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Evaluation of Anthelmintic Activities of *Persea americana* and *Musa sikkimensis*

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ABSTRACT

The goal was to know about the anthelmintic properties of the leaves of *P. americana* and peels of *M. sikkimensis*. An ethanolic extract from the leaves of *P. americana* and aqueous extract of *M. sikkimensis* was used to screen for phytochemicals and analyse the anthelmintic activity on Indian earthworm *Pheretima posthuma* to determine its therapeutic capabilities. The experiment was carried out by preparing standard drug solution (Albendazole), *P. americana* leaf extract and *M. sikkimensis* peel extract in a concentration range of 5mg/ml, 10mg/ml and 20mg/ml respectively for each. The earthworms were placed in a beaker with solutions of varying concentrations. The time of paralysis and death was noted and plotted in a graph. From the results, it was found that *P. americana* and *M. sikkimensis* extract caused paralysis in less time in a dose-dependent manner, as well as the death was caused much earlier compared to the control group. This suggests that *P. americana* and *M. sikkimensis* extract exhibited strong anthelmintic activity. Furthermore, a link has been found between anthelmintic activity and high levels of flavonoids, terpenoids and tannins. Flavonoids, tannins and terpenoids found in various extracts have been proven to have anthelmintic properties in earlier investigations. *Persea americana* have been reported to have alkaloids, flavonoids, glycosides, saponins, steroids, tannins, proteins, and Musa sikkimensis have been reported to have alkaloids, glycosides, saponins, steroids, terpenoids, tannins, proteins. These results lead to the conclusion that *P. americana* and *M. sikkimensis* extract may possess anthelmintic activity.

Keywords: Anthelmintic activity; Helminth; Musa; Avocado

INTRODUCTION

In developing countries helminths, this includes nematodes, whipworm, and trematodes becoming a huge global health burden. The infection caused by these helminths results in chronic illness among human population. This parasitic infection can cause significant effect on day-to-day activities of humans like school performance, work productivity, human growth, which can severely deteriorate the quality of life. Either due to weak immune system or nutritional imbalance, there has been a significant rise in such cases. WHO estimates that 1.5 billion human population or 24% of the world's total population are infected by these parasitic worms[1]. In agricultural animals, diseases caused by parasites led to losses of about billions of dollars per year throughout the world[2].

An area of the body afflicted with helminthiasis has been colonized with nematodes, including tapeworms, roundworms, or pinworms. The worms usually dwell in the gastrointestinal tract, but they can also pass through bodily cavities and penetrate the liver and other organs. Although the great majority of worm-related infections are usually restricted to tropical regions, some of them might manifest in temperate climates. There are three different kinds of parasitic worms: nematodes, sometimes referred to as roundworms, cestodes, also known as tapeworms or tape, and flukes or trematodes[3,4].

Gastrointestinal nematodes (GI), such as hookworms, whipworms, and roundworms affected under 15years most[5]. Approximately more than 10% of the population is infected by GI nematodes worldwide[6].

Anthelmintics are the drugs used to treat the illnesses in humans and animals brought on by parasitic worms[7].

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These drugs work to eradicate intestinal worms locally or systematically to eliminate adult helminths or evolving forms that penetrate tissues and organs. Most anthelmintic drugs on the market nowadays have adverse effects that include vomiting, diarrhea, headaches, nausea, discomfort in the abdomen, and appetite loss[8].

For centuries, ethnic healers and traditional medical systems have used herbal remedies to treat a variety of illnesses. Currently, it is believed that 80% of people use herbal traditional remedies to treat a variety of illness. This is in part because of its accessibility, and lack of negative side effects[8].

Helminthic infections are traditionally treated using a variety of plants and medicines. For the treatment, various plant parts like leaves, roots, rhizomes, etc. are utilized. Compared to contemporary treatments, these are less poisonous and more potent[3].

It has been found that the plants and herbs with anthelmintic activities contain bioactive compounds such as flavonoids, terpenoids and tannins which have therapeutic effect in human body.

