kg concentration showed significant ($p<0.001$) increase in the paralysis time to 43.00±5.5. The increase was found to be 616%. Treatment with the crude extract of 20mg/kg concentration showed significant ($p<0.01$) increase in the paralysis time to 23±1.52. The increase was found to be 283%. Treatment with the crude extract of 40mg/kg concentration showed significant ($p<0.05$) increase in the paralysis time to 11.67±3.38. The increase was found to be 94.5%. Treatment with the crude extract of 10mg/kg concentration showed significant ($p<0.001$) increase in the death time to 50.0±3.05. The increase was found to be 689%. Treatment with crude extract of 20mg/kg concentration showed significant ($p<0.01$) increase in the death time to 20.67±3.48. The increase was found to be 226%. Treatment with crude extract of 40mg/kg concentration showed significant ($p<0.05$) increase in the death time to 7.66±0.66. The increase was found to be 21.12%.

Acid and Basic fractions Musa Paradisaca-

Treatment with acid fraction of Musa Paradisaca showed non-significant increase in the paralysis time. The increase was found to be 19.96% as compared to the standard group. Treatment with fraction (pH 8) showed significant ($p<0.05$) increase in the paralysis time to 28.67±0.88 minutes and the increase was found to be 244%. Treatment with fraction (pH 11) showed significant ($p<0.01$) increase in the paralysis time to 56.00±1.15 minutes. The increase was found to be 572%. Treatment with acid fraction showed significant (0.05) increase in the death time. The increase was found to be 210% as compared to the standard group. Treatment with fraction (pH 8) showed significant ($p<0.001$) increase in the death time to 50.0±3.21 and the increase was found to be 49.23%. Treatment with fraction (pH 11) showed significant (0.01) increase in the death time to 39.67±3.71 minutes. The increase was found to be 526%.

Results

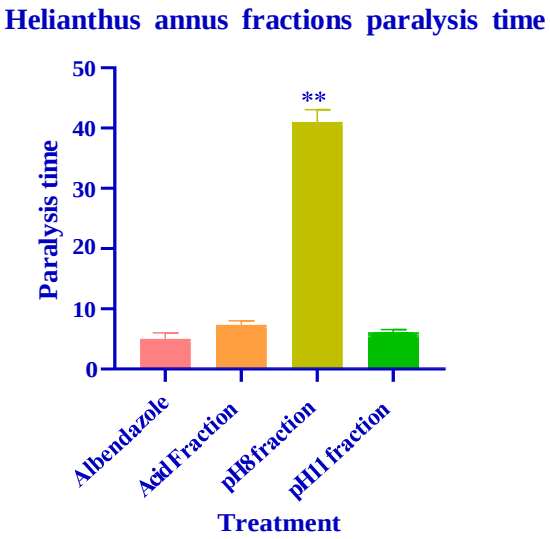


Figure 8: Paralysis time of Helianthus annuus Linn acid and basic fractions.

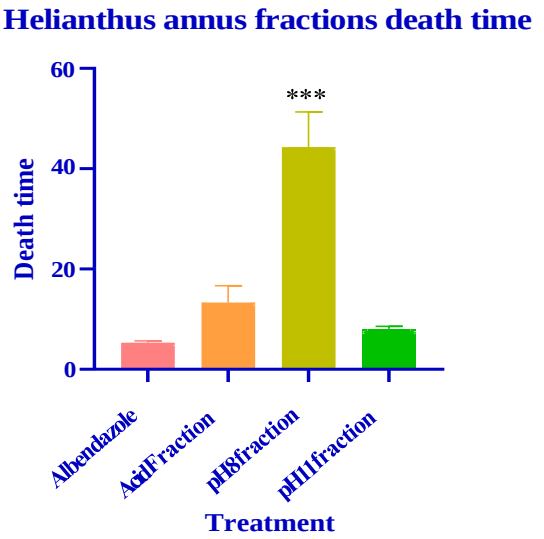


Figure 9: Death time of Helianthus annuus Linn acid and basic fractions.

All values were expressed as mean ± SD (n=6). The data was analysed by one way ANOVA followed by Tuky's multiple comparison test.

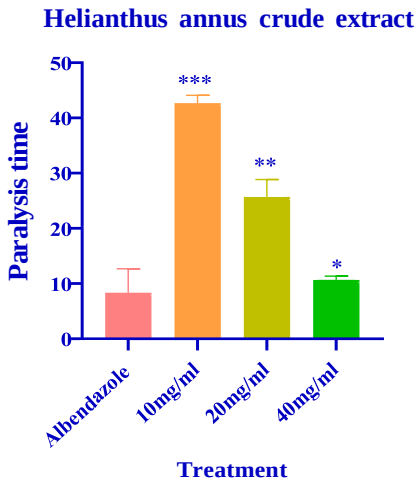


Figure 10: Paralysis time of Helianthus annuus Linn crude extract at 10, 20 and 40mg/ml concentrations.