Persea americana is a dicotyledonous tree of family Lauraceae. Since ancient times, different components of the *Persea americana* possess various activities such as anti-oxidant, anti-inflammatory, anti-diabetic, anti-convulsant, anti-ulcer, anti-arthritic etc.[9–12]. From the literature review, it has been evident that this plant contains tannins and flavonoids in abundant amount which may be responsible for the various activities[10].

Musa spp. has been used traditionally for various ailments. They have various therapeutic uses such as antihypertensive activity, antimicrobial activity, antidiabetic activity, anti-ulcer activity, antidiarrheal activity etc. It is evident that other species of Musa spp. has potent anthelmintic activity. *Musa sikkimensis* also contains tannins and terpenoids in abundant amount which may be responsible for the various activities[13–20].

As of now, no research has been done to assess the anti-anthelmintic action of *Persea americana* leaves and *Musa sikkimensis* peel. Hence, this study was designed to study the potential anti-anthelmintic activity of *Persea americana* and *Musa sikkimensis* using earthworms (*Pheretima posthuma*).

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and purchased from standard companies. All the ingredients of solution were prepared freshly before the experiment.

Standard drug

Albendazole IP 400 mg was purchased from Cadila Pharmaceuticals Ltd.

Plant collection

The leaves of *P. americana* and the peels of *M. sikkimensis* were collected from Majhitar, East Sikkim and authenticated by BSI, Gangtok and received the authentication no (SHRC-5/02/2023-24/TECH/458) (SHRC-5/02/2023-24/TECH/459) respectively. After the collection both leaves and peels were washed and shade dried for 1 week. The dried leaves of *P. americana* were cut into small pieces and was grinded by using Grinder. The dried peels of *M. Sikkimensis* were initially grinded in mortar & pestle and later were transferred into grinder to form coarse powder. Then, they were prepared for extraction process.

Preparation of extract

For the extraction process, the maceration extraction method was followed for both *P. americana* leaves and *M. sikkimensis* peels.

The powdered form of dried avocado leaves was weighed & then extracted by maceration by using 96% ethanol as solvent for 72 hours. The content was stirred frequently to guarantee full extraction. After extraction, using filter paper the final filtrate was collected by passing through a Whatman filter paper (grade 1). Then it was evaporated to dryness on a rotatory evaporator under reduced pressure to a thick and dark green material, later it was evaporated over water bath maintaining a temperature of 45-50°C[21].

The powdered form of dried *M. sikkimensis* peels were weighed & then extracted by maceration by using double distilled water as solvent for 72 hours. The content was stirred frequently to guarantee full extraction. After extraction, using filter paper the micelle and marc were separated. The micelle and solvent were separated by evaporation over water bath maintaining a temperature of 45-50°C[22].

Calculation of yield

The yield calculated was = (wt. of the dried extract /wt. of dried powder) x100.

Qualitative Phytochemical Screening[23]

Both the ethanolic extract of *P. americana* leaves and *M. sikkimensis* peels were separately subjected to qualitative analysis for the following Phytoconstituents: alkaloids, flavonoids, carbohydrates, glycosides, saponins, steroids, proteins, tannins and terpenoids.

Evaluation of Anthelmitic Activity

Indian earthworm *Pheretima posthuma* [Annelida] of similar sizes were collected from the water- logged areas of soil. They were washed with tap water to remove the unwanted particles like dirt. The anthelmintic assay was performed on Indian earthworm *Pheretima posthuma*,

due to its anatomical and physiological resemblance with intestinal roundworms parasites of human beings. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug[24].

Briefly, 50 ml formulations containing three different concentrations, each of aqueous extract of *Musa sikkimensis* (AEMS) and alcoholic extract of *Persea americana* (AEPA) (5, 10, 20mg/ml in double distilled water), standard drug (albendazole) solution (5 mg, 10 mg, 20 mg/ml) were prepared, and six earthworms of same size were placed in it. Both the standard and test solution were freshly prepared and 'time for paralysis' was noted when there was no movement detected except when the worms were vigorously shaken. The 'time for death' of worms was recorded when the worms neither moved when was shaken vigorously nor did they show any movement when dipped in warm water at 500°C (932°F). A maximum time period of 120 min was taken for the paralyzing as well as death time of *Pheretima posthuma* worms. Albendazole (5 mg, 10 mg, 20 mg/ml) was used as reference standard with distilled water as the vehicle control. Dose selection was based on earlier literature. All experiments were repeated thrice[24].

Statistical analysis

The mean ± SEM were analyzed statistically by ANOVA followed by Tukey Kramer multiple comparison test, $p<0.05$ being considered as significant.

RESULTS

The percentage yield of the extracts was calculated as follows:

The percentage yield of *P. americana*:

Weight of dried extract/weight of dried leaves powder x 100 (w/w)

= (wt. of the dried extract /wt. of dried powder) x100

= (5.874/50) x 100

=11.748%

The percentage yield of *M. sikkimensis*:

Weight of dried extract/weight of dried leaves powder x 100 (w/w)

= (wt. of the dried extract /wt. of dried powder) x100

= (12.53/150) x 100

=08.36%

Preliminary qualitative phytochemical analysis

Results of the preliminary phytochemical investigation of ethanolic extract of *P. Americana* exhibited presence of alkaloids, flavonoids, glycosides, saponins, tannins, steroids, and proteins, whereas phytochemical investigation of aqueous extract of *M. sikkimenses* exhibited presence of alkaloids, glycosides, saponins, steroids, terpenoids, tannins and proteins.

Evaluation of anthelmintic activity of M. sikkimensis and P. Americana

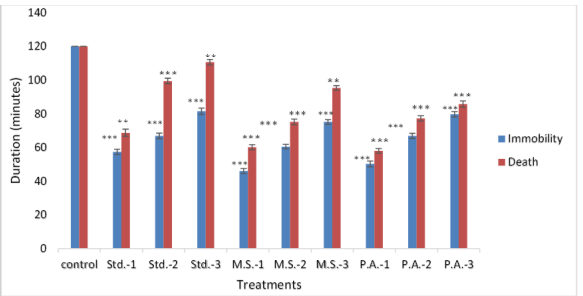
From the results, it was evident that the aqueous extract of the peel of *Musa sikkimensis* in a dose of 20mg/ml, caused paralysis at 46.07 ± 1.45 min and death at 60.10 ± 1.54 min for earthworm, whereas the alcoholic extract of the leaves of *P. Americana* in a dose of 20mg/ml, caused paralysis at 50.27 ± 1.69 min and death at 68.76 ± 2.10 min respectively.

From the observations, it was evident that the treatment groups caused paralysis in less time in a dose dependent manner, as well as the death was caused much earlier compared to the control group (Table 1, Figure 1). Although, both extracts showed anthelmintic activity in a dose-dependent manner, but the aqueous extract of *M. sikkimensis* appeared to be more effective for anthelmintic activity using earthworms. Evaluation of anthelmintic activity was compared with standard drug Albendazole.

Table 1: Effect of *P. americana* and *M. sikkimensis* on death and paralysis time

Groups	Concentration (mg/ml)	*Pheretima posthuma* Paralyzing time (min)	*Pheretima posthuma* Death time (min)
Distilled water	-	120	120
Avocado leaf extract (alcohol)	5mg/ml	79.78±1.58***	85.72±1.89***
	10mg/ml	66.86±1.51***	77.30±1.59***
	20mg/ml	50.27±1.69***	57.96±1.40**
Banana Peel extract (aqueous)	5mg/ml	75.22±1.40***	95.25±1.54***
	10mg/ml	60.48±1.35***	75.25±1.67***
	20mg/ml	46.07±1.45***	60.10±1.54***
Albendazole	5mg/ml	81.47±1.92***	110.60±1.63***
	10mg/ml	66.88±1.62***	99.44±1.62***
	20mg/ml	57.38±1.55***	68.76±2.10***

***p≤0.001, **p≤0.01, when compare with control group, Test: *P. americana* and *M. sikkimensis*, Std: Albendazole



***p≤0.001, **p≤0.01, when compare with control group, Test *P. americana* and *M.sikkimensis*, Std: Albendazole

Fig. 1: Effect of *P. Americana*& *M. sikkimensis* on paralysis and death time

DISCUSSION

The emergence of anthelmintic resistant spurred the search for plant-based antCics[25]. The medicinal plants contain various phytoconstituents and secondary metabolites that exhibit anthelmintic activity[26].