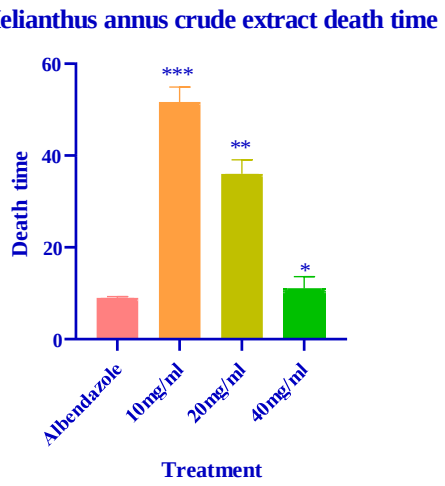


Figure 11: Death time of Helianthus annuus Linn crude extract at 10, 20and 40mg/ml concentrations.

All values were expressed as mean ± SD (n=6). The data was analysed by one way ANOVA followed by Tuky's multiple comparison test.

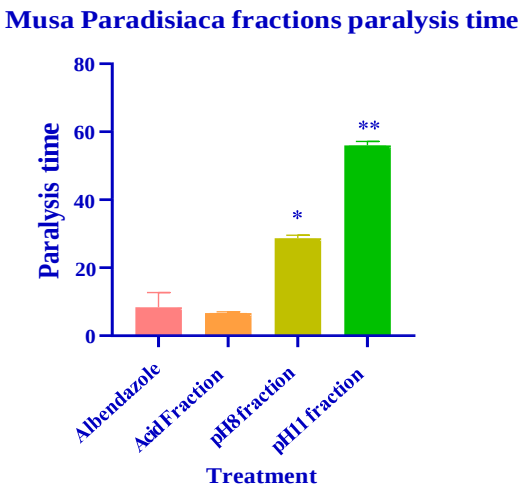


Figure 12: Paralysis time of Musa Paradisaca acid and basic fractions.

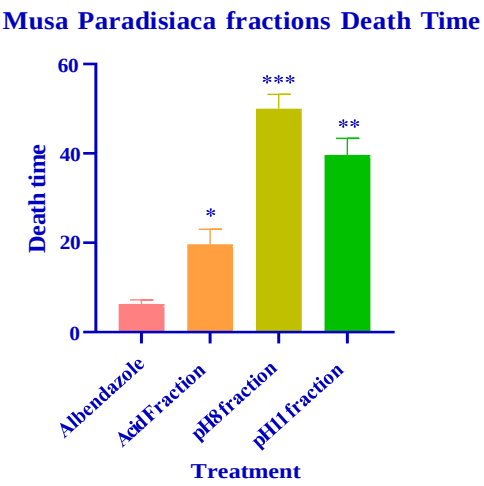


Figure 13: Death time of Musa Paradisaca acid and basic fractions.

All values were expressed as mean ± SD (n=6). The data was analysed by one way ANOVA followed by Tuky's multiple comparison test.

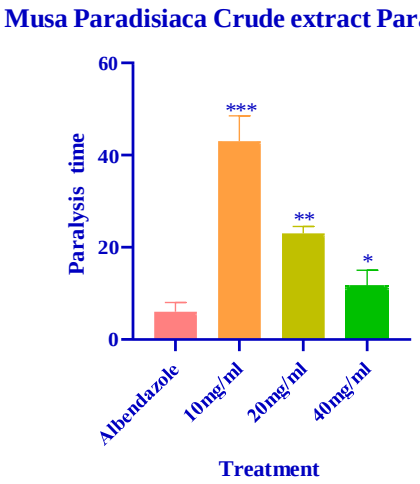


Figure 14: Paralysis time of Musa Paradisaca at 10, 20 and 40mg/ml concentrations.

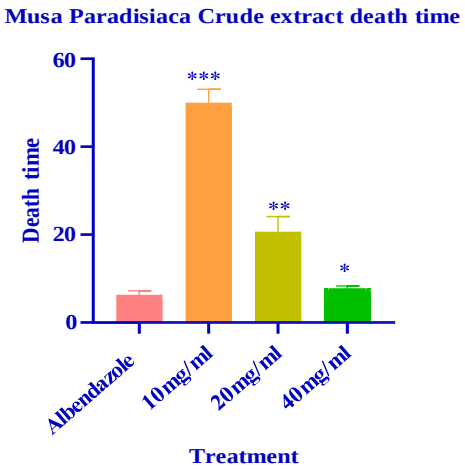


Figure 15: Death time of Musa Paradisaca crude extracts at 10, 20 and 40mg/ml concentrations.

All values were expressed as mean ± SD (n=6). The data was analysed by one way ANOVA followed by Tuky's multiple comparison test.