The primary objective of this study was to assess theanthelmintic properties of the ethanolic extract of *P. americana* leaves and aqueous extract of *M. sikkimensis* peel. The experiment was conducted using Indian earthworm *Pheretima posthuma* in order to determine the therapeutic capabilities of the extracts.

The experiment was carried out by preparing standard drug solution (Albendazole), *P. americana* leaf extract and *M. sikkimensis* peel extract, maintaining a concentration range of 5mg/ml, 10mg/ml and 20mg/ml respectively for each. The earthworms were kept in a beaker containing different solution of different concentration. All the concentrations of the extracts lead to paralysis and fatality of the earthworm.

It was evident that the aqueous extract of the peel of *Musa sikkimensis* in a dose of 20mg/ml, caused paralysis at 46.07 $\pm$ 1.45 min and death at 60.10 $\pm$ 1.54 min for earthworm, whereas the alcoholic extract of the leaves of *P. Americana* in a dose of 20mg/ml, caused paralysis at 50.27 $\pm$ 1.69 min and death at 68.76 $\pm$ 2.10 min respectively. While the standard drug albendazole it was recorded as 57.38 $\pm$ 1.55 min and 68.76 $\pm$ 2.10 min respectively.

From the results, it was found that *P. americana* and *M. sikkimensis* extract caused paralysis in less time in a dose dependent manner, as well as the death was caused much earlier compared to the control group. This suggests that *P. americana* and *M. sikkimensis* extract exhibited strong anthelmintic activity.

Furthermore, a link has been found between anthelmintic activity and high levels of flavonoids, terpenoids and tannins. Flavonoids, tannins and terpenoids found in various extracts have been proven to have anthelmintic properties in earlier investigations[10].

Persea americana have been reported to have alkaloids, flavonoids and tannins and *Musa sikkimensis* have been reported to have terpenoids and tannins. These results suggested that *P. americana* and *M. sikkimensis* extract may possess anthelmintic activity indicating its potential use as an anthelmintic medication.

Some of the compounds present in different plant extracts can be primarily responsible for their GABA-mimetic action, causing reversible paralysis of body wall muscle in the earthworm[26].

It may be also due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis[27].

The possible mechanism of the anthelmintic activity cannot be completely explained on the basis of our present results. While there needs further study to isolate and reveal the active compound contained in the crude extract of *P. americana* leaves and *M. sikkimensis* peel as well as to establish mechanisms of action. The current research outcome is very encouraging and may offer an alternative to anthelmintic medication, after further investigation and can be established clinically.

CONCLUSION

Results from this work has signified that that the aqueous extract of *M. sikkimensis* & the alcoholic extract of *P. americana* have exhibited potent anthelmintic activity when compared to commonly used medicine albendazole.

These findings suggested that the treatment groups caused paralysis in less time in a dose dependent manner, as well as the death was caused much earlier compared to the control group. This suggests that *P. americana* and *M. sikkimensis* extract exhibited strong anthelmintic activity.

Flavonoids, tannins and terpenoids found in various extracts have been proven to have anthelmintic properties in earlier investigations. *Persea americana* and *Musa sikkimensis* have been reported to have these phytoconstituents. These results suggested that *P. americana* and *M. sikkimensis* extract may possess anthelmintic activity indicating its potential use as an anthelmintic medication.

From these findings it can be concluded that *P. americana* and *M. sikkimensis* may serve as the potential treatment for parasitic disorder such as helmintic infections. Further, studies can be carried out to establish the fact clinically.

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