There was no paralysis and death time in the saline treated group. Treatment with the standard drug Albendazole (1mg/ml) showed paralysis time as 5.00±1.00 minutes. Treatment with Helianthus annuus Linn acid fraction showed non-significant increase in the paralysis time. The increase was found to be 46.6% as compared to the standard group. Treatment with fraction (pH 8) showed significant ($p<0.001$) increase in the paralysis time to 41.00±2.08 minutes and the increase was found to be 720%. Treatment with fraction (pH 11) showed non-significant increase in the paralysis time to 6.00±0.52 minutes. The increase was found to be 20%. Treatment with acid fraction showed non-significant increase in the death time. The increase was found to be 150% as compared to the standard group. Treatment with fraction (pH 8) showed significant ($p<0.001$) increase in the death time to 44.33±6.98 and the increase was found to be 731%. Treatment with fraction (pH 11) showed non-significant increase in the death time to 6.00±0.52 minutes. The increase was found to be 50.09%.

Treatment with the crude extract of Helianthus annuus Linn of 10mg/kg concentration showed significant ($p<0.001$) increase in the paralysis time to 42.67±1.45. The increase was found to be 412%. Treatment with crude extract of 20mg/kg concentration showed significant ($p<0.01$) increase in the paralysis time to 25.67±3.18. The increase was found to be 208%. Treatment with crude extract of 40mg/kg concentration showed significant ($p<0.05$) increase in the paralysis time to 10.67±0.66. The increase was found to be 28.04%. Treatment with the crude extract of 10mg/kg concentration showed significant ($p<0.001$) increase in the death time to 51.67±3.28. The increase was found to be 474.1%. Treatment with crude extract of 20mg/kg concentration showed significant ($p<0.01$) increase in the death time to 36.00±3.05. The increase was found to be 300%. Treatment with crude extract of 40mg/kg concentration showed significant ($p<0.05$) increase in the death time to 11.00±2.64. The increase was found to be 22.22%.

Treatment with acid fraction of Musa Paradisaca showed non-significant increase in the paralysis time. The increase was found to be 19.96% as compared to the standard group. Treatment with fraction (pH 8) showed significant ($p<0.05$) increase in the paralysis time to 28.67±0.88 minutes and the increase was found to be 244%. Treatment with fraction (pH 11) showed significant ($p<0.01$) increase in the paralysis time to 56.00±1.15 minutes. The increase was found to be 572%. Treatment with acid fraction showed significant (0.05) increase in the death time. The increase was found to be 210% as compared to the standard group. Treatment with fraction (pH 8) showed significant ($p<0.001$) increase in the death time to 50.0±3.21 and the increase was found to be 49.23%. Treatment with fraction (pH 11) showed significant (0.01) increase in the death time to 39.67±3.71 minutes. The increase was found to be 526%.

Treatment with the crude extract of Musa Paradisaca of 10mg/kg concentration showed significant ($p<0.001$) increase in the paralysis time to 43.00±5.5. The increase was found to be 616%. Treatment with the crude extract of 20mg/kg concentration showed significant ($p<0.01$) increase in the paralysis time to 23±1.52. The increase was found to be 283%. Treatment with the crude extract of 40mg/kg concentration showed significant ($p<0.05$) increase in the paralysis time to 11.67±3.38. The increase was found to be 94.5%. Treatment with the crude extract of 10mg/kg concentration showed significant ($p<0.001$) increase in the death time to 50.0±3.05. The increase was found to be 689%. Treatment with crude extract of 20mg/kg concentration showed significant ($p<0.01$) increase in the death time to 20.67±3.48. The increase was found to be 226%. Treatment with crude extract of 40mg/kg concentration showed significant ($p<0.05$) increase in the death time to 7.66±0.66. The increase was found to be 21.12%.

Discussion

Helianthus annuus Linn stem extracts revealed saponins, tannins, flavonoids, and phenols in the initial phytochemical investigation; in contrast, Musa paradisiaca has flavonoids, tannins, phenols, and other compounds. Secondary plant metabolites called flavonoids have strong antiviral, antibacterial, anti-inflammatory, and antioxidant properties. Musa paradisaca's strong anthelmintic properties are indicated by its high flavonoid concentration. Strong anthelmintic compounds, such as flavonoids found in nature, have the ability to eliminate or severely damage helminthes infections in the gastrointestinal tract. (22) The current study revealed that the Musa Paradisaca crude extract had a much greater concentration than the acid and basic fractions. As a significant phytoconstituents, saponins has antibacterial, antiviral, anti-inflammatory, anticancer, antioxidant, and immunomodulatory properties, (4-10). The primary constituent of Helianthus annuus Linn, saponins, exhibit activity. Current study indicates that the stem crude extract has more potency than the acid and basic fractions. It paralyses the worms by acting directly on their central nervous system, which ultimately causes the worms to die. (3) In South-East Asia, particularly India, helminthiasis is a dangerous illness that may affect both people and poultry raised for food.

While there are a number of commercially available remedies, they all have detrimental side effects. Additionally, they could be the cause of some cumulative toxicity in the body if they are administered repeatedly. It has been discovered that using natural substances is better, safer, and more affordable. Thus, the plants may provide a different supply of anthelmintic medications. The study revealed that the Musa Paradisaca crude extract had a much good effect than the acid and basic fractions. Furthermore, the crude extract from the stem of Helianthus annuus Linn is more effective than the acid and basic fractions.

Conflict of interest statement: We declare that we have no conflict of interest.

